Maize for Asia:
Emerging Trends and Technologies

Proceedings of The 10th Asian Regional Maize Workshop

Makassar, Indonesia
October 20-23, 2008

Editor in Chief
Pervez H. Zaidi, Muhammad Azrai, and Kevin Pixley

Ministry of Agriculture Republic of Indonesia

CIMMYT
International Maize and Wheat Improvement Center

Asian Development Bank

S. M. Sehgal Foundation

10th ARMW
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The Indonesian Agency for Agricultural Research and Development (IAARD) (http://iaard.go.id) is the research arm of the Indonesian Ministry of Agriculture. There are 11 Research and Development Centers in IAARD, each of them has main function to manage research and development on food crops, horticulture, estate crops, livestock, veterinary, soil and agro-climate, agro-socio economics, machinery development, post-harvest, biotechnology and agricultural technology assessment. Within these centers, IAARD manages 15 research institutions, 3-research station and 31 assessment institution located throughout provinces in the country. IAARD has the vision to become a world class research and development institution in producing and developing agricultural innovations in supporting the realization of the industrial agriculture system.

Correct citation: Zaidi, P.H.; Azrai, M. and Pixley, K.V., eds. 2010. Maize for Asia: Emerging Trends and Technologies. Proceeding of The 10th Asian Regional Maize Workshop, Makassar, Indonesia, 20 - 23 October 2008. Mexico D.F.: CIMMYT

Abstract: This is proceeding of the 10th Asian Regional Maize Workshop held in Makassar, Indonesia during 20 - 23 October 2008, and co-organized by International Maize and Wheat Improvement Center (CIMMYT), Mexico and The Indonesian Agency for Agricultural Research and Development (IAARD), Indonesia. The theme of the workshop was "*Maize for Asia: Emerging Trends and Technologies*". The 10th ARMW brought together in Makassar, Indonesia, over 300 maize scientists, researchers and students from public and private sectors, including participants from China, Philippines, Thailand, Cambodia, Myanmar, Bangladesh, Nepal, Bhutan, India, Iran, Indonesia, Vietnam, Laos, Australia, Kenya and Mexico. The workshop had 65 oral and 108 poster presentations, and included invited lectures, research paper presentations, scientific deliberations and discussions on maize in Asia. Papers of the proceeding deals with molecular tools, for maize improvement, genetics and breeding, crop management, biotic and Abiotic stresses affecting maize, technology dissemination and country reports.

ISBN: 978-979-1159-41-8

Printed in Indonesia

FOREWORD

Maize has shifted from traditional food crop to industrial commodity. Use of maize for food and processed food industries has steadily increased. In recent decade, major maize producing countries reduced their export due to increased domestic demand for ethanol industry. This has affected maize supply in global trade. The global maize production is around 750 million ha. The US has the largest harvested area and contributed about one fifth of the global maize area, followed by China, Brazil, India, Mexico, and Argentina. East and South east Asian countries such as Japan, S. Korea, Taiwan, Malysia, Indonesia, Thailand, and Philippines are the major importers of maize. About 50% of the world maize is consumed by those countries. Indonesia, in fact is the number one maize producing country in South East Asia.

Substantial progress in maize production has been made in Asia. The success has been mainly attributed to the adoption of of improved technologies resulted from research and development. It is realized that the role of maize workers (researchers, extensions, policy makers, businessman) is important in the increased maize production in Asian region. In order to keep up with strong commitments for steady increase in maize production, the Asian Regional Maize Workshop was organized in Makassar, Indonesia during 20-23 Oct 2008. The workshop was a communication for a among maize scientist s and workers in the regions. The purpose of the ARMW were to gather new information and technologies, to exchange idea and to share experience.

The Asian Regional Maize Workshop was co-organized by the IAARD and the CIMMYT and by strong support by the governments of South Sulawesi province, Gowa district, Maros district. The theme of the workshop was Maize for Asia: Emerging Trends and Technologies.

The workshop was attended by some 400 participants from 22 countries including USA, Australia, Mexico, Switzerland, Cuba, China, India, Bangladesh, Nepal, Vietnam, Philippines, Iran. All papers presented in the workshop are published in this proceeding. In this special occasion, I would like to acknowledge the participation and contribution of all people who organized and attended the workshop and publish this proceeding.

Bogor, June 2010

Indonesian Center for Food Crops
Research and Development

Director,

Prof. Dr. Suyamto

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Section 1

Maize in Asia

Asian maize market opportunities small and large [1]

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Abstract. Asia's maize sector has shown rapid growth over the last decades. This paper reviews the evolving maize sector in Asia, focusing on the eight main maize producing countries: China, India, Indonesia, Nepal, Pakistan, Philippines, Thailand, and Vietnam. Taken together, these eight Asian countries now produce 98% of Asia's maize and 26% of global maize, but show major geographic variation in terms of maize economy indicators and dynamics. The paper flags some of the implications of Asia's maize sector for the poor and the evolving roles for the public and private sector this implies. Challenges and opportunities to accelerate maize production in Asia are reviewed. There remain significant growth opportunities for maize in Asia, and this calls for an integrated research and development approach to strengthen the innovation pathways needed to sustainably reduce poverty, enhance incomes and conserve natural resources.

Keywords: Maize; corn; Asia; markets; research and development; policy

Introduction

The second half of the 20th century has seen continuous growth in global maize production and this has converted maize into the leading global cereal in terms of production over the last decade (USDA-FAS, 2008). Despite the growth, the global maize market had two distinguishing and enduring features (Wada et al., 2008). First, some two-thirds of global maize was used as feed over the last 40 years. Second, the share of maize traded globally remained relatively constant.

The main global cereal markets – maize, wheat and rice – have seen some major adjustments in recent years. In early 2008 this resulted in a global food crisis as a result of a major spike in cereal prices (particularly rice and wheat), which particularly affected the poor, being net cereal consumers. Various factors have contributed to the food crisis. On the demand side there were the high oil prices that spurred an interest in bio-fuels and increased demand due to dietary change and income and population growth (e.g. Asia). High oil prices also put upward pressure on crop production costs (e.g. fertilizer, tillage). On the supply side there were low cereal stocks, supply shocks and declining agricultural research and development (R&D) investments exacerbated by restrictive cereal trade policies. Other contributing factors include the slide in the value of the U.S. dollar and speculation in commodity markets. Whereas the final word is still out on the relative importance of each factor, it is important to put the 2008 food crisis into perspective. For a realistic assessment of global cereal price trends one needs to: (i) consider real prices and not the headline nominal prices, so as to correct for inflation; and (ii) consider an extended time horizon (particularly beyond 2000 as cereal prices were historically very low, Gulati, 2008). The food crisis has since been overtaken by the global financial crisis in the latter half of 2008, with cereal prices again coming down.

Since the turn of the century, the global cereal markets have been affected by the resurgence of ethanol as biofuel in the USA. The rebound was spurred by subsidies, high oil prices, concerns about national fuel security and concerns about the environmental effects of fossil fuel use (e.g. Hazell and Pachauri, 2006). In the U.S., ethanol was of particular interest as it could be produced from maize, maize prices were relatively low, and the U.S. is the global leader in maize production and exports (Baker and Zahniser, 2006). By 2007-08, 82 million tons of maize were used for ethanol, representing a quarter of U.S. production and 12% of global production. The increasing ethanol demand is largely met by higher production. Still, the size and speed of the expanding use of maize by the ethanol industry is affecting the U.S. agricultural sector and has sparked a debate over the use of grain for fuel instead of grain for food or feed and the adequacy of future grain supplies (Economist, 2007; Hoffman et al., 2007).

[1] A slightly adapted version of the present conference paper is published as Erenstein, O., 2010. The evolving maize sector in Asia: Challenges and opportunities. Journal of new seeds, 11(1), 1-15.

Asian maize markets are variously integrated in the global economy and have shown even more rapid growth and dynamics. Diverting maize grain to ethanol production does not seem a viable political or economical option for now in the case of Asia. Still, two important implications for Asia should be noted. First, the demand for ethanol in the U.S. is significantly adding to the global demand for maize and thereby putting upward pressure on global maize prices. Second, 2nd generation technologies are under development to generate cellulosic ethanol (Baker and Zahniser, 2006); these new technologies are less contentious and are likely to lower the production costs of ethanol in the future and thereby increasingly displace starch ethanol from maize grain.

The remainder of the paper focuses on the maize sector in the Asian situation. The next section provides a brief review of the evolving maize sector in Asia – focusing on the eight main maize producing countries. The subsequent section addresses some of the implications of Asia's maize sector for the poor and reducing poverty. The last section flags some of the challenges and opportunities to accelerate maize production in Asia.

Asia's maize sector

The Asian maize sector has seen some rapid growth over the past 25 years. Several key factors supported these dynamics (Falcon, 2008). The economic policies of the Asian nations were overall favorable, providing the basic fundamentals for long-run economic growth. Asia's population growth slowed whereas income growth went up. There were radical changes in the maize sector in terms of technology development and use (rapid advent of hybrids), technology delivery (a larger role for the private sector) and surging demand (linked to a rapidly growing livestock sector).

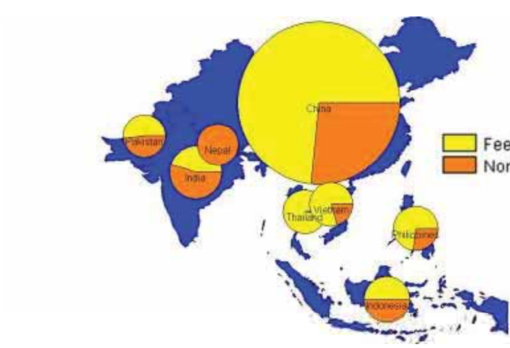

Figure 1 Geography of maize consumption in Asia-8 (TE 2006-07)
Source data: USDA-FAS, 2008

Geography is an important consideration in understanding the Asian maize sector. The Asian maize sector is dominated by China (Figure 1). China not only dominates regional production, China also stands out by having primarily (81%) temperate maize. China is followed at a distance by an array of seven other countries: India, Indonesia, Nepal, Pakistan, Philippines, Thailand and Vietnam.[2] Taken together, these eight Asian countries now produce 98% of Asia's maize and 26% of global maize.

Fueling the growth in the Asian maize sector is overall economic development and growth, particularly in terms of income growth per capita and increasing urbanization. This has two contrasting effects for maize demand (Figure 2). On the one hand, there is a surge in the indirect

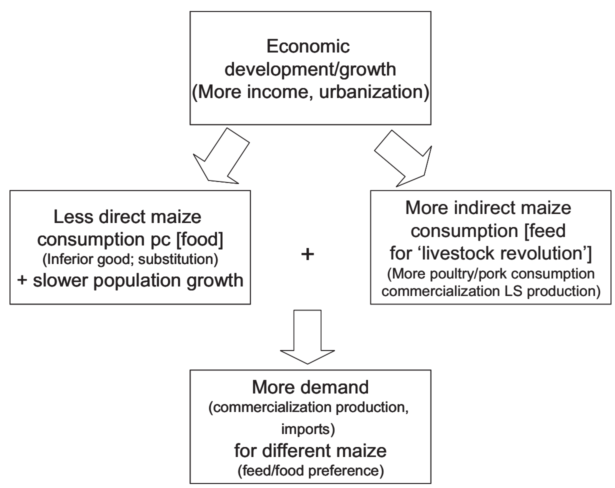

Figure 2 Drivers of maize sector in Asia

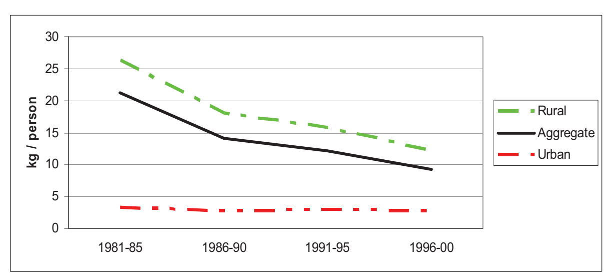

Figure 3 Declining maize food consumption, China
Source: Adapted from Huang & Rozelle, 2008

[2] These 7 countries are hereafter referred to as Asia-7 [ex-China], whereas Asia-8 includes the same plus China. The present paper differs from Gulati and Dixon, 2008 by including Pakistan, Asia's seventh largest maize producer.

consumption of maize, reflecting increases in poultry and pork consumption, the commercialization of livestock production and the associated use of maize as animal feed. Asia now produces more than half the world's pork and more than a quarter of global poultry. On the other hand, the growth in direct consumption of maize as food slows as population growth itself slows, and also as direct per capita consumption typically decreases, maize being an inferior good and hence substituted by superior products (Figure 3). The aggregate result is an increase in demand for maize met by commercialization of maize production and imports. It also implies shifting preferences for feed and food maize (Figure 2).

The implications for Asia's maize consumption over time are shown in Figure 4. Whereas aggregate consumption has surged in both China and Asia-7, non-feed use of maize has remained relatively constant and the growth primarily came from increasing feed use of maize. This implies an increasing share of maize as feed in overall maize consumption. The maize feed shares show considerable geographic variation (Figure 1), and there appears to be an inverse relation between maize feed shares and the incidence of poverty.

Figure 4 Changing maize consumption, Asia-8 (total consumption = non-feed [top, yellow] + feed [bottom, blue]).
Source data: USDA-FAS, 2008

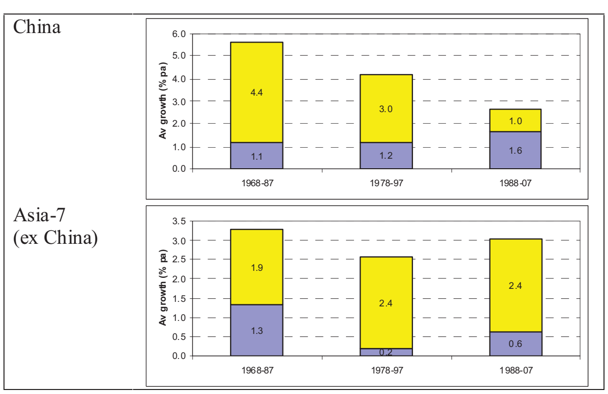

Figure 5 Diverse maize growth, Asia-8 (total growth = yield growth [top, yellow] + area growth [bottom, blue]). Source data: USDA-FAS, 2008

A number of maize sector indicators for the Asia-8 countries are presented in Table 1. These reiterate the significant regional variation in maize area, yields, production and net trade. The underlying dynamics for

Table 1 Maize indicators by country, Asia-8 (triennium average ending 2006-07)

Country	Area (M ha)	Yield (t/ha)	Production (M t)	Net trade (M t)
China	26.6	5.3	140.4	5.5
India	7.6	1.9	14.7	0.5
Indonesia	3.3	2.1	6.8	-1.0
Nepal	0.8	2.0	1.7	0.0
Pakistan	1.0	2.9	3.0	0.0
Philippines	2.5	2.3	5.7	-0.2
Thailand	1.1	3.7	4.0	0.1
Vietnam	1.0	3.8	3.9	-0.5
Asia-7 (ex China)	17.4	2.3	39.8	-1.0
World	146.9	1.8	709.1	

Source data: USDA-FAS, 2008

Table 2 Maize indicators over time, Asia-8

Country	Period	Area (M ha)	Yield (t/ha)	Production (M t)	Net trade (M t)
China	1968-77	17.2	2.2	38.2	-0.2
	1978-87	19.3	3.4	65.4	0.9
	1988-97	21.7	4.6	99.3	5.2
	1998-07	25.5	5.1	129.1	6.9
Asia-7	1968-77	13.5	1.1	15.0	1.6
(ex China)	1978-87	15.4	1.3	20.7	2.2
	1988-97	15.7	1.7	26.7	-0.3
	1998-07	16.6	2.2	36.3	-0.8

Source data: USDA-FAS, 2008

Table 3 Maize growth by country, Asia-8 (% pa, 1968-2007)

Country	Area	Yield	Production
China	1.3	2.8	4.1
India	0.6	2.1	2.7
Indonesia	0.6	2.1	2.8
Nepal	2.2	0.1	2.4
Pakistan	1.5	2.2	3.7
Philippines	-0.3	3.1	2.8
Thailand	0.3	1.9	2.3
Vietnam	7.2	3.5	11.3
Asia-7 (ex China)	0.7	2.3	3.0
World	0.7	1.8	2.5

Source data: USDA-FAS, 2008

China and Asia-7 are presented by period in Table 2 and growth rates by country in Table 3. Yield growth typically contributed most to overall production growth in Asia-8, except for Nepal where yields were stagnant and Vietnam where area increase provided an even bigger contribution. A breakdown of growth rates per period further highlights the diverse maize growth between China and Asia-7 (Figure 5). China's production growth slowed from its earlier high levels to its current rate more at par with Asia-7, albeit with a declining contribution from yield growth. Asia-7's production growth was relatively more constant and remained primarily dependent on yield growth.

Gerpacio and Pingali (2007) characterize maize production environments and systems in China, India, Indonesia, Nepal, the Philippines, Thailand, and Vietnam, synthesizing the findings from 7 more detailed country studies (e.g. Joshi et al., 2005; Meng et al., 2006). These studies conducted village-level and farmer-group surveys in the main maize production systems across Asia to examine technological and policy prospects for its sustainable intensification and to derive the corresponding R&D priorities.

Gulati and Dixon (2008) present a detailed analysis of government support and protection of the maize sector in the same seven Asia countries, with their results summarized in Table 4. Their results show major policy induced market distortions, both in terms of subsidies and taxes, with market liberalization likely to particularly hurt maize producers in Vietnam and the Philippines, and only China showing export competiveness.

Maize & poverty in Asia

In 2000 there were some 500 million poor people in Asia (including 200 M in E/SE Asia and 300 M in S Asia), which were expected to come down to 340 million by 2020 (including 100 M in E/SE Asia and 240 M in S Asia, Falcon, 2008). The declining poverty count is primarily associated with overall economic growth in Asia, with an estimated poverty elasticity of -1.0% for E Asia and -0.6% for S Asia per % change in real per capita GDP (Besley and Burges, 2003 as cited in Falcon, 2008). What are the implications of Asia's dynamic maize sector for the poor and reducing poverty?

Table 4 Government protection of maize, Asia, 1997-2000

	Importable hypothesis	Exportable hypothesis
China	Undistorted [Heavily taxed]	Subsidized [taxed]
India	H. taxed [ib.]	Subsidized [ib.]
Indonesia	Taxed	Subsidized
Nepal	Undistorted [ib.]	H. Subsidized [ib.]
Philippines	H. Subsidized [ib.]	H. Subsidized [ib.]
Thailand	Undistorted [ib.]	H. Subsidized [ib.]

Undistorted: Nominal protection coefficient (NPC): 0.95-1.05.
Using official exchange rates [shadow exchange rates].
Source: Gulati and Dixon, 2008

There are at least four important considerations in terms of maize-poverty linkages. First, the poor are net purchasers of food and thereby benefit from lower staple prices. At the same time, low staple prices limit the income for smallholders producing a marketable surplus. Second, direct maize consumers are typically poor. Indeed, maize is an inferior good and its consumption as food is associated with poverty. It has been estimated that more than 75 million poor people in Asia eat significant amounts of maize directly (Falcon, 2008). Third, there is an overlap between maize food consumption and a tradition of maize production (e.g. Nepal and tribal areas in India). Fourth, there is an overlap between poverty and maize production constraints. These linkages pose major challenges to poverty alleviation through the maize sector. It also highlights the need for targeted public goods and intervention in maize-poverty areas, including the supply of appropriate improved germplasm.

Maize production systems in Asia show a marked dichotomy (Table 5). On the one hand there is the commercial/non-traditional maize production to feed the livestock revolution, while on the other there is subsistence/traditional maize production feeding poor producers. The dichotomy is a simplification and in reality there is more of a continuum from subsistence/traditional to commercial/non-traditional systems. However, the dichotomy is useful as it raises important strategic

Table 5 Maize production system dichotomy

Orientation	Subsistence	Commercial
Maize tradition	Traditional	Non-traditional
Maize produced for	Food	Market/Cash
Poverty	High	Low
Production area	Marginal and/ or remote	Well endowed and/ or connected
Maize varieties	Open pollinated varieties (OPV)	Hybrid
Driver	Population growth (food demand)	Market (feed: livestock demand)
Growth potential	Low (stagnation)	High (dynamic)
Role private sector	Limited (no incentives)	Seed, inputs
Role public sector	High (provision public goods)	Limited

Source: Adapted from Falcon, 2008

questions. What are the opportunities and threats for livelihoods, poverty alleviation and natural resource management in each system? The two systems imply different roles for maize in poverty alleviation, particularly in terms of a direct role or indirect role (trickle down). What are the corresponding implications for R&D? This directly affects the relative role of the public and private sector. Where should NARES and CIMMYT focus their efforts: on both subsistence and commercial systems; on only one; or should there be a gradient of relative emphasis? The answer to these questions is context specific and involves potential trade-offs between equity and efficiency. The present paper would argue that the public and private sector should not compete head on; instead they have clear complementary roles (e.g. Gerpacio, 2003).

The complementary public-private roles should reflect the continuum of system needs, the continuum of basic to applied research, varying product needs (food vs. feed), and should be dynamic – i.e. there is a need to reassess the roles as systems evolve (e.g. relative strength of private sector in Nepal vs. India). As highlighted by Morris et al. (1998:55): "Profit-oriented firms are unlikely to assume functions that cannot easily be exploited for commercial gain, so government agencies will continue to play an important role in supporting basic research, conducting applied research targeted at marginal environments, collecting and disseminating market information, and establishing and enforcing industry standards. Public-sector involvement in commercial seed production seems destined to decrease, however, as the private seed industry gains in strength." This calls for a reassessment of public-private sector roles and linkages to avoid duplication and competition. Whereas the private sector increasingly caters for the more favorable/commercial maize regions, there will be an increased role of public sector agricultural research for less favorable/subsistence maize areas (Heisey and Edmeades, 1999; Pal et al., 1998; Singh, 2001). Indeed, the traditional maize growing areas have been relatively neglected and bypassed by the recent developments in the maize sector, thereby enhancing disparity. Their lesser market integration and tendency to recycle seed pose particular challenges. A real challenge will be alleviating poverty in Asia's poor subsistence maize producing areas, like India's tribal areas.

Challenges and opportunities

Asia's land frontier is effectively exhausted. Any increase in the maize area would therefore need to come from either an increase in the cropping intensity or substitution of existing crops. There are some prospects for increasing cropping intensity in single cropped areas (e.g. rice-fallow systems). However, these tend to be conditioned by having access to sufficient water to grow a second crop like maize, whereas most areas that can exploit (ground)water relatively easily have already done so.

Substitution of existing crops provides the biggest opportunity for increasing maize area. Such substitution is largely driven by the relative profitability of maize cultivation, thereby particularly aided by the high productivity of hybrids and the increased demand for maize. Environmental factors are providing further impetus to such a substitution, for instance in the case of India's two major cereals, rice and wheat. The impending water crisis will particularly affect the ability to continue to grow ponded rice. In the quest for economically viable water saving alternatives, maize emerges as an attractive option (e.g. in rice-rice systems in southern India). In response to the opportunities provided by the Green Revolution and public support for wheat, wheat cultivation has extended into areas which are relatively marginal for wheat. For instance, in the Eastern Indo-Gangetic Plains, wheat productivity is relatively low due to amongst others a short crop cycle and terminal heat stress. This again makes maize with its relatively high productivity an attractive option for the *rabi* season. Recent years have indeed seen a surge in maize area in for instance W Bengal and Bangladesh. Global warming is likely to aggravate these environmental stresses for wheat and rice in the future, and may imply an increased role for maize, a tropical C4 plant, in India's future agricultural landscape.

Improved varieties have transformed maize production in Asia and were a major contributor to the observed growth in maize production. In non-traditional maize producing areas improved varieties already are near universal and hybrids widespread. The increased seed cost of hybrids thereby seems more than off-set by the increased productivity, at least in the more favorable non-traditional maize environments. In the case of India, seed laws were liberalized in the late 1980s. Subsequently the private investment in maize research has risen sharply and seed companies have captured a significant share of the market (Morris et al., 1998; Pal et al., 1998; Singh et al., 1995; Singh and Morris, 1997). There is a need to extend the use of high yielding material into traditional maize environments. Incorporating tolerance and resistance against the main biotic and abiotic (particularly drought) stresses offers particular promise.

Increasing maize productivity in Asia's farmer fields, however, implies more than hybrids. One may argue that such increases would depend equally on germplasm, agronomy and policy (Braun, pers. comm.). Indeed, a major challenge relates to narrowing the gap between potential and realized yields. Crop improvement has boosted potential maize yields, but average farmer yields for most of Asia-7 are still relatively low (Table 1). Enhanced crop management practices offer particular scope to enhance the expression of genetic potential – but require an enabling environment that provides farmers the incentives to do so. For instance, in India weed management has proven problematic, and problematic weeds emerge as the main economic loss in traditional and non-traditional maize growing areas alike (Joshi et al., 2005). Again in India, the principal socio-economic constraints to maize production included ineffective technology dissemination and, in traditional maize growing areas, the non-availability of quality seeds (Joshi et al., 2005:23). Often farmers' needs are served by integrated combinations of technologies/ practices; for instance, integrated water management through genetic drought resistance and agronomic practices will reduce drought risk.

Quality protein maize (QPM) grain contains enhanced levels of two essential amino acids, lysine and tryptophan. These amino acids make more of the protein in the maize useful for humans and livestock who consume it. QPM may therefore offer a less expensive source of high protein animal feed which would be of particular interest to the poultry industry, as feed is the single largest cost item in poultry production, accounting for 55-64% of variable costs in India (Landes et al., 2004). The product is being further developed, tested, and promoted. For instance, in India five QPM hybrids (2 white and 3 yellow) have been released drawing from parental materials developed and supplied by CIMMYT. To address seed constraints, monitored seed production of yellow hybrids has been taken up by small-scale farmers in the Eastern Indo-Gangetic Plains. Although recent QPM hybrids produce yields that are competitive with other modern varieties, a number of issues remain for its widespread use (Lauderdale, 2000). Production, identification and distribution problems present particular challenges for the delivery of QPM to the target group, and may call for some novel institutional arrangements (Hellin et al., 2008).

Resource conserving technologies allow for savings in production costs and enhanced incomes that improve livelihoods, and can provide a stepping stone towards conservation agriculture. For instance, zero and reduced tillage are increasingly popular with wheat farmers in the Indo-Gangetic Plains. Some of these technologies can be readily transferred or adapted to maize systems, and particularly promising options for maize include zero-tillage and raised bed systems (Gupta et al., 2004). This is perhaps easiest in aerobic systems, like maize-wheat systems, but also offers promise in rice-maize systems (IRRI and CIMMYT, 2007).

The limited use of maize stover as animal forage after seed setting poses a further opportunity. Only in special situations are green maize plants used, for instance when grown as green fodder and after the harvest of maize cobs or babycorn. A recent study in southern India highlighted that amongst cereal stovers, maize stover was the least preferred feed due to its perceived high wastage and low palatability (Biradar, 2004). This is in stark contrast with maize growing areas in Latin America and Africa where maize stover is an important off-season livestock feed. Increased use of maize stover could ease forage deficits and rationalize crop residue management practices over space and seasons, and thereby allow for the retention of more sustainable levels of crop residue in the fields. Maize stover quality is one neglected research area that may merit further scrutiny.

Addressing these challenges and opportunities would benefit from a shift in the agricultural R&D paradigm. To enhance the client orientation and efficiency of Asia's agricultural R&D there is a need to broaden from reductionist, crop/plot level research to people centered, participatory and holistic system methods and to inter-disciplinary, multi-institutional approaches. This will assist in enhancing the targeting of interventions and priority setting of R&D and add to our knowledge base on maize livelihoods, value chains and impact pathways.

In conclusion

Asia's maize sector has shown an impressive performance in the recent past. Significant growth opportunities remain for maize; as well as some major challenges. This calls for an integrated R&D approach in terms of:

1. Thrusts, i.e. more than just hybrids, including agronomy and policy.
2. Partners, i.e. with complementary roles for the public and private sector;
3. Clients, i.e. enhancing and ensuring equity.

In the end, such an integrated R&D approach will strengthen the innovation pathways needed to sustainably reduce poverty, enhance incomes & conserve natural resources in much of Asia.

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Agriculture for nutrition: Maize biofortification strategies and progress

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Abstract. Nearly half of the world's population suffers from micronutrient malnutrition. Biofortification of staple food grains is a viable approach that complements fortification and supplementation strategies where balanced, healthy diets are unlikely to be accessible and solve malnutrition problems in the near future. CIMMYT, HarvestPlus, and several global partners conduct research programs to develop and disseminate maize varieties biofortified with provitamins A, lysine, or tryptophan. Recent research findings have made possible the use of marker assisted selection for favorable alleles of important genes in the carotenoid biosynthetic pathway, which is expected to increase the efficiency and effectiveness of future breeding work to increase provitamins A. Successful quality protein maize (QPM) cultivars have already been developed and released in many countries, and recently convincing evidence been published documenting the nutritional benefit to children of consuming QPM. This paper outlines current strategy and recent progress in developing biofortified maize at CIMMYT, which is paralleled at several partner institutions.

Keywords: Maize; nutrition; biofortification, quality protein maize, QPM, provitamins A, micronutrients

Introduction

CIMMYT, HarvestPlus, and many partners are developing biofortified maize to help alleviate malnutrition, which afflicts nearly half of the world's population. Micronutrient malnutrition, caused by inadequate consumption or utilization of iron, zinc or vitamin A, is known to be widespread, but protein and essential amino acid deficiency also compromises the health of many populations. While the preferred solution is for all to consume balanced, healthy diets, the reality is that many do not currently enjoy access to such diets. Furthermore, recent global trends, such as the "food crisis" of 2008, suggest that as food prices increase, consumption of nutrient rich meats and vegetables may decrease among poor consumers who are driven to consume relative more of cheaper, staple grains.

Biofortification of staple food grains can be an effective way to provide essential nutrients to consumers whose diets rely heavily on these grains. Other effective strategies include supplements and fortification of foods, but these are costly interventions that require repeated or continuous investments, whereas biofortified crops could be produced and regularly consumed, as long as appropriate varieties are available. The development and promotion of biofortified maize varieties has been an important effort at CIMMYT for more than 30 years, in the case of quality protein maize, and for about 5 years in the case of provitamins A.

Quality Protein Maize (QPM)

The history of QPM development and the present status of QPM adoption in Africa have been recently reviewed by Krivanek et al. (2007). In brief, QPM is maize that contains a naturally-occurring mutation, the *opaque-2* gene, which results in a changed ratio of protein types in the endosperm of maize grain. While the total protein content is no different between normal and QPM, the concentrations of lysine and tryptophan, which are the most limiting amino acids in the protein of maize, are about double in QPM than normal maize. CIMMYT and partners have developed numerous QPM hybrids and open-pollinated varieties (OPVs), and several have been released in various African, Latin-American and Asian countries.

Although the nutritional benefits of QPM have been amply demonstrated using animal models (particularly for pigs and chickens), many in the nutrition community remain skeptical about the value of QPM for human nutrition. With this in mind, Gunaratna et al. (In Press) conducted a meta-analysis of nine effectiveness studies conducted in Ethiopia, Ghana, India, Mexico and Nicaragua, with children consuming either QPM or normal maize. They found that consumption of QPM instead of normal maize led to a 12% (95% confidence interval: 7-18%) increase in weight gain, and 9% (95% confidence interval: 6-15%) increase in height of the children. These results clearly demonstrated the nutritional value and potential impact of QPM for populations that are deficient in lysine or tryptophan and rely on maize as an important source of dietary protein.

Micronutrient Biofortification of Maize

Pixley et al. (2007) reported about the opportunities and strategies for biofortified maize during the ninth Asian regional maize workshop held in Beijing, 2005. Since then, excellent review papers have been published by Ortiz-Monasterio et al. (2007) and Pfeiffer and McClafferty (2008). Therefore, the objective of this paper is to report selected highlights of recent progress at CIMMYT and within the HarvestPlus maize team.

Conventional breeding to increase iron concentration has been mostly discontinued at CIMMYT because of very limited natural variation found for this trait. However, collaborative research with the USDA Soil and Plant Nutrition Laboratory at Cornell has found significant genetic variation for bioavailability of iron in maize, opening a possible strategy which we thus far have not pursued due to prioritization of efforts as well as complexity and cost of the bioavailability assay. Selection and breeding to increase zinc concentration in grain is progressing, and genotypes have been found or developed with zinc levels that exceed the target level (about 40 parts per million (ppm)) set by HarvestPlus. One important complication applies to both iron and zinc: Because these minerals can not be synthesized by plants, they must be taken-up from the soil and translocated within the plant to the grain. This implies that the soil content of iron or zinc, and soil pH may be limiting factors, and even “efficient” genotypes may have low concentrations of these minerals when grown on certain soils.

Most of the biofortification work at CIMMYT concerns breeding of maize with increased concentrations of provitamins A carotenoids. Three cycles of intra-population S1 recurrent selection have been completed in three African OPVs, ‘Obatanpa’, ‘SAM4’ and ‘ZM301’. Yield trials have been grown or are growing at more than 10 locations in Mexico and Africa, and provitamins A concentrations will be quantified to calculate realized heritability for provitamins A, as well as to estimate correlated effects, if any, of selection for provitamins A on other traits (e.g. disease resistance, maturity or grain yield).

CIMMYT’s maize biofortification program is almost entirely devoted to inbred line and hybrid development, while developing OPVs as a spin-off of this work. The general breeding strategy is to cross elite lines from our breeding programs in Africa or Mexico with source lines that have 6-10 ppm of provitamins A. These F1’s are backcrossed to the elite line, and BC1F1 kernels are visually selected for intense orange or yellow color. Experience has convinced us that BC1-derived lines are unlikely to have >5 ppm of provitamins A, so we now routinely perform a second cross (“2nd dose”), BC1 by provitamins A source line. Best hybrids from this program have been obtained with H”8 ppm of provitamins A.

We have recently begun using marker-assisted selection (MAS) in the breeding program for provitamins A. This became possible after Harjes et al. (2008) published results of association mapping research, and PCR-based markers were subsequently developed to enable selection for favorable alleles of *LycE* (lycopene epsilon cyclase), a crucial gene in the carotenoid biosynthetic pathway. More recently, unpublished collaborative research between CIMMYT, the University of Illinois, and Cornell University, has found important allelic variation and developed useful markers for alleles of a second crucial gene in the carotenoid pathway, *CrtR-B1* (carotene beta-hydroxylase 1). We are now using MAS to develop better source lines, combining both of these favorable alleles together with above-average concentrations of provitamins A. Use of MAS promises to increase the efficiency and effectiveness of our breeding program for provitamins A, and further work is ongoing to validate the effects of these alleles in diverse genetic backgrounds and to increase the through-put and cost-effectiveness in our molecular laboratory.

Challenges and opportunities

The greatest challenges in this work are to develop biofortified cultivars that combine: 1) meaningfully enhanced levels of one or more nutrient, 2) competitive agronomic performance (e.g. yield, stress tolerance), 3) farmer acceptance, and 4) consumer acceptance. Data and evidence to date indicate that the technical goals can be achieved, at least in developing agronomically competitive maize with enhanced levels of provitamins A. At the same time, participatory variety selection programs, education and marketing efforts must be combined in a coordinated strategy that paves the way for adoption and consumption of biofortified maize varieties.

For further information, or to request seed of experimental biofortified germplasm, the reader is invited to contact the corresponding author of this manuscript.

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Utilization of lowland rice in increasing maize production in Indonesia

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Abstract. To meet the maize demand, maize area is expanded to new agricultural land. Besides this, maize cropping is also shifted to lowlands, where water is not adequate to support the growth of rice. Almost all lowland rice area in Indonesia is located in Java, Sumatera, Bali, Nusa Tenggara, Kalimantan and Sulawesi islands. On those islands, lowlands cover 7,391,384 ha, comprising 2,361,792 ha of technical irrigation, 1,119,615 ha semi technical irrigation, 1,758,280 ha non-technical irrigation, and 2,151,697 ha of rainfed lowland. Cropping intensity of rice in lowlands is tending to decrease, which indicates that there is an opportunity to increase cropping intensity in the lowlands by planting maize. Rice and maize in lowlands can be planted as rice-rice-maize, rice-maize-maize, or rice-maize-fallow cropping patterns. Lowland areas planting rice twice per year were 4,558,568 ha, located 2,327,647 ha in Java, 1,162,130 ha in Sumatera, 232,687 ha in Bali and Nusa Tenggara, 133,502 ha in Kalimantan, and 702,602 ha in Sulawesi islands. Lowland areas planted with rice once per year were 2,832,816 ha, scattered 995,871 ha in Java, 779,756 ha in Sumatera, 200,652 ha in Bali and Nusa Tenggara, 476,747 ha in Kalimantan, and 379,880 ha in Sulawesi islands. Planting maize after rice provided high profit because maize prices increased to their highest level. High yield obtained by planting hybrid varieties, along with high prices are resulting in expansion of maize area in the lowlands, which is increasing every year.

Key words: Lowland production, rice, maize

Introduction

Maize is an important food crop in Indonesia, and is also important as feed and industrial raw material for various industries. The demand for maize, especially for feed, has been steadily increasing over the years. Maize demand for feed has exceeded 50% of the national requirement (Suryana *et al*., 2008). This demand is larger than production, and has forced the government to import maize. In 2005, Indonesia imported 1.80 million tons of maize and in 2010 it is projected that imports will reach 2.71 million tons (Swastika, 2007). Therefore, national production must be pushed significantly to meet domestic requirements.

In 2007, the national maize harvested area was around 3.63 million hectares, with productivity of 3.66 t/ha (Deptan, 2008). In efforts to increase maize production, besides increasing productivity, the maize planting area was extended by using available land. Lowlands are agricultural lands that are compartmentalized and have small dikes to resist water, with rice typically as their main crop. The planting of maize on lowland areas usually happens in the dry season, and is a strategic step that can reduce the maize production deficit and help meet the demand. The grain quality produced in the dry season is much higher, and its price usually increases to its highest level.

One million hectares of lowland is usually neglected and left fallow after rice harvest. Those lowlands can be used to expand the maize growing area. Maize can be grown on lowlands more than once, especially for green maize. Thus, increased maize production on lowlands can be achieved through proper cropping systems and increasing cropping intensity.

A part of the irrigated lowlands can not be planted with rice because there is insufficient water for irrigation due to climate change, less water held in soil as a result of deforestration, and damage to some irrigation facilities. Irrigation water has become a main problem faced in rainfed lowlands. The irrigation water problem can be solved by use of surface water and underground water. The availability of engined pumps is very useful for utilization of water for maize irrigation. Maize grown on lowland can be damaged by unpredictable rains, resulting in water logging that can kill the maize crop. To anticipate that problem, drainage must be prepared.

Lowland area available

Lowlands are devided into irrigated and rainfed lowlands. The two lowland systems are identified based on the degree of water control. Irrigated lowlands have permanent irrigation infrastructure, and rainfed lowlands are irregularly flooded by rainfall runoff. Irrigated lowlands

Table 1. Irrigation systems of lowland area in Indonesia

Island	Lowland area (ha)			
	Technical irrigation	Semi-technical irrigation	Non-technical irrigation	Rainfed irrigation
Java	1,528,625	408,025	606,368	780,410
Sumatera	357,942	335,385	595,706	652,853
Bali and Nusa Tenggara	100,973	185,030	76,382	70,954
Kalimantan	24,569	33,748	194,303	357,629
Sulawesi	349,683	157,427	285,521	289,851
Total	2,361,792	1,119,615	1,758,280	2,151,697

BPS (2004)

are more uniform and generally have higher productivity. Meanwhile, large parts of the rainfed lowlands have low productivity potential.

Technical irrigated lowland is a rice field where the input drainage is separated from the output drainage to enable controlled water usage. Generally this type of irrigation consists of main drainage, secondary drainage and tertiary drainage. The main and secondary levels are completely controlled by the government.

Semi-technical irrigation lowland is a rice field where the input or output drainage are not controlled by the government. Non-technical irrigated lowland is a rice field with simple irrigation built by farmers. Rainfed lowland is a rice field where the irrigation depends upon the rainfall.

In efforts to meet the maize demand, maize cropping can be shifted to lowlands, where water in lowlands is not adequate to support the growth of rice. Almost all lowland rice area in Indonesia is located in Java, Sumatera, Bali, Nusa Tenggara, Kalimantan and Sulawesi islands. On those islands, lowlands cover 7,391,384 ha, consisting of 2,361,792 ha technical irrigation, 1,119,615 ha semi-technical irrigation, 1,758,280 ha non-technical irrigation, and 2,151,697 ha of rainfed lowlands (Table 1).

Lowland areas planted with rice twice per year were 4,558,568 ha, located 2,327,647 ha in Java, 1,162,130 ha in Sumatera, 232,687 ha in Bali and Nusa Tenggara, 133,502 ha in Kalimantan, and 702,602 ha on Sulawesi islands. Lowland areas planted with rice once per year were 2,832,816 ha, scattered 995,871 ha in Java, 779,756 ha in Sumatera, 200,652 ha in Bali and Nusa Tenggara, 476,747 ha in Kalimantan, and 379,880 ha in Sulawesi islands (Table 2).

These data indicate that vast lowland areas are available to be utilized for maize cropping. Since rice is a main crop for the lowlands, cropping systems must be arranged accordingly.

Table 2. Rice cropping and cropping index in lowland area in Indonesia

Island	Lowland area (ha)		Cropping index 2006*
	Rice cropping intensity		
	Once	Twice	
Java	995,781	2,327,647	1.7
Sumatera	779,756	1,162,130	1.6
Bali and Nusa Tenggara	200,652	232,687	1.54
Kalimantan	476,747	133,502	1.22
Sulawesi	379,880	702,602	1.65

BPS (2004)
*BPS (2006)

Cropping system

The rice cropping intensity in the lowlands has been tending to decrease (BPS, 2006), which indicates that there is an opportunity to increase cropping intensity on lowlands by planting maize. Rice and maize in lowlands can be planted as rice-rice-maize, rice-maize-maize, or rice-maize-fallow cropping patterns. Extremely, on irrigated lowlands in Baranti village and rainfed lowlands in Kulo village, Sidenreng Rappang district, farmers did not plant rice on irrigated lowlands due to lack of irrigation water. Farmers in those villages grow maize as maize-maize-maize cropping pattern (Fadhly *et al.*, 2008).

The degree of water control and availability determines whether maize is grown once or twice in this lowland system. Flooding for rice is assured only in the first season for much of Malang and Kediri, where the rice crop is followed by two maize seasons. Where better water control permits two rice harvests, only one maize crop is grown. It is not unusual to find a dry-season maize crop on lowlands that are technically irrigated (with full water control), which could grow three rice crops a year. In system with technical irrigation, the Department of Agriculture often reduces water supplies to farmers in the dry season to permit canal maintenance and to control pest build-up through enforced breaks in continuous rice cultivation. Residual moisture and limited irrigation supply in certain areas are sufficient for maize (Mink, Dorosh and Perry, 1987). On irrigated lowlands, farmers grow maize for one or two crops. The introduction of short duration rice varieties made this possible. The choice of rice variety determines the time available for the other two crop seasons. After choosing a short-duration rice variety, farmers usually grow two hybrid maize crops. It will be used at least for the first maize crop following rice. These cropping patterns are still practiced in those areas, and even have been developed in many provinces such as Lampung, North Sumatera, South Sulawesi and West Nusa Tenggara.

This cropping pattern is also found on a limited basis in South Sulawesi, primarily on the inland plain around Lake Tempe. Lack of irrigation on rainfed lowland and otherwise fertile soils, limits rice to one crop per year, during the time of peak rainfall, which floods the level, bounded plains. Rains during the first and third crop seasons are insufficient to grow flooded rice, but they permit growing maize on either end of the main rice crop. Hence two maize crops per year are possible in this area (Mink, Dorosh and Perry, 1987; Bahtiar *et al*., 2006).

In terms of better management technologies, production recommendations should include practices such as hybrid varieties, adjusting population densities, no tillage or minimum tillage, using animal manure and inorganic fertilizers, ridging systems to reduce run-off, planting with the onset of the first good rains, dry planting, and controlling weeds early in plant development. Planting maize after rice provides high profit because at that time maize prices tend to increase to their highest level. High yield obtained by planting hybrid varieties along with high prices are resulting in expansion of maize area in the lowlands, increasing every year.

Irrigation

Water is the main limitation to maize production in the lowlands. In general, maize needs a least 500-700 mm of well-distributed rainfall during the growing season. Drought during the crop establishment stage can kill young plants, reducing the plant density. The main effect of drought in the vegetative period is to reduce leaf growth, so the crop intercepts less sunlight. Around flowering, from about two weeks before silking to two weeks after silking, maize is very sensitive to moisture stress. Grain yield can be seriously affected if drought occurs during this period. During the grain-filling period, the main effect of drought is to reduce kernel number and size (Lafitte, 1994).

If the soil profile is at field capacity at the time of planting, 350 to 400 mm of well-distributed rain throughout the growing cycle is sufficient to produce a good crop. For optimum growth, soil moisture content should be at about 60 to 70 percent of the field capacity. Good deep soil, permitting the roots to grow down to 1.5 m, may have a moisture capacity of 1 cm of water per 6 cm of soil, i.e. about 250 mm of water. The main water source for the maize crop may be moisture stored in the soil before planting, from rainfall during the crop season, from irrigation and, in much lower amounts, from dew condensed on the leaves that is funneled by the leaf blades and stems to the base of the plants. In certain areas, water from rainfall and its distribution throughout the year is adequate for more than one maize crop per year (Violic, 2000).

There were 4,558,568 ha of lowlands planted with rice twice per year, and almost half of those were technically irrigated lowlands. Lowland area planted with rice once per year was 2,832,816 ha, and more than half of that were rainfed lowlands (Table 3).

Generally maize crops are grown early or during the dry season. The majority of maize crops planted in the dry season require irrigation water; although expensive but very important. If water is available, high input levels can be used with low risk, especially fertilizers, which are very effective when moisture is sufficient. In conditions of high natural soil fertility, water availability throughout the growing cycle permits the exploitation of this natural advantage.

Irrigation is an important factor in maize farming on lowlands. Various water sources for irrigation can be used, such as coverage rivers, dikes, dams, irrigation canals, and wells. Generally, farmers use drilled wells as a water source and irrigate their maize crops by using engined pumps. Farmers drill wells by themselves and they only purchase

Table 3. Rice cropping intensity in lowland areas by irrigation system

Irrigation system	Lowland area (ha)	
	Rice cropping intensity	
	Once	Twice
Technical	352,673	2,009,119
Semi technical	271,188	848,427
Non-technical	614,034	1,144,246
Rainfed	1,594,921	556,776

BPS (2004)

pipes, pipe glue and other necessary materials. Usually one drilled well is used for one hectare.

Drainage

Maize is very sensitive to excess soil moisture conditions; hence it is important that fields where this crop is grown be well drained. If the water-table is high, the development of the crop will be considerably hampered, and the crop will suffer due to the inability of the root system to adsorb adequate amounts of moisture. Maize is particularly sensitive to excess moisture at the seedling stage, when the growing point is below ground level, and will show a reduction in plant stand and retarded growth with consequent final yield reduction. At the knee-high stage of growth, an excess of moisture for three to six days may cause a yield reduction of up to 30 to 50 percent, but if this excess moisture occurs at he flowering stage, yield reduction will be less. In many cases, surface drainage may help reduce the excess of water (Violic, 2000).

To avoid excessive water due to unpredictable rain, drainage must be prepared. Drainage ditches are made all around and every 10-20 m across and/or lengthwise of area planted, depending on rainfall. The drainage ditches are prepared before planting.

Conclusions

Overall there were 7,391,384 hectares of lowlands consisting of 2,361,792 hectares with technical irrigation, 1,119,615 with semi-technical irrigation, 1,758,280 hectares with non-technical irrigation, and 2,151,697 rainfed hectares. Lowland area planted with rice twice per year was 4,555,568 hectares and planted with rice once per year was 2,832,816 hectares.

In efforts to increase maize production, maize cropping systems could be shifted to lowlands by promoting increased cropping intensity using rice-rice-maize, rice-maize-maize, or rice-maize-fallow cropping patterns.

Irrigation is an important factor in maize farming on lowlands. Various water sources for irrigation can be used, but farmers generally use drilled wells and irrigate their maize crops by using engined pumps. To avoid excess water due to unpredictable rains, drainage must be prepared.

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Yield potential and gap analysis of maize production in Sulawesi, Indonesia

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Abstract. Maize production in Indonesia is not stable, especially in areas of Sulawesi. Production and productivity achieved at the farmer level fluctuates and are still very low, so that a yield gap exists between yield achieved and yield potential. Based on time series data for 7 years (2001-2007), it was indicated that the average production and productivity vareid greatly, i.e: in North Sulawesi the average production was 200,985 t with productivity of 2.57 t/ha, in Central Sulawesi production was 64,664 tons with productivity of 2.45 t/ha, in South Sulawesi production was 698,198 tons with productivity of 3.26 t/ha, in Southeast Sulawesi production was 77,023 tons with productivy of 2.21 t/ha, and in Gorontalo production was 297,990 tons with productivity of 3.39 t/ha. Across Sulawesi, the average annual production was 1,338,862 tons with productivity of 2.78 t/ha. Thus, maize productivity in Sulawesi was still very low compared with yield potential and average yield of several superior varieties, both hybrid and composite, such that a 3-6.5 t/ha yield gap occurred. This yield gap could be overcome by the availability of a packet of locally specific planting technologies that could be easily and quickly adopted by farmers.

Key words: production, productivy and yield gap.

Introduction

In the regional development of agriculture, maize is a secondary crop after rice, and is mainly planted in the rainy season on dry fields and to a lesser extent during the dry season.

Growth rates in various regions are very different, and there is a prosperity gap in the society of every region. These differences are due, for instance, to differences in income across society, provided socio and economic infrastructure, and ownership of resources. Regional development is intended to equalize the distribution, it means that participatory planning and society involvement in all regions in the provincial development process to improve the strength of growth capacity in the region of a province, and also able to encourage the development of other provinces that are relatively less developed. It is necessary to increase the cooperation amongst provinces, and for producers and development efforts to be coordinated to benefit regional development.

Sulawesi, regionally, consists of five provinces, one of which is a very young province with different potential, particularly in agriculture and especially for maize.

There are two types of approached to regional development: sectoral and regional (Yusuf G, 1999). The sectoral approach is initiated with some questions: 1) Which sector must be improved to handle national development, 2) For each produced material, how is it produced, what technology is needed, and when it is produced, 3) When will the sector activity occur, 4) What policy, strategy and steps must be taken? Meanwhile, the regional approach focuses on the question, which region should receive the priority for improved, and what sector is most suitable to achieve improvement in region's society?

One of the regional approach policies supports the national program to production 2000 million tons of maize can be seen from the potential data in each region, particularly the capacity of the land, production, and productivity then. It can then be seen that there is a gap resulting from differences in natural resources, soil, climate/season, the availability of water, human resources, technology, technology adoption and management, and a number of problems in the region.

Based on the explanation above, it is necessary to know the yield potential of maize and the gap that occurs in farmers' fields, with the result of this research identifying the causal factors of the yield gap and how to handle them.

Methodology

The research was held by collecting data for the time series from 2001-2007 in five provinces in Sulawesi: South Sulawesi, North Sulawesi, Central Sulawesi, South East Sulawesi, and Gorontalo. The data were taken from BPS Indonesia and Gorontalo, Seed Certification Bureau of South Sulawesi, ICER, and other tabulated information. The collected data comprised: harvested area, production, seed distribution and productivity. The data were analyzed using tabular and descriptive methods.

Result and Discussion

General Potential of the Region

The landmass of Sulawesi is approximately 190-405 km^2, the largest province is South Sulawesi: 67,960 km^2, and the smallest one is Gorontalo: 12,214.44 km^2.

Of the total landmass, lands are used for yard, garden/ field, meadow, fishpond, state garden, field, and some is not used. Some of the land is used for food plants, particularly maize on 889,852 ha after rice and a secondary maize crop in rainy season on 1,711,251 ha.

Harvested Maize Area

The maize area in Sulawesi region, based on 2001-2007 data, was approximately 429,913.86 ha. The largest area is in South Sulawesi, followed by North Sulawesi, Gorontalo, South East Sulawesi and the smallest area is in Central Sulawesi. This can be seen in Table 1, which shows that the extent of harvested area in Sulawesi region increased with a growth rate of 9.35%; the highest rate was in Gorontalo, followed by Central Sulawesi, North Sulawesi, South East Sulawesi, and the smallest was in South Sulawesi. This shows that Gorontalo is the province with most rapid growth in maize area.

Maize Production

Based on the data for 2001-2007, the maize production in Sulawesi region always increased year by year, with an average production of 1,338,862.14 tons. The greatest maize production was obtained in South Sulawesi, followed by Gorontalo, North Sulawesi, South East Sulawesi, and the least was in Central Sulawesi (Table 2).

Table 1. The Harvest Area of Maize in Sulawesi Region, 2001 – 2007

No	Year	Harvested Area (ha)					Sulawesi Region
		North Sulawesi	Central Sulawesi	South Sulawesi	Southeast Sulawesi	Gorontalo	
1	2001	68443	20330	191960	28771	36610	346114
2	2002	55659	21850	205909	33789	45718	362925
3	2003	65656	22309	213818	37927	58716	398426
4	2004	66250	22657	199310	35827	60773	384817
5	2005	88854	26553	210336	32658	88376	446777
6	2006	82189	25587	206387	33343	109792	457298
7	2007	115664	40371	262214	40975	118816	578040
Average		77531	25665	212848	34756	74114	424914
Growth Rate (%)		11.26	13.75	5.84	6.71	22.45	9.35

Sources: BPS, Statistik Indonesia (2002, 2005 dan 2008), BPS, Statistik of Gorontalo Provence, 2002 and Gorontalo in Figures 2008

Table 2. The Maize Production in Sulawesi Region, 2001 – 2007

No	Years	Production (ton)					Sulawesi Region
		North Sulawesi	Central Sulawesi	South Sulawesi	Southeast Sulawesi	Gorontalo	
1	2001	150459	49095	515405	60358	131420	906737
2	2002	116867	48498	661005	68148	130251	1024769
3	2003	144668	49177	650832	87650	183998	1116325
4	2004	150124	53450	674716	78147	251214	1207651
5	2005	195305	67101	719139	73154	400046	1454745
6	2006	242714	66433	696984	74672	416222	1497025
7	2007	406759	118897	969306	97037	572784	2164783
Average		200985	64664.4	698198	77023.7	297990.714	1338862.14
Growth Rate(%)		3.53	3.12	2.03	1.56	4.94	2.72

Sources: BPS, Statistik Indonesia (2002, 2005 dan 2008), BPS, Statistik of Gorontalo Provence, 2002 and Gorontalo in Figures 2008

Tabel 3. Maize Productivity in the Sulawesi Region, 2001-2007

No	Years	Productivities (ton/ha) North Sulawesi	Central Sulawesi	South Sulawesi	Southeast Sulawesi	Gorontalo	Sulawesi Region
1	2001	2.19	2.41	2.68	2.10	2.23	2.32
2	2002	2.10	2.22	3.21	2.02	2.85	2.48
3	2003	2.20	2.20	3.04	2.31	3.13	2.58
4	2004	2.27	2.41	3.44	2.22	3.46	2.76
5	2005	2.72	2.52	3.42	2.24	3.51	2.88
6	2006	3.00	2.59	3.33	2.24	3.70	2.96
7	2007	3.51	2.94	3.69	2.37	4.81	3.47
Average		2.57	2.45	3.26	2.21	3.39	2.78
Growth Rate		1.42	0.60	0.98	0.37	2.36	1.16

Sources: BPS, Statistik Indonesia (2002, 2005 dan 2008), BPS, Statistik of Gorontalo Provence, 2002 and Gorontalo in Figures 2008

Maize Productivity

Maize productivity in the Sulawesi region always increased based on the data for 2001-2007, and averaged approximately 2.78 t/ha. The highest productivity was in Gorontalo; with 3.39 t/ha, followed by South Sulawesi (3.26 t/ha), North Sulawesi (2.57 t/ha), Central Sulawesi (2.45 t/ha), and the lowest was in Southeast Sulawesi (2.21 t/ha), as seen in Table 3.

From Table 3 it can be seen that the average productivity growth rate in the Sulawesi region was approximately 1.16% per year. Among the five provinces, the highest productivity growth rate was in Gorontalo (2.36%), followed by North Sulawesi (1.42%), South Sulawesi (0.98%), Central Sulawesi (0.60%) and the slowest growth was in Southeast Sulawesi (0.37% per year).

Table 4. OVP Improved Varieties Released Since 1980 by IAARD

No	Varieties	Years Released	Yield potential (t/ha)	Productivity (t/ha)
1	Arjuna	1980	-	4.3
2	Bisma	1995	7.5	5.7
3	Gumarang	2000	8.0	5.0
4	Kresna	2000	7.0	5.2
5	Lamuru	2000	7.6	5.6
6	Sukmaraga	2003	8.5	6.0
7	Srikandi Kuning	2004	7.9	5.4
8	Srikandi Putih	2004	8.1	5.9
9	Arjuna	2006	6.6	4.6

Sources: BPS, Statistik Indonesia (2002, 2005 dan 2008), BPS, Statistik of Gorontalo Provence, 2002 and Gorontalo in Figures 2008

Yield Gap

The 2001-2007 data show that maize productivity remains much lower in farmers' fields (Table 3) than the yield potential determined in several multi location tests by the Indonesian Agency for Agricultural Research and Development (IAARD) (Table 4). IAARD has released a total of 63 high yielding varieties, both OPV's and hybrids. In 2008, the planted area for maize in Indonesia was covered 43.7% with hybrids, 30.0% with composites and 26.3% with local varieties (ICER, 2008)

The yield gap is caused by several constraints, including: 1. Biophysical constraints, 2. Economic and Socio-cultural constraints, and 3. Institutional constraints.

Biophysical constraints comprise: Biotic constraints (pests, diseases and weeds) and abiotic constraints (soil fertility, drought, floods, etc.)

The most frequent constraints to maize growth are pests and diseases either in the field or in storage. The loss of yield caused by pests varies from 20-80% (Baco et al, 1983) and the damage caused by *downy mildew disease* can reach 100% (Sudjono, 1988), spoiled stalks 50% (Shurtleff, 1980). Generally, maize planting in particular months is often attacked by pests, for instance in May-August corn borer (*Ostrinia furnacalis*), October-March corn ear worm (*Helicoverpa armigera*) and April-September army worm (*Spodoptera sp).*

Maize varieties that are resistant to *downy mildew*, *ear rot*, and *sheath blight* have been known (Wakman et.al, 2001; Wakman and Hasanuddin, 2004); nonetheless, not all farmers plant such varieties. Also, there are many maize varieties released recently for which their resistance against pests and diseases in unknown.

The most common abiotic constraint is drought, which is caused by poor temporal distribution of rain. Yield decrease occurs if drought occurs during the reproductive phase, particularly on annual planting pattern: Rice – Palawija is very risky because of the lesser availability of plants, especially maize.

Socio Cultural and Economic Constraints

Maize production and productivity in farmers' fields is still much lower than the yield potential for several varieties released by ICER because of the use of poor production facilities; and failure to use high quality seed, fertilizer, herbicide and pesticides according to recommendations. This is caused by expensive input prices and limited farmers' capital and knowledge. Moreover, from the social and cultural perspectives, the ownership of land is narrow and widespread, and most farmers do not own the land, so they are very dependent on the owner of the land. Besides, labor is expensive and quality of education is poor, so the mastery of knowledge and technology affects the technology adoption.

Institutional Constraints

Economic organizations in the rural region are often lacking or distant. This makes it difficult for farmers to obtain production facilities and working-capital. Therefore, it is necessary to establish credit organizations and production facility stores in every village, where these are easily reachable by the farmers. It is necessary to form or activate farmer groups and extension services in delivering technologies, so that farmers can easily adopt and apply technologies to increase their production and income, and create a prosperous society.

Conclusion and Suggestions

1. Global maize production has risen in Sulawesi, despite fluctuations during the seven years 2001-2007.
2. The increase in harvested area, production and productivity of maize has the greatest potential in Gorontalo among all Sulawesi Provinces.
3. In general, there remains a large yield gap between farm level yields and yield potential for several varieties both composites and hybrids.
4. The constraints to production include biophysical, economic, socio-cultural, and institutional (e.g. extension services, etc).
5. Cooperative research and development should be conducted on Zea mays production and utilization.
6. Efficient databases should be developed to facilitate the sharing of information.

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Current status of maize development in Vietnam and strategies to meet demand by 2020

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Abstracts. Vietnam was a maize exporting nation until 1994; however, Vietnam now imports 0.5-0.7 million metric tons (MT) of maize for animal feeds. By 2020, the Vietnamese population is projected to be 100 million people, and the demand for maize for animal feeds is projected to be 8 – 9 million MT. At present, the average yield of maize in Vietnam is 4 t ha^{-1}, lower than potential yield in trials of 12-14 t ha^{-1}. The reasons result from: 1) maize production in Vietnam is mostly under difficult conditions, in which 80% of maize area is under rainfed, acid soils, sloping lands, small-scale farmers, difficult transportation, low level of social and economic conditions; and 2) inadequate investment in research on cultural (crop management) techniques. To meet the demand for maize by 2020, strategies of the maize program in Vietnam are set up to: 1) Develop hybrids with high and stable yield (12-13 t ha^{-1}) for intensive farming maize areas by combining conventional and modern biotech breeding; 2) develop maize hybrids tolerant to abiotic (drought, acid soil, water logging, poor soils) and biotic stresses for difficult regions; 3) develop transgenic (genetically modified, GM) maize hybrids not only for herbicide and BT, but also resistant to abiotic stresses (drought, low temperature, acid soils...); 4) develop maize hybrids with short growth duration (7-9 t ha^{-1}) suitable for crop rotations and additional crop per year; 5) create vegetable corn (waxy, sweet, baby and QPM maize hybrids); 6) study cultural techniques for maize on slopping lands; and 7) Study ICM/IPM for the main maize growing areas.

Keywords: Vietnam, maize production, maize demand, , maize cultural techniques

Introduction

Maize production in Vietnam has achieved great progress since 1994, doubling total production every six-year period (over 1 million MT in 1994, 2 million MT in 2000 and 4 million MT in 2007 - Figure 1). However, demand for maize for animal feed is projected to be 6.5-7.1 million MT by 2015 and 8-9 million MT by 2020. This is an ambitious target given the situation of limited maize growing land (difficult to exceed the 1 million hectares grown in 2007), and the diverse ecological, social and economic zones of Vietnam. This big gap is being solved by developing high yielding hybrids by combining conventional and modern maize breeding methodologies, issuing flexible policies for multinational and domestic maize seed companies, more investment in post harvest technologies, and investing more effort in developing cultural techniques to increase profits from production. Following is a description of the current status of maize development in Vietnam and strategies to meet demand by 2020.

Current status of maize development in Vietnam

Progress and obstacles to maize production

The average yield of maize in Vietnam (Figure 1) has rapidly climbed from 34% of the world average (11/32 quintals ha^{-1}) in 1980, 42% in 1990 (15.5/37 quintals ha^{-1}), 60% in 2000 (25/42 quintals ha^{-1}), 73% in 2005 (36/49 quintals ha^{-1}), to 77% in 2007 (38.5/49.7 quintals ha^{-1}) (FAOSTAT, 2007). However, this is still lower than the world average, and the production has not met the national demand of 6.5-7.1 million MT by 2015 and 8-9 million MT by 2020. So, the importation of maize in Vietnam has increased from 50 thousand MT in 2001 to 700 thousand MT in 2007 (Table 1).

The reasons why our maize yield is lower than the world average can be summarized as follows:

Objective obstacles:

- Maize production in Vietnam is mainly based on rainfed conditions (>80%), of which >60% is on slopping land or acid soils, and is conducted by small-scale farmers, with difficult access to transportation, and low level of social and economic conditions;

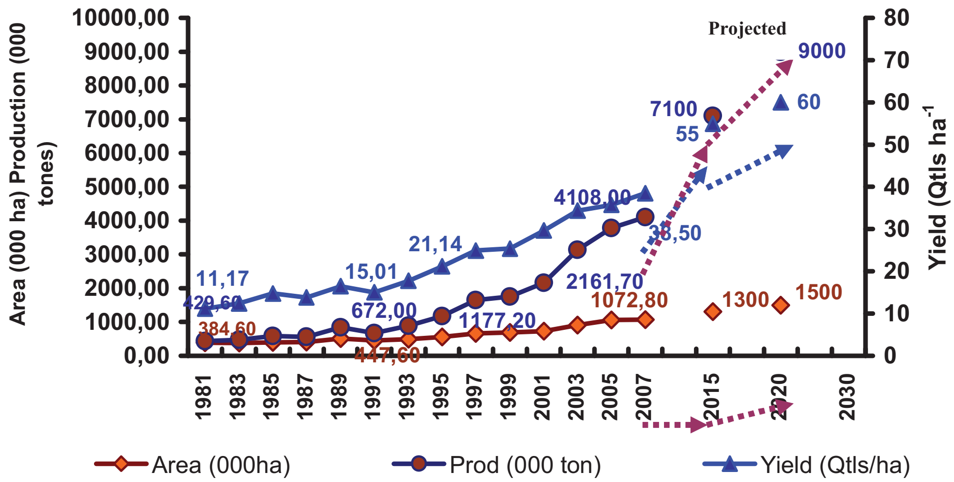

Figure 1. Maize production from 1981-2007; projected for 2015 and 2020

Table 1. Maize production and trading in Vietnam from 1985 to 2007

Year	Total production (Tones)	Import (Tones)	Exportation (Tones)
1985	584.9	0	
1990	584.9	2,000	
1996	1,536.7	35,500	227,000
2000	2,005.9	197,430	8,814
2001	2,161.7	50,000	24,000
2002	2,511.2	300,000	4,774
2003	3,136.3	200,000	50,000
2004	3,453.6	300,000	80,041
2005	3,757.0	236,292	2,515
2006	3,810.0	500,000	
2007	4,108.0	700,000	

Source: (Department of animal husbandary, 2007); (Thanh and Neefjes, 2005)

- Eight ecological zones in Vietnam are very distinct and variable in terms of temperature, rainfall, sunshine, storms and typhoons in the tropical monsoon climate;
- Farmers' cultural techniques and agro-input investment for intensive maize farming production are variable from region to region and generally are at low level.
- Low or inadequate investments in research on cultural techniques;

Subjective limitations:

For several decades there has been inadquate investment in research on cultural techniques to determine suitable population densities and spacing, doses of N, P, K, efficiency of rainfed and irrigated maize, herbicide utilization, etc. Therefore, cost of production was higher than that in other countries in the region (In 2005, cost of one ton of maize was US$84.35 in Thailand, US$116.11 in the Philippines, but was US$120 in Vietnam (Thanh and Neefjes, 2005)).

Strength of maize breeding and extension in Vietnam

Our local and imported maize germplasm, as well as inbred line nurseries suitable for Vietnam, are rather diversified. Maize breeders at the National Maize Research Institute and seed producers in several seed companies who understand well our climate and weather conditions are rich in maize breeding as well as extension experience. Our maize biotechnology laboratory is sufficiently equipped for genetic diversity analysis, doubled haploid production by anther culture, and genetically modified corn (GMC). Therefore, our germplasm products are comparable to those of other countries in the region.

Our maize extension workers are proficient in training farmers, demonstrating, and organizing field days and workshops.

Opportunities and challenges

Opportunities

Many foreign seed companies are locating in Vietnam, which facilitates exchanges of information, materials, and advanced methods of breeding and marketing. Multi-national maize hybrids are good checks for Vietnamese maize hybrids. Cycles of new maize hybrids which are adapted for large-scale production are generally 2-3 years, much faster than before.

Challenges

- There has been strong competition among national maize breeding programs and foreign maize organizations.
- Introduced maize hybrids may spread diseases for production.
- Maize in Vietnam is still under small scale production (each household in the Red River delta plants maize on 0.03 - 0.05 ha) with high costs of production.
- Technologies of post harvest and product processing are still very weak and not yet systematic.
- If maize cultivation on slopping land is not done sustainably, soil will be degraded rapidly.
- It will be difficult to meet the demand for maize by expanding maize growing area due to strong competition with other crops such as soybean, cassava, sugarcane and land reservation for industry. This is demanding maize hybrids with yield of 10-12 t ha^{-1} on favorable area and 6-7 t ha^{-1} on difficult land, with stable yield across regions. On the other hand, open pollinated maize varieties (OPVs) are still needed for mountainous regions.
- Application of maize biotechnologies such as Bt maize and drought tolerant maize are just at initial stages.
- 80% of the maize area is under rainfed and in the mountainous regions with difficult communication system, poor and low knowledge level of producers.

Strategies for production and research

Point of view

The Vietnamese agricultural ministry approved our point of view:

1) Enhancing commercial maize development which is competitive in the world market, with a view to meeting demand of animal feeds, processing industry and participating in the grain exportat market;
2) Maize R & D are for sustainable food program at national as well as household level, toward nutrition security and food safety;
3) Breeding maize hybrids which are able to participate in hybrid seed exportat market;
4) Finding solutions for higher economic efficiency of maize production, more profitable for producers, while maintaining environments;

Table 2. Proposed maize production in Viet Nam by 2015 and 2020

		2005			2010			2015			2020		
Region	Area	Yield	Prod	Area	Yield	Prod	Area	Yield	Prod	Area	Yield	Prod	
Whole country	1043.3	36	3756.3	1200	45	5400	1300	55	7150	1500	60	9000	Justification
Northeast	220.8	29.7	655.8	250	35	875	270	45	1215	280	49	1372	additional maize autumn crop and maize on spring fallow land
Northwest	156.4	25.7	401.9	170	42	714	170	56	952	170	65	1105	As above
Red river delta	81.9	40.9	335	70	45	315	80	67	536	90	70	630	Decreased due to expanding vegetable area
North central coast	149.4	34.7	518.4	160	42	672	170	48	816	180	50	900	Increased thanks to enlarging autumn maize and more maize replacing rice with limited water
South central coast	39.1	37.6	147	70	45	315	90	55	495	100	57	570	Increased thanks to expanding summer autumn maize (avoiding flooding)
Central Highland	231.2	40.9	945.6	260	50	1300	280	60	1680	280	60	1680	Enlarged by extending second maize crops
Southeast	129.5	43.5	563.3	130	52	676	140	57	798	280	68	1904	Expanded thanks to widening drought tolerant maize on second maize crop and spring – winter maize.
Mekong river delta	35	54.2	189.7	90	60	540	100	66	660	120	70	840	Increased by shifting 3 rice crops into 2 rice – 1 maize crop

Area: 000 hectares; Yield: Quintals ha^{-1}; Production: 000 tones

5) Closely cooperating with other domestic and foreign institutions on maize R & D in order to quickly transfer better hybrids to farmers.

Target

General target

The Vietnamese population is projected to be 100 million people by 2020. Thus by 2015 maize production is planned to be expanded to 1.3 million ha with yield of 5.0-5.5 t ha^{-1}, 6.5 – 7.1 million MT of maize, and by 2020 to 1.5 million ha with yield of 5.5-6.0 t ha^{-1}, total output of 8-9 million MT of maize grain.

Technical targets

For basic research

The Government is intensively investing in research on GMC for drought tolerance, herbicide resistance, and Bt genes; recombinant DNA to accumulate favorable genes into high yielding maize inbred lines with yield of > 6 t ha^{-1}; and application of male sterility on hybrid seed production.

For applied research

Toward targets for each region, we are trying to develop high yielding maize hybrids tolerant to diseases, drought and suitable to cropping systems: 5-7 hybrids (12-13 t ha^{-1}) for favorable areas, 8-10 hybrids (6-7 t ha^{-1}) for unfavorable regions, 1-2 QPM hybrids, 1-2 sweet corn hybrids, 4-6 waxy maize hybrids, 2-3 early maturing hybrids for additional crops and avoiding flooding. Besides, a small percentage of maize producers also need some OPVs for mountainous regions and 2-3 hybrids with high biomass yield for animal husbandry. The duties of our maize researchers are to study for:

1) Establishing intensive crop management (ICM) (NPK doses, densities, times of irrigation, rotation, intercropping, relaying etc) and site specific nutrient management (SSNM) for each hybrid, with lower cost of production for each region;
2) Determining sustainable maize cultivation methods for slopping land; applying mechanization with a view to lowering costs of production, increasing profitability and enhancing commercial maize production;
3) Improving hybrid seed technologies to enhance the quality of seeds and lower cost of production;
4) Opening markets and technologies for post harvest, drying, processing of seeds and commercial grain, food grain.

Specific production target for each region

The specific plan of area, yield and production of maize, and justification for each region are detailed in Table 2.

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Review of current high yielding hybrid corn research and development in Myanmar

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Abstract. Maize is the second most important cereal crop in Myanmar, where it is used in the poultry and livestock feed industries, as human food in rice deficit areas and its surplus contributes to foreign exchange earnings. In most major maize growing countries, high yielding hybrid corn varieties are increasingly replacing OPVs and hence the productivity of corn in those countries is much higher than in Myanmar. With rapid development of the livestock and feed industries in the country, and rising domestic demand, there is scope for expansion of the maize growing area in the future. In order to increase productivity in Myanmar, there is a strong need to develop hybrid corn and gradually replace OPVs with hybrids. To develop superior locally adapted inbred lines, hybrid corn research has been conducted at the Department of Agricultural Research (DAR), Yezin since 1975. Furthermore, DAR has collaborated with CIMMYT in maize research development activities since 1978. In this respect, DAR successfully developed a high yielding local hybrid, yielding 35-40% over the OPVs, in 1990. Up to now, six hybrid maize varieties have been released, including two varieties for the tropics, two for the sub-tropics and two for both tropical and sub-tropical regions. The current maize cultivated area is approximately 300,000 ha, and the national average yield was 3.16 MT/ha in 2006-07. Hybrid corn varieties which are more productive than the existing hybrids are in the breeding pipeline at DAR, and drought resistant and low nutrient tolerant varieties will be released in the near future.

Keywords: Hybrid corn, Research & Development, OPVs, Productivity, Myanmar

Introduction

Among the cereal crops grown in Myanmar, maize is the second most important after rice. The bulk of corn grain produced in the country is mainly used in the poultry and livestock feed industries. A small portion is also used as human food, and for export, contributing to foreign exchange earning. The area under corn has gradually increased from 133,000 ha in 1989/99 to more than 327,000 ha in 2006/07 (table-1). During the same period, the annual production of corn grain has increased from 194,000 MT to 1,032,000 MT, which is partly attributed to increasing area and partly due to larger adoption of high yielding hybrid corn varieties. Presently, the hybrid corn area in Myanmar has reached 30% of the total corn area in the country. Corn cultivation is mainly concentrated in Shan State, Sagaing Division, Chin State and Magway Division (Table 2). Although, the annual grain yield went up from 1710 kg/ha in 1996 to 3455 kg/ha in 2006, with an annual growth rate of 7.3 %, the present national yield of 3455 kg/ha is still lower than the Asia-Pacific regional average yield (Table-3). The major causes for this low productivity of corn in Myanmar are: 1) inbreeding depression of local as well as released OPVs due to low frequency of quality seed replacement, 2) slow rate of hybrid adoption, 3) lack of seed production markets and distribution system, 4) lack of knowledge by farmers about the use of quality seeds, 5) high cost of inputs (mainly chemical fertilizers), 6) use of traditional post-harvest operations (drying, shelling, cleaning and storage) resulting in loss and poor quality of grain or seed, 7) isolation problems that prevent expanding the area of hybrid seed production on farmers' fields, 8) abiotic stresses (drought, low soil fertility, high soil acidity, high temperature at reproductive stage, water logging stress), and 9) biotic stresses (diseases-turcicum

Table 1. Total sown area, production, and productivity of corn in Myanmar.

Year	Sown ('000 Ha)	Harvested ('000 Ha)	Yield (MT/Ha)	Production ('000 MT)
1989-90	133	126	1.58	194
1995-96	167	162	1.70	275
1999-00	210	203	1.72	349
2001-02	251	251	2.12	532
2003-04	284	284	2.48	704
2005-06	321	320	2.87	918
2006-07 (Prov)	327	327	3.16	1032

Source: Myanmar Agriculture at a Glance, 2007. Department of Agricultural Planning, Ministry of Agriculture and Irrigation, Yangon, Myanmar.

Table 2. Total sown area (Acres) of corn cultivation in States/Divisions (1999-2005).

State and Division	1999-2000	2000-2001	2001-2002	2002-2003	2003-2004	2004-2005	2005-2006
Kachin	12052	17071	18229	12916	14667	18036	20720
Kayah	25600	18786	20779	15453	15654	16928	16824
Kayin	4996	5178	5416	1548	2630	6085	22541
Chin	68655	69726	76731	77736	84187	85200	90037
Sagaing	167662	175582	197341	116983	125754	137531	180048
Tanintharyi	367	536	693	287	136	284	50
Bago	29407	32608	32842	4805	5184	4322	5238
Magway	238840	248689	276545	82808	83850	85036	85868
Mandalay	94074	117658	114451	48200	47195	42930	42114
Mon	1606	1900	2299	1	54	12	-
Rakhine	1107	1000	1434	-	-	-	20
Yangon	2261	2370	3477	150	72	95	16
Shan	209875	235264	272277	256622	275806	289494	298625
Ayeyarwady	23851	47939	64150	46150	47742	38237	30613
Union total	880353	974307	1091643	663659	702931	724190	792764

Source: Statistical Year Book, 2006, Central Statistical Organization, Nay Pyi Taw, Myanmar, 2008.

Table 3. Corn yield (kg/ha) in Myanmar and other Asia countries (1996-2006)

No.	Country	1996	2003	2004	2005	2006	Annual growth rate (1996-2006)
1	China	5204	4815	5122	5289	5289	0.2%
2	Rep. of Korea	4030	4140	4260	4200	4730	1.6%
3	Thailand	3778	3667	3749	3913	4116	1.8%
4	Vietnam	2498	3436	3462	3600	3702	4.0%
5	Indonesia	2480	3241	3344	3428	3470	3.4%
6	Myanmar	1710	2477	2630	2842	3455	7.3%
7	India	1709	2041	1880	1886	1938	1.3%
8	Asia Pacific Average	3862	3372	3555	3912	4409	1.3%
9	Rest of the World	4365	4817	5423	5305	5180	1.7%
10	World	4214	4520	5000	4937	4871	1.5%

Source: Selected Indicators of Food and Agriculture Development in the Asia-Pacific Region 1996-2006

blight, banded leaf and sheath blight, stalk rot disease complex, and pests - grasshoppers and cutworms in the seedling stage, and maize borer at the late stage). However, field insects do not pose many hazards to the crop and the use of plant protection measures against any of these pest hazards is almost negligible. Therefore, hybrid corn research is needed to enhance the use of high yielding hybrid corn varieties in Myanmar.

Current Status of Hybrid Corn Research and Development Activities

In order to increase the productivity and total production of corn in Myanmar, a long term hybrid corn research and development program was initiated at the Agricultural Research Institute (ARI) (at present DAR), Yezin in 1974-75 with the following objectives.

- To produce hybrids promising in terms of yield, resistant to pests, with good agronomic characters and broad adaptability.
- To promote and extend hybrid corn seed production programs to fully meet the local demand.
- To strengthen human resources in hybrid seed production technology through training.
- To gradually replace the OPVs with hybrids in major corn growing regions.

Furthermore, for improving the maize productivity in Myanmar, ARI has been collaborating with CIMMYT in the area of maize research activities since 1978.

Development of High Yielding Hybrid Corn Varieties in Myanmar

For the development of high yielding hybrid corn varieties in Myanmar, the following steps are being taken to achieve the national target.

Development of homozygous inbred lines

Development of homozygous inbred lines is the most important initial step in hybrid production. Genetically diverse exotic and local germplasm are used as source materials for the extraction of inbred lines. Exotic germplasm ranges from open-pollinated varieties, synthetics, inbreds and hybrids with adaptation to the tropics and subtropics. Most of the exotic germplasm has been introduced from CIMMYT, Mexico. The plant breeding methods used for inbred line development are standard methods. Plants with good agronomic characteristics, free from diseases and insect pests are selected, self-pollinated and separately harvested. Selection and self-pollination has to be carried out for 6-7 years or seasons (i.e. 6-7 generations for selection and self-pollination) to obtain fixed inbred lines. Since 1974, DAR's corn breeding program has developed and tested more than forty thousand homozygous inbred lines for their agronomic characters and combining ability. Currently, DAR has five thousand six hundred and twenty inbred lines from different selfing generations (i.e. S_1 to S_7 generation) which are undergoing selection and self-pollination to develop homozygous inbred lines.

Testing of homozygous inbred lines and hybrids

After the development of fixed inbred lines, they have to be tested for their combining ability. A testcross program is initiated by crossing the established inbred lines with a common tester. The resultant testcross hybrids are evaluated against outstanding check varieties at different locations. The best 10-15% of the testcross hybrids is selected based on multi-location evaluations. The parental inbred lines of these test crosses are put into a diallel mating scheme in which every individual inbred line is manually crossed with every other inbred line to produce experimental hybrids. These hybrids are evaluated at research stations across several locations to identify superior hybrids. Development of inbred lines and hybridization to produce testcross hybrids and experimental hybrids are being conducted at DAR, Yezin. Evaluation of testcross hybrids and experimental hybrids are being carried out at DAR, Yezin and Tatkon, which represents lowland tropics, and at Aung Ban, Kyauk Mae, Naung Mon and Loikaw Research Farms, which represent the highland subtropical agro climatic region. Promising hybrids identified from the multi location testing program are put into demonstration trials in the following year on farmers' fields in the major corn growing regions. Hybrids require two years of on-farm testing/demonstrations before they can be released to the farmers. Each year more than 36 on-farm demonstrations are carried out in collaboration with MAS (Extension Division) to identify the hybrid(s) most preferred by farmers.

Hybrid corn release

The hybrid corn research program was initiated in 1974-75 as a complementary and parallel program with the population improvement program. The program was successful in developing Yezin Hybrid Corn No.1 and No. 2 in 1990 (CARI 1992). Yezin Hybrid No. 3 was released in 1993 (CARI 1995), and Yezin Hybrid No. 4, No. 5 and No. 6 in 1996, 2000 and 2005 (CARI 1998, 2002 and DAR 2007), respectively. Yezin Hybrid No.1 and No. 2 are medium early hybrids with lowland tropical adaptation, and Yezin Hybrid No.2 is resistant to Banded Leaf and Sheath Blight. Yezin Hybrid No. 3 and No. 5 are medium maturity varieties with mid altitude subtropical adaptation. These varieties are highly resistant to Helminthosporium Leaf Blight, which is prevalent and serious in the Shan State. Yezin Hybrid No. 4 and No. 6 are early maturing hybrids and highly adaptable to both lowland tropics and hilly Shan State. In addition to their resistance to Helminthosporium Leaf Blight, these three hybrids are highly resistant to Banded Leaf and Sheath Blight, which is serious in the lowland tropics. Yezin Hybrid No. 4 is a three way cross (TWC) hybrid, and Yezin Hybrid No.6 is a double cross hybrid, whereas all other hybrids are single crosses (SXs). The released hybrids have a yield advantage of 35-40 % over the existing OPVs. The area under these hybrids has increased from 5,300 ha in 1999-2000 to over 60,000 ha in 2007-2008. There are good prospects for expansion of the area planted with Yezin hybrids provided that there is sound infrastructure for hybrid seed production and an efficient marketing system.

Hybrid corn seed production

Inbred line seed increase

Seeds of female (seed) and male (pollen) parental lines have to be multiplied to the quantities required for hybrid production. The two parental lines have to be grown separately in complete isolation to maintain a high degree of genetic purity. At present, parental seed production is mainly done at DAR, Yezin and Tatkon Research Farm.

F1 hybrid seed production

In the hybrid seed production fields, six rows of female are alternately planted with two rows of male parent. Removal of tassels (detasseling) by hand has to be done for the female rows before they start shedding pollen. Rouging, or the removal of off-type plants, has to be done before flowering. Seeds produced on the female parent are harvested for use as F1 hybrid seed. In the early years of hybrid development, until 1996, hybrid corn seed production was carried out at Yezin, Tatkon Research Farm, Heho Seed Farm and Myaemon and Wettoe Extension Farms under Myanmar Agriculture Service (MAS). From 1998 to 2001, in addition to those farms, hybrid corn seed production was carried out jointly between DAR and Extension Division on farmers' fields in Pyinmana, Leway and Tatkone Townships. At present, F_1 hybrid seed production is being carried out at DAR, Yezin and Tatkon research farm for the lowlands, Naung Mon and Kyauk Mae for Northern Shan State, and Aung Ban for Southern Shan State, based on the seed demand by the growers in the respective regions.

Training Programme

DAR conducts a one week training course on "Hybrid Corn Seed Production Technology" for junior technicians and field assistants from the Myanmar Agriculture Service (MAS) farms and Township Extension Service. The training program is conducted two times per year.

Requirements for Research Development

In order to increase corn production in Myanmar, there are essential requirements to expand the hybrid corn development program, including: 1) more trained persons in the area of hybrid corn research and seed production, 2) physical facilities on the farms for quality hybrid seed production, 3) adequate seed processing and storage facilities on the research farms, 4) isolation techniques in hybrid seed production, and 5) participation of the private sector in hybrid seed production and marketing. The two essential elements for a hybrid development and utilization program are the genetic resources and a viable seed production, distribution and marketing system. At present, DAR and its satellite farms produce F_1 hybrid seed and sell directly to the growers at a fixed price. The leftover or unsold seeds are collected to be sold as grain at a very low price.

CP Thailand is one of the few hybrid corn seed companies that has been involved in hybrid corn seed marketing since 1999. The company can afford to sell seed on consignment basis in addition to offering other incentives to clear its stock. Today, CPDK 888, CP's most outstanding corn hybrid, occupies some 30% of the total hybrid corn area of the country.

Other topics that require further research are the development of appropriate seed treatment to improve stand establishment, particularly at sub-optimal conditions, identification of cost-effective packaging materials, and treatments to minimize seed deterioration.

Future Outlook

In boosting total production and productivity of corn in Myanmar, it is imperative that Myanmar should gradually replace OPVs with the hybrids in major corn growing regions. The development of locally adapted superior parental lines and hybrids is now being carried out at DAR, Yezin. Hybrid corn varieties which are more productive than the existing hybrids are in the breeding pipeline and will be released in the near future. DAR, along with MAS, produces F_1 seeds annually only to a limited extent. Without a strong seed industry, hybrid corn varieties will not be spread to local farmers effectively and rapidly. In many developing countries, indigenous companies (without R & D) are producing hybrids bred by the public sector, whereas companies having their own R & D produce their own hybrids as well as the seeds of hybrids bred by the public sector. About 90% of seed production is being undertaken by private sector organizations and this sector is playing a very important role in increasing corn production in developing countries. In summary, it is critically important that hybrid corn R & D is given a favorable policy environment to ensure a significant increase in private sector investment in the seed sector, which is the delivery vehicle for improved technology in agriculture. DAR would like to continue firmly in collaboration with CIMMYT and other organizations to increase the momentum of the maize sector development in Myanmar.

Conclusion

Myanmar is an agricultural country and its economy will continue depending on agricultural production in the foreseeable future. At present, maize is one of the important commodities in earning foreign exchange as well as generating income for farmers. In this context, it is clear that maize research and development plays a critical role for increasing productivity in terms of hybrid variety development, hybrid seed production, and agronomic practices. Among other things, involvement of the private sector needs to be encouraged in hybrid seed production and distribution of hybrid varieties to sufficiently cover the area under expansion with maize. Maize farmers should be provided enough working capital and institutional arrangements for provision of inputs. Since Myanmar's maize started entering the international market, it has been mandatory to ensure that its quality specification meet the needs of international markets. Thus, high yielding hybrid corn research and development is needed as a priority for the development of the agricultural sector in Myanmar.

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Maize: An emerging crop of Bangladesh

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Abstract. Maize (*Zea mays* L.) is the 3rd most important cereal crop of Bangladesh. From the trends of its increase in demand, yield, acreage and production in recent years it seems that maize is going to hold the 2nd position next to rice in a few years. The crop has several advantages over other crops, like ability to grow two crops in a year, flexibility in planting time, and lesser vulnerability to stress and wind conditions. Although maize research started in the early 1970's, its acreage and production did not increase much until the mid 1990's. With the introduction of hybrid varieties and recent growth of poultry industries, maize cultivation has expanded faster than any crop in the past. The present production is above 23 million tons from around four million hectares of land producing at a rate of 6 tons per hectare. The Bangladesh Agricultural Research institute (BARI) performs research (variety and technology), the Bangladesh Agricultural Development Corporation (BADC) produces seed, and the Department of Agricultural Extension (DAE) conducts technology transfer activities. Besides, several NGOs and private seed farms are introducing exotic varieties from multi-national companies and directly marketing them to farmers. BARI has released 11 hybrid varieties in recent years and eight OP varieties earlier. CIMMYT is giving all sorts cooperation, like material supply, manpower development, etc. BARI-released varieties are competing well with the multinational varieties. At this moment, there do not seem to be many problems with maize cultivation, but in future there may be some threats like price hike of fertilizers, sheath blight, leaf blight and water stresses.

Introduction

Bangladesh is a small but densely populated country. More than 150 million people live on 144,000 sqm (1045 persons per sqm). This once highly resource-full country now has become depleted due to high population, frequent natural disasters, man made blockages of natural water resources, global warming and climate change. The mighty rivers passing through Bangladesh before falling to the sea have become silted and cause over flow and flash flooding during monsoon, causing crop damage and sometimes loss of human and animal shelters and lives. During the dry season, salt water rises and causes crop damage and scarcity of irrigation water.

The country has mostly flat land, uniform climatic conditions and two cropping seasons, an 8-month hot and humid monsoon , and a 4-month dry and mild winter . The dry period starts in November, the hot and humid season starts in March, and the monsoon generally starts in April or May. Dry season crops (called Rabi crop) are completely irrigated, while early monsoon crops (Kharip 1) are partly irrigated. Full monsoon crops (Kharif 2) are water loving, grow well under full time water and some times even get submerged. Full monsoon starts in late May or early June and continues to October.

Rice is the sole crop in the Kharip 2 season. Irrigated rice is also grown on the major part of the lowlands during the Rabi season, but the higher lands are covered with potato, wheat, pulses, oilseeds and vegetables. The Kharip 1 season is covered with irrigated rice (called Boro rice), maize and vegetables.

Maize had not gained much popularity in Bangladesh until the mid 1990's, although research and extension work had been done since 1970 (Table 1). After the booming of the poultry industry and introduction of hybrid maize along with shelling and processing technologies, maize production has increased faster than any other crop in the country. Farmers of Bangladesh have accepted the maize crop next to rice because of its year round production,

Table 1. Area, acreage and yield of maize in Bangladesh

Year	Area (000 ha)	Production (000 tons)	Yield (t/ha)
1971-72	2.80	2.20	0.79
1975-76	2.50	2.00	0.80
1981-82	2.40	1.00	0.42
1985-86	4.05	3.00	0.74
1991-92	3.60	3.00	0.83
1995-96	10.13	32.00	3.16
2001-02	30.05	172.37	5.74
2002-03	45.00	258.75	5.75
2003-04	70.00	400.00	5.70
2004-05	93.00	479.00	5.15
2005-06	137.00	784.00	5.72
2006-07	221.62	1268.61	5.40
2007-08	382.00	2361.00	6.18

Source: DAE

flexibility in planting time and lesser susceptibility to high temperatures and other natural hazards like wind, rainfall, weeds, pests and diseases. The intensive efforts of researchers, seed producing agencies, extension agents and international cooperation from institutions like CIMMYT have made it possible to take the crop to the grass root level of Bangladeshi people. The Plant Breeding Division of BARI deserves the major share of the credit because it has been trying to introduce and improve the crop since the early 1970's.

Maize can be grown twice yearly in Bangladesh. Its greatest advantage is its flexibility of planting time. Two weeks early or two weeks delay of planting do not matter much, so farmers can harvest their previous crop at full maturity and plant maize at the most convenient time for them. During Rabi season, maize can be planted starting from October and until mid December, when cold waves come. During Kharip 1, maize can be planted starting from early February until late March, but planting later than March may be risky because of heavy rains during harvest time. Seed production and research under poly cover may be possible if needed.

Research Progress

Variety development

Systematic research on introduction and improvement of maize started just after the liberation. During the 1970's and 1980's research activities were limited to OP variety development, production technologies and adaptation to local climatic conditions. Shelling, marketing and utilization were the big challenges during that time. Three OP varieties were released during the 1980's, three more in the 1990's and two in 2002. Yields of these varieties ranged from 5 to 7 tons per hectare. Although the yield was low, farmers could maintain their varieties and produce their own seed. There was not much risk due to lack of isolation distances, maintenance of inbred lines, or pollination control.

Attention was given to introduction and development of hybrid varieties in the mid 1990's. There was lot of debate on whether we should go for hybrid varieties or not. The Plant Breeding Division of BARI took the challenge and started research on hybrid varieties in concurrence with the OP variety programs. CIMMYT took the pioneer opportunities to support BARI in all research aspects of hybrid variety development including research material supply, manpower development, and technical and financial support. The first hybrid variety of Bangladesh was released in 2002, and euring the last six years, 11 hybrids (BHM 1 to 11) were released by BARI. These include single cross, double cross, three way cross and top cross hybrids (Table 2). Recently released BARI hybrids are competing well with the varieties of multinational companies. The yield potential of the BARI released hybrids varied from 9 to 12 t/ha. The yield of the Kharip season crop is generally 2 to 3 tons less compared to Rabi crop. Crop duration is also 20 to 30 days less in Kharip season.

Most of the research activities at present consist of three major categories, namely (i) Development of inbreds, (ii) Development and evaluation of hybrids, and (iii) Maintenance and seed production of inbreds and hybrids. Availability of isolation fields is a limiting factor for seed production and maintenance of inbreds and parental lines.

It is also worth mentioning that the Plant Breeding Division has developed a new laboratory capable of conducting molecular research. A small study has been completed lately, and we hope to continue with elaborate and extensive programs in the coming seasons.

Table 2. BARI maize hybrid and their parents (CML= CIMMYT Maize Line, BIL= BARI Inbred line)

Name of the hybrid	Type of hybrid	Parents
BARI hybrid maize 1	3 way cross hybrid	(Ki32 ×Ki31) Ki 42
BARI hybrid maize 2	Single cross hybrid	CML 287 ×CML 298
BARI hybrid maize 3	Single cross hybrid	CML 285 ×CML 287
BARI hybrid maize 4	Top cross hybrid	BML 1 × Barnali
BARI hybrid maize 5	Single cross hybrid	CML 161 × CML 165
BARI hybrid maize 6	Single cross hybrid	CML 431 × CML 486
BARI hybrid maize 7	3 way cross hybrid	(CML 431 ×CML 486) ×CLO 2450
BARI hybrid maize 8	Single cross hybrid	BIL 77 × BIL 31
BARI hybrid maize 9	Single cross hybrid	BIL 79 × BIL 28
BARI hybrid maize 10	Single cross hybrid	BM7 × BML 36
BARI hybrid maize 11	Single cross hybrid	BIL 111× BIL 113

Production technology

Scientists of the Plant Breeding Division have been conducting studies in collaboration with other Divisions of BARI like Soil Science, Agronomy, Pathology, Entomology and Economics. Planting at 70 x 20 cm spacing at a fertilizer rate of 250, 120, 120, 40, 5 and 1 kg N, P_2O_5, K_2O, S, Zn and B per hectare has been identified as the best practice for hybrid maize. Grain filling is severely affected in acid soil due to unavailability of boron. Short duration and shallow rooted crops can be successfully grown as intercrops of maize. Relay cropping with potato and vegetables can save 15 to 25 days for the maize crop. Potato-maize-rice or vegetables-maize-rice is a very good cropping pattern in Bangladesh. It has also been found that transplanting of maize seedlings or planting seed under zero tillage with mulch can be easily practiced if needed.

On-farm verification trials

Before releasing a variety, promising varieties are evaluated in farmers' fields in order to see their adaptability and farmers' preferences. Trials are done through our research substations and On-Farm Research Division of BARI in collaboration with Agricultural Extension Department (DAE). Each year 50 to 100 trials are done throughout the country covering all agro-ecological zones.

Technology transfer

Released varieties are distributed to farmers through DAE. Each year, 200 to 400 samples are distributed per variety to farmers throughout the country. The farmers are selected by DAE. Private seed companies, NGOs and cooperatives are also involved in the extension programs for seed production and popularization of the crop. Twenty to forty field days per season are conducted each year at the demonstration trials in order to motivate the farmers in the vicinity. Last year, 450 demonstration trials and 15 field days were conducted. Training of farmers and DAE officials is a regular practice of BARI. Whole family training has been found very effective for adoption of the new technologies. Last season, 232 whole family training events and 2524 farmers were trained by BARI scientists (Table 3). Private seed companies and NGO workers are also included in the training programs.

Table 3. Training on production technology

Year	No. of whole family training	No. of farmers
2001	28	225
2002	228	1821
2003	389	3110
2004	370	3684
2005	250	2000
2006	280	2456
2007	232	2524

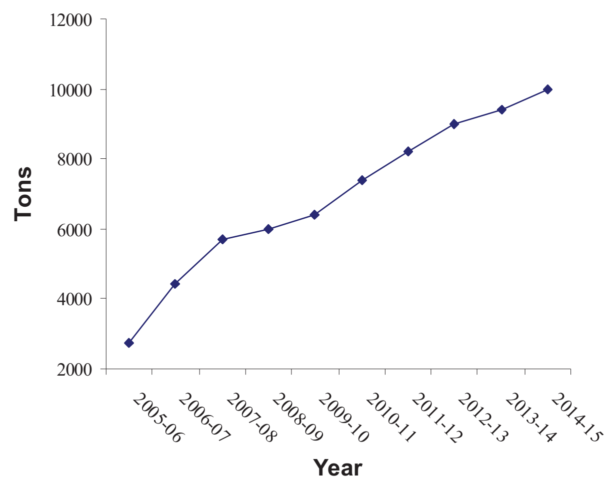

Fig. 1. Hybrid maize requirement in Bangladesh

Seed production

Unavailability of seed is one of the major limiting factors for expansion of maize production throughout the country. BARI is producing limited quantities of Breeder seed and distributing to BADC, private seed companies, NGOs and other private organizations. BARI also provides training and technical assistance to them for hybrid seed production and commercialization. BARI's contribution towards seed supply to farmers does not exceed 7%. Private seed companies are taking the major share.

Several seed companies are importing seeds of different hybrid varieties from Thailand and India. Bangladesh needs to import about 5000 tons of hybrid seed per year, and the demand is increasing day by day (Fig. 1). Local production of hybrid seed is also increasing, but progress is too slow to meet the demand. During 2007, BARI distributed 14 tons of Breeder Seed to different organizations and farmers. BRAC, Lal Teer Seeds, Supreme Seed, RDRS and a few elite farmers have started producing hybrid seeds of BARI released varieties as well as their own selected varieties, but that does not exceed 2000 tons in total. The rest is imported.

Marketing

Bangladesh needs about 2 to 3 million tons of maize grain per year. The maximum production was last year (around 2 million tons) because of rice crop damage due to two flash floods in the central north part and a tidal bore in the southern part of Bangladesh. Price fall significantly affected maize farmers. Bird Flu significantly reduced the demand from the poultry industry. During the previous years, 1.5 to 2.0 million tons of grain was imported.

Utilization

Maize is well known for its versatile uses. In Bangladesh, the principal use of maize is in the poultry industry. It is also a good source of nutrition for animals and fish. Human consumption as food is not significant, but rural people are using maize in various forms. Maize flour is partially mixed with wheat and used as chapati. Roasted and boiled cobs are popular among tribal people. Baby corn is still limited to hotel consumption. Popcorn is gaining popularity among urban people. The demand of maize as fodder is increasing very fast.

Challenges

BARI released varieties are competing well with the multinational varieties. At this moment, we do not seem to have many problems with maize cultivation, but in future there may be some threats like price hikes of fertilizers, sheath blight, leaf blight and water stresses. Breeding for stress tolerance is an important need to address the future problems of global warming and climate change. Breeding white grained varieties is a demand for use of maize as a substitute for wheat.

Two open pollinated maize varieties released in Cambodia

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Abstract. Maize was possibly introduced and grown in Cambodia during the late 17th to 18th century. It is the second food crop after rice in terms of cultivated area and economical importance. Maize is grown in the wet season on the fertile soil along the river delta and the red soil of upland slopes. Experiments were conducted in three provinces of Kandal, Kampong Cham, and Pailin, and at the CARDI field station in Phnom Penh. Main selection criteria were days to male and female flowering, plant height, ear height, and grain yield. Seventy maize genotypes, including open pollinated populations, hybrids and inbred lines of normal and quality protein maize introduced from CIMMYT were tested along with four local checks during the wet season of 2003, and dry season 2004. Ten outstanding germplasms, including three QPMs were selected for further testing in advanced yield trials at CARDI, Kampong Cham, Kandal and Pailin for two seasons and two years. As the result, two well adapted populations, namely COTAXTLA-S0031 and S-99TLWQ-HG-AB, which were the most preferred by farmers and out-yielded their own varieties by 5-6%, were recommended to farmers under the names of Loeung Mongkul for COTAXTLA-S0031 and Sar Chey for S-99TLWQ-HG-AB.

Introduction

The introduction of maize to Cambodia is possibly associated with the visits of European missionaries to the country during the late 17th and early 18th centuries. Maize was quickly adopted by the Cambodian farmers and became one of the most important food crops in the country. Presently, maize ranks second after rice and is cultivated in a wide range of production environments stretching across the country, from upper to lower Mekong delta and from upland to lowland fields. However, its production is concentrated mainly in Pailin, Battambang, Kampong Cham and Kandal (MAFF, 2007). Its production systems vary greatly depending on various factors including crop growing environment, cropping patterns and socio-economic conditions of the farmers. It is grown as a single crop, as an intercrop or mixed crop with others, or in the backyard of farmers' homes. In the areas along the river delta of Mekong and Basac, maize is grown in the late dry season just before the onset of rains. The planting time is critical as the crop should be harvested before the arrival of the flood waters to the field. In these areas, the crop is generally planted in March to April and harvested in August or September. Farmers can also plant another maize crop when the flood waters completely recede from the field or approximately in January. However, in the upland regions of the country, maize production starts either late during the dry season or in the early wet season, depending on the location. In Pailin, the northwest region of the country, farmers plant as early as February, but in Kampong Cham, in the central region of the country, farmers plant as late as June and July. In some places, although it is not common; farmers irrigate the crop, especially when the demand is high.

In Cambodia, maize has traditionally been grown for food and to a lesser extent for feed. It is quite common and popular for maize to be eaten fresh (boiled) when it is still in the early dough stage. For this case, glutinous maize is mostly preferred. Some times maize is cooked with rice as a staple food, especially when the harvest of rice is not sufficient. In addition, maize is also used to prepare several kinds of desserts. However, with the recent expansion of animal production systems, plus the growing demand in international markets, a shift from food to feed maize has been observed. Consequently, the demand for feed maize has been rapidly rising during the last nine years (1997-2005, Table 1), leading to a vast expansion of maize

Table 1. Cambodia maize production statistics, 1997-2005 (MAFF, 2006)

Year	Total maize production		
	Area (000 ha)	Production (000 t)	Yield (t/ha)
1997	49.4	42.4	0.9
1998	44.9	48.5	1.1
1999	59.8	95.3	1.6
2000	71.5	156.9	2.2
2001	80.2	185.6	2.3
2002	80.5	148.9	1.8
2003	93.4	314.6	3.4
2004	91.2	256.7	2.8
2005	90.7	247.8	2.7

Table 2. Maize demand projections, 1995-2020 (Pingali and Pandey, 2001)

Region	Demand		% change
	1995	2020	
Global	558	837	50
Developing world	282	504	79
East and Southeast Asia	150	280	46
South Asia	12	23	92
Sub-Saharan Africa	27	52	93
Latin America	76	123	62
West Asia/North Africa	16	26	63

production, especially in the northwest region of the country (MAFF, 2006, Nou Keosothea, 2005). This rising demand apparently will continue for at least another 20 years. As projected by Pingali and Pandey (2001), the global demand for maize will at least double by 2020 compared to 1995, but this will vary by regions (Table 2). According to this projection, demand from developing countries will grow by about 80 % from that in 1995. As Cambodia is situated within this group, where the demand will rise dramatically, there is an urgent challenge for the Cambodian maize improvement program to meet this growing demand.

At the country level, critical attention needs to be given to increase maize production; however, Cambodia still lacks most improved technologies available elsewhere. One of these is the absence of appropriate varieties with high yield and market-demanded quality. Due to this reason and because of high market demand, most maize farmers are cultivating varieties developed in other countries. Hybrid seeds of some commercial varieties popular in Thailand and Vietnam are widely distributed in the country. Production of hybrid maize has been rising rapidly in the country, especially in the north-western region close to the border with Thailand. On the other hand, new problems have occurred along with the spread of hybrid seeds. First, the high cost of imported hybrid seeds is problematic because Cambodian maize growers are generally small scale and subsistence, and are highly dependent on money borrowed from moneylenders in their villages. Unfortunately, those are not formal credit provider organizations, and interest rates they charge are very high, from 5 to 10 % per month (Nou Keosothea, 2005). Due to this reason, preference for OPVs over hybrids, as they can save their own seed for sowing in the next planting seasons, is overwhelming. Second, as varieties have been developed in different growing environments, different levels of management practices, and to some extent different socioeconomic situations in the other countries, they are not always well adapted to the local growing conditions of the country. This consequently results in poorer yield, poorer quality and in many occasions with high incidence of seed-borne diseases. The problem further deepened because no proper testing had been conducted and no sensible recommendations were made. Disputes between local growers and foreign traders frequently occurred due to the inferior market quality of maize produced by Cambodian farmers. Another problem that has often happened relates to seed quality. As hybrid seed distributed in the country usually comes from direct contacts between growers and cross-border seed dealers, it is not always under any seed importation regulations. Therefore, the seed is usually of poor quality, with poor gemination and purity, and is often infected by some harmful pests.

Realizing the problems, and in response to the challenge ahead, the Plant Breeding Department of the Cambodian Agricultural Research and Development Institute (CARDI), with financial support from the Cambodian Agricultural Research Fund (CARF), conducted a number of experiments throughout the country in 2003 to identify broadly adapted, high yielding and high quality open pollinated maize varieties for their release to farmers in Cambodia.

Materials and Methods

Series of trials and experimental design

Four types of trials have been routinely used by the breeding program to select crop varieties for release (Men Sarom *et al*., 2000 & 2001). First, all 70 introduced germplasms (hereafter referred to as genotypes) from CIMMYT were evaluated in an observational yield trial (OYT) without replications during the wet season at two sites. The best performing genotypes were advanced to preliminary yield trials (PYT), with two replications and for two seasons, to select the best ten genotypes for testing further in advanced yield trials (AYT) with three replications and for three seasons. Plot size of OYT, PYT and AYT was 1.5 m x 5.0 m. Both PYT and AYT at each location and year were arranged in randomized complete block designs. Promising genotypes identified from AYTs were tested in farmer's fields with farmer's management before their release. This type of trial, called pre-on-farm adaptive trial (Pre-OFAT), had no replications and plot size was 7.5 m x 20 m.

There were 10 sets of AYTs conducted in the Cambodian Agricultural Research and Development Institute (CA), Kandal (KD), Pailin (PL), and Kampong

Cham (KC) in two seasons and for two years (Table 3). The experiments at KC in dry season (DS) 2004 failed due to drought after crop establishment, and at PL in dry season (DS) 2005 failed due to damage by wild pigs. The two failed experiments were not included in the analysis.

Table 3. Location, season and year of advanced yield trial

Location	Year 2004		Year 2005	
	Dry season	Wet season	Dry season	Wet season
CARDI	1	1	1	1
Kandal (CA)	1		1	
Kampong Cham (KC)	1 (failed)			1
Pailin (PL)	1		1 (failed)	

Genotypes used

Seventy open pollinated genotypes introduced from CIMMYT were evaluated in OYT (Table 4). Among the 70 genotypes, 59 were normal maize (15 had yellow and 44 white colored kernels) and 11 were quality protein maize (QPM). CIMMYT scientists have successfully transferred the opaque-2 mutant gene found in 1963 to cultivated maize as it has proteins that are nearly twice as nutritious as those found in non-opaque-2 maize. The improved opaque-2 maize is called QPM, while maize without the opaque-2 gene is simply knonw as normal maize (Brown *et al*., 1988). The QPM genotypes were comprised of 10 white and one yellow kernel type. Twenty eight genotypes selected from OYT (No. 1-28 in Table 4) were tested in PYT in dry season (Jan. – Jun. 2004), and 18 of those genotypes (No. 1-18 in Table 4) were repeated again in PYT wet season (Jul. –

Table 4. Genotypes, kernel type (Y=yellow, W=white) and quality protein maize (QPM)[A] used in experiments

No	Genotype	Discription	No	Genotype	Discription
1	COTAXTLA-S0031	Y	36	DTPWC8F175-1-1-B	W
2	S-99TLWQ-HG-AB	W, QPM	37	DTPWC8F204-2-B	W
3	S-00TLWQ-B	W,QPM	38	DTPWC8F224-2-B	W
4	POZA-RICA S9627	Y	39	DTPWC8F231-2-B	W
5	S-99TLYQ-AB	Y,QPM	40	DTPWC8F317-1-1-B	W
6	AGUA-FRIA S0031	Y	41	DTPWC8F324-1-1-B	W
7	S-0128	Y	42	DTPWC8F353-1-1-B	W
8	S-99TLW-BN SEQ(1)	W,QPM	43	G16BNSEQC0F118-1-1-4-2-B-B-B-B	W,QPM
9	AGUA-FRIA S0030	W	44	G16BNSEQC0F118-1-2-1-2-B-B-B-B	W,QPM
10	AGROSS S0030	W	45	G16SeqC1-15-2-1-2-2-2-B-B-B-B-B	W
11	DTPWC8F31-1-1-B	W	46	SOEC6F74-1-4-1-1-1-B-B-B-B-B-B	W
12	DTPWC9-F5-4-1-1//CML-449	W	47	DTPWC8f324-1-1-1#-B	W
13	DTPWC9-F55-1-1-1//CML-449	W	48	DTPWC8F61-1-B	W
14	DTPWC9-F67-2-2-1//CML-449	W	49	DTPWC8F76-1-B	W
15	DTPWC9-F70-4-4-1//CML-449	W	50	DTPWC8F31-1-2-1-#-B	W
16	DTPWC9-F104-5-4-1//CML-449	W	51	DTPWC8F184-1-B	W
17	DTPWC9-F115-1-2-1//CML-449	W	52	DTPWC8F107-2-B	W
18	DTPWC9-F120-3-3-1//CML-449	W	53	G16BNSEQC0F118-1-1-4-1-B-B-B-B	W,QPM
19	DTPWC9-F131-1-3-1//CML-449	W	54	G16BNSEQC0F118-1-1-3-1-B-B-B-B	W,QPM
20	DTPYC9-F46-1-2-1//CML-451	Y	55	DTPWC8F321-1-B	W
21	DTPYC9-F46-1-6-1//CML-451	Y	56	G16BNSEQC0F118-1-1-4-5-B-B-B-B	W,QPM
22	DTPYC9-F463-4-1//CML-451	Y	57	DTPWC8F255-1-B	W
23	DTPYC9-F46-3-5-1//CML-451	Y	58	DTPWC8F204-1-B	W
24	DTPW C9	W	59	DTPWC8F317-1-1-1-#-B	W
25	DTPY C9	Y	60	DTPWC8F100-1-2-B	W
26	G16BNSEQ. C3	W,QPM	61	DTPWC8F126-1-B	W
27	G18SEQ C5	W,QPM	62	DTPWC8F31-1-B	W
28	SPE C8	W	63	DTPWC8F128-1-B	W
29	DTPWC8F31-1-3-1-B	W	64	DTPWC8F266-1-1-1-#-B	W
30	DTPWC8F266-1-1-1-B	W	65	DTPYC9-F69-3-6-1//CML-451	Y
31	DTPWC8F347-1-3-1-B	W	66	DTPYC9-F74-1-1-1//CML-451	Y
32	DTPWC8F100-1-2-B	W	67	DTPYC9-F86-2-2-1//CML-451	Y
33	DTPWC8F76-1-1-B	W	68	DTPYC9-F103-5-1-1//CML-451	Y
34	DTPWC8F107-2-B	W	69	DTPYC9-F134-2-3-1//CML-451	Y
35	DTPWC8F128-1-B	W	70	DTPYC9-F43-5-1-1//CML-451	Y

Dec. 2004). Selection of genotypes from OYT and PYT were based on kernel yield and other agronomic traits (data are not shown). Ten best genotypes (No. 1-10 in Table 4), comprising four QPM (one is yellow kernel), four yellow kernel genotypes and two white kernel genotypes, were then tested in AYTs along with two open pollinated checks (Composite 1 and Supper sweet corn, both from Kbal Koh station). Four promising genotypes including two QPM were selected from AYT and were tested in Pre-OFAT. Management and results of AYT and Pre-OFAT are shown and discussed in the following sections.

Cultural practices

In Arts, soils were plowed twice and harrowed after the second plowing. Raised beds were made by hand with 40 cm width and 25-30 cm height. Two seeds were hand-planted at 2-3 cm depth in hills in mid bed, with 75 cm between rows and 25 cm within row. Thinning was done to one plant per hill at 10-15 days after planting (DAP). Missing hills were found in a few plots in each experiment and were replanted with seedlings removed during the thinning operation. Crops were irrigated in dry season and whenever there was a dry period during the wet season. Weeds were controlled by hand twice or thrice depending on needs. No pesticides or herbicides were used.

The fields were fertilized with 100:80:60 kg/ha of N:P_2O_5:K_2O. Nitrogen was applied as urea (46% N) and diammonium phosphate (18% N) at three times (30% as basal, 30% at 20 DAP and 40% at 40 DAP), while P_2O_5 and K_2O were applied once as a basal application of diammonium phosphate (46% P) and potassium chloride (60% K), respectively.

Pre-OFATs were managed by farmers but they followed a protocol using 10 rows wide and 20 m long. Most farmers planted with 75 cm between rows and 25 – 35 cm within row. Soils were plowed two times and harrowing was done in some fields depend upon each farmer. A few farmers applied chemical fertilizer in very small amounts. Weeds were controlled by hand and no pesticides were used.

Data Measurement

Days-to-male flowering (DTMF) was measured as the time taken from seeding to 50% pollen shed of all plants in the plot. Days-to-female flowering (DTFF) was recorded from planting to 50% of the plants in the plot having silks two to three cm long. At maturity, plant and ear heights were measured from soil surface to the point where the tassel starts to branch and to the node bearing the upper-most ears, respectively. Kernel yield was calculated on a dry weight basis harvested from the whole plot.

Analysis of variance

Three types of analysis were performed for kernel yield from the eight environments of AYT. First, a combined analysis was performed to see the effects of year (Y), location (L) and genotype (G), and their interactions for 12 genotypes tested in dry season at two sites (CARDI and Kandal) for two years [1]. Second, a combined analysis was performed to see the effects of year, genotype and their interactions tested at CARDI in wet season for two years [2]. The last analysis was performed to see the environment (E = location-year), genotype, and their interaction effects as a whole [3]. The G x E analysis also was performed for the days-to-male and female flowering, plant height and ear height for the 12 genotypes (data are not shown). The three types of analysis of variance were conducted using IRRISTAT4.04. Also, the components of variance (õ^2) for kernel yield were estimated using REML 3.6 (Residual Maximum Likelihood).

$$y_{ijkl} = \mu + y_k + l_j + (ly)_{jk} + (r/ly)_{ljk} + g_i + (gl)_{ij} + (gy)_{ik} + (gly)_{ijk} + \mathring{a}_{ijkl}$$

where μ is the grand mean, y_k, l_j and $(ly)_{jk}$, $(r/ly)_{ljk}$, g_i, $(gy)_{ik}$, $(gl)_{ij}$, $(gly)_{ijk}$ and $\mathring{a}_{ijkl}$ are the random effects of year (Y), location (L) and location-by-year (LxY), respectively, replicates, genotype (G) and genotype-by-year (GxY), genotype-by-location (GxL), genotype-by-location-by-year (GxLxY) and residuals, respectively.

$$y_{ijkl} = \mu + y_k + (r/y)_{lk} + g_i + (gy)_{ik} + \varepsilon_{ljk}$$

where μ is the grand mean, y_k, $(r/y)_{jk}$, g_i, $(gy)_{ik}$ and ε_{ljkl} are the random effects of year (Y), replicates, genotype (G) and genotype-by-year (GxY) and residuals, respectively.

$$y_{ijkl} = \mu + e_m + (r/e)_{jm} + g_i + (ge)_{im} + \varepsilon_{ljm}$$

where μ is the grand mean, e_m, $(r/e)_{jm}$, g_i, $(ge)_{im}$ and ε_{lijm} are the random effects of environment (E), replicates, genotype (G) and genotype-by-environment (GxE), and residuals, respectively.

Stability analysis was performed for mean kernel yields of genotypes across eight environments to see the consistency of these genotypes by ranking of kernel yields accordingly from the highest to the lowest in each environment. For Pre-OFAT, mean over trials and its standard deviation (SD) was calculated with kernel yield advantage of tested genotypes over farmers' varieties. Farmers' first preference for all genotypes (including farmer's variety) is also provided.

Results

There Y, L and G effects and heir interactions, were significant (P<0.01) for kernel yield of the 12 genotypes tested in dry season at two locations (CARDI and Kandal) and for two years, with the L effect being the greatest effect ($\tilde{o}^2$ = 3.7), which was 7.7 times that of the genotype component (Table 5a). However, the genotype effect ($\tilde{o}^2$ = 0.76) was significant (P<0.01) and only slightly greater than the year component when combined analysis was performed for wet season for two years for kernel yield of genotypes tested in CARDI (Table 5b). When Location-year experiment was considered as environment (E), then there were effects of E and G and also their interaction (G x E) (P<0.01) (Table 5c). The environment component was 5.4 and 4.5 times greater than the genotype and genotype-by-environment components, respectively. The results, in general, indicate instability of tested genotypes for kernel yield across environments.

Table 6 shows kernel yield of genotypes at each environment and their mean across eight environments, as well as environmental means. In general, kernel yield in wet season (average over three environments = 6.38 t/ha) was higher than in the dry season (average over five environments = 4.61 t/ha). At CARDI, crops were grown in both dry seasons on Prateah Lang soil with low fertility (White *et al.*, 1997); therefore, kernel yields were the lowest among the eight environments. In wet season, however, crops were grown on upland areas in CARDI, and kernel yields were intermediate compared to KC05WS.

Days-to-male (DMF) and female (DFF) flowering, plant height (PH) and ear height (EH) for eight environments are shown in Table 7. The four variables differed significantly among the environments. In general, the crop grown at CARDI flowered later and had shorter plants than crops grown at Pailin, Kandal and Kampong Cham, because CARDI's soil was less fertile than soils at the other three locations. Crops grown on fertile soils (KC05WS and KD04DS) flowered about 11 days earlier and had larger kernel yield (Fig. 1, R^2 = 0.79**) than crops grown on poor soils (CA04DS and CA05WS).

Table 5. Components of variance ($\tilde{o}^2$) and standard error (SE), and level of significance for kernel yield of 12 genotypes tested from 2004-2005

Year (Y)	Location (L)/E	Y x L	Genotype (G)	G x Y	G x L/E	G x L x Y
a) Combined across CARDI and Kandal over years for dry season						
0.50±0.18**	3.70±0.13**	1.25±0.18**	0.48±0.21**	0.28±0.13**	0.00±0.12ns	0.87±0.39**
b) Combined across years for CARDI in wet season						
0.72±0.69ns			0.76±0.26**	0.00±0.28ns		
c) Combined across all location-year (environment) experiments						
	3.51±1.39**		0.65±0.23**		0.78±0.18**	

** Significant at P<0.01, ns = Not significant at P<0.05

Table 6. Kernel yield of tested genotypes, and genotypic mean and environmental mean kernel yield over eight environments

No	Genotype	CA04DS	KD04DS	PL04DS	CA04WS	KD05DS	CA05DS	KC05WS	CA05WS
1	S-99TLYQ-AB	3.66	8.35	4.73	6.68	5.12	2.13	8.63	4.32
2	COTAXTLA-S0031	4.19	7.88	7.14	8.01	5.45	3.70	10.90	5.18
3	AGUA-FRIA-S0031	4.00	7.72	6.88	6.48	5.31	3.30	10.83	5.28
4	PR S9627	2.55	8.95	4.76	4.52	4.55	3.33	8.87	4.99
5	S-0128	2.93	8.18	5.00	5.25	4.46	3.50	8.47	3.74
6	S-99TLWQ-HG-AB	4.42	8.66	4.76	6.68	4.58	2.53	8.20	4.32
7	S-00TLWQ-B	3.12	9.12	4.76	7.33	5.13	2.43	10.50	5.25
8	S-99TLW-BN-EQ(1)	3.33	7.78	5.57	7.10	6.19	3.60	10.07	6.81
9	AGUA-RRIA S0030	3.76	7.32	4.94	6.82	5.85	3.60	8.23	5.02
10	ACROSS-S0030	3.06	8.26	4.83	6.93	5.27	2.97	8.03	4.88
11	Composite (loc.1)	1.25	3.05	1.97	3.33	2.15	2.00	3.90	2.41
12	Super sweet corn (loc.2)	2.68	3.18	3.91	4.17	1.77	1.23	4.73	2.71
	Mean	3.25	7.37	4.94	6.11	4.65	2.86	8.45	4.58

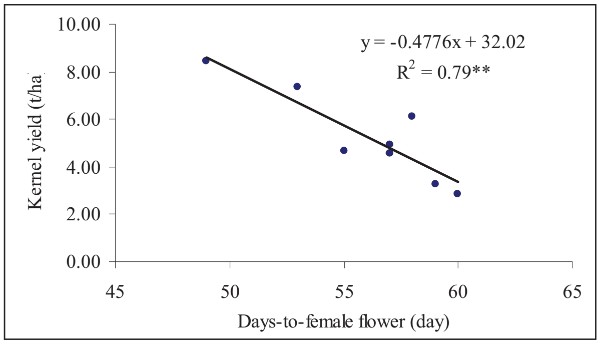

Fig. 1, R^2 = 0.79**

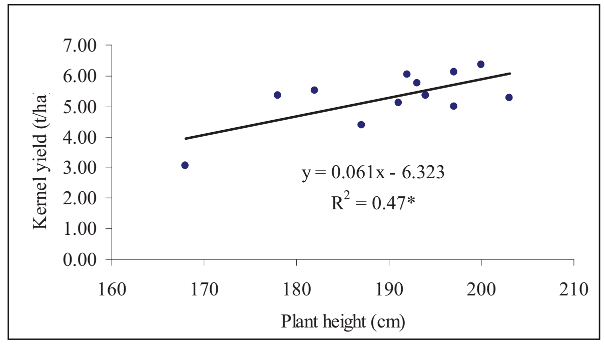

Fig. 2, R^2 = 0.47*

Table 7. Environmental mean values of days-to-male and -female flowering, plant and ear height across 12 genotypes

Environment (E)	Day to male flowering (day)	Day to female flowering (day)	Plant height (cm)	Ear height (cm)
CA04DS	55	59	185	86
KD04DS	51	53	221	103
PL04DS	56	57	230	120
CA04WS	55	58	165	72
KD05DS	49	50	221	107
CA05DS	57	60	145	51
KC05WS	47	49	202	105
CA05WS	56	57	152	77
Mean	53	55	190	90
5%LSD	1**	1**	11**	10**
G x E	**	**	ns	ns

CA04DS = CARDI dry season 2004, KD04DS = Kandal dry season 2004, PL04DS = Pailin dry season 2004, CA04WS = CARDI wet season 2004, KD05DS = Kandal dry season 2005, CA05DS = CARDI dry season 2005, KC05WS = Kampong Cham wet season 2005, CA05WS = CARDI wet season 2005.** Significant at P<0.01, ns = Not significant at P<0.05.

There were significant differences among the genotypes for DMF, DFF, PH and EH (P<0.01), but these differences were small; being six days for DMF, five days for DFF, 35 cm for PH and 24 cm for EH (data not shown). In general, kernel yield was slightly associated with plant height, with taller genotypes yielding more than short ones (Fig. 2, R^2=0.47*).

Since there was an effect of G x E interaction, stability analysis was performed by ranking genotypes based on kernel yield for all environments (Table 8). Two genotypes ranked most consistently across environments, and one of them (COTAXLA-S0031) had the highest kernel yield (6.56 t/ha) while the other (S-99TLWQ-HG-AB) had intermediate kernel yield (5.52 t/ha). COTAXLA-S0031 ranked consistently highest across environments (2.0±1.4). By contrast, Composite (Check 1) yielded the lowest, and ranked consistently across environments (11.8±0.5). There were some genotypes that ranked well in better environments and intermediate in the others. Genotype S-00TLWQ-B yielded the highest in KD04DS and 3rd in CA04WS and KC05WS, but intermediate in other environments. A similar trend was observed for POZA-RICA S96127.

Two highly stable genotypes were identified as the best genotypes. COTAXTLA-S0031 had the highest kernel yield, performed the most consistently across environments, and has yellow kernels; it was released and renamed as Loeung Mongkul. S-99TLWQ-HG-AB was intermediate for kernel yield, but was highly consistent across environments and was released as Sar Chey; it is QPM with white kernels. Both varieties were tested in on-farm adaptive trials along with the farmers' varieties (in most cases these were hybrids) at 45 sites in 2005-2007. Both varieties had kernel yield a bit higher than the farmers' varieties, being 6% higher for Loeung Mongkul and 5% for Sar Chey. Among the 45 farmers, 18 preferred Loeung Mongkul, 12 preferred Sar Chey and only three still preferred their varieties.

Discussion

One of the main reasons for decreasing area of hybrid maize in recent years, being 73,000 ha in 2003 and 67,000 ha in 2005 (Sakhan Sophany *et al.*, 2006; MAFF, 2006), was the high price or impurity of hybrid seeds. Therefore, many farmers preferred open pollinated maize with high yield and quality so they can keep the seeds from season to season and make good profits (unpublished survey data). On average, ten of the initial 70 open pollinated genotypes yielded more than the average of two check varieties (2.41 t/ha to 3.78 t/ha). There were significant year, location, environment and interaction effects for kernel yield,

Table 8. **Mean kernel yield (MKY) and ranking of genotypes and their mean across eight environments**

No	Genotype	MKY	CA04DS	KD04DS	PL04DS	CA04WS	KD05DS	CA05DS	KC05WS	CA05WS	Mean ± SD
2	COTAXTLA-S0031	6.56	1	4	1	1	3	1	1	4	2.0±1.4
8	S-99TLW-BN-EQ(1)	6.31	6	8	3	3	1	2	4	1	3.5±2.4
3	AGUA-FRIA-S0031	6.22	2	9	2	8	4	6	2	2	4.4±2.9
7	S-00TLWQ-B	5.96	7	1	8	2	6	9	3	3	4.9±3.0
9	AGUA-FRIA S0030	5.69	3	10	5	5	2	3	8	5	5.1±2.7
10	ACROSS-S0030	5.53	8	3	6	4	5	7	10	7	6.3±2.3
6	S-99TLWQ-HG-AB	5.52	5	5	7	6	8	8	9	8	7.0±1.5
1	S-99TLYQ-AB	5.45	4	7	10	7	7	10	6	9	7.5±2.1
4	POZA-RICA S9627	5.31	11	2	9	11	9	5	5	6	7.3±3.2
5	S-0128	5.19	9	6	4	9	10	4	7	10	7.4±2.5
11	Composite (loc.1)	2.51	12	12	12	12	11	11	12	12	11.8±0.5
12	Super sweet corn (loc.2)	3.05	10	11	11	10	12	12	11	11	11.0±0.8
	Mean kernel yeild (t/ha)	5.27	3.25	7.37	4.94	6.11	4.65	2.86	8.45	4.58	
	SD	0.89	0.86	2.06	1.32	1.27	1.11	0.79	1.68	0.97	
	5% LSD	0.55									

CA04DS = CARDI dry season 2004, KD04DS = Kandal dry season 2004, PL04DS = Pailin dry season 2004, CA04WS = CARDI wet season 2004, KD05DS = Kandal dry season 2005, CA05DS = CARDI dry season 2005, KC05WS = Kampong Cham wet season 2005,CA05WS = CARDI wet season 2005.** Significant at *P*<0.01.

Table 9. Kernel yield (t/ha) and its standard of deviation (SD), kernel yield advantage (KYA) and 1st preference given by 45 farmers of released varieties tested in 45 sets of on-farm adaptive trial, 2005-2007.

Variety	KY±SD	KYA (%)	1st preference
Loeung Mongkul	8.07±1.85	6	18
Sar Chey	8.00±1.76	5	12
Farmer's variety (Hybrid)	7.59±2.45		3

indicating that selecting maize genotypes requires testing across locations and years.

However, results obtained from stability analysis showed that some genotypes yielded consistently (in ranking) across the environments. Among the normal maize, COTAXTLA-S0031 yielded the highest and performed the most consistently across eight tested environments (6.56 t/ha, 2.0±1.4). This genotype flowered early (male51 days and female 53 days), had intermediate EH (87 cm), but tall plant type (200 cm). COTAXTLA-S0031 is a normal open pollinated maize with semi- upright leaves; it matures in 105 days, has 12 to 14 ear-rows, yellow semi-flint kernels, and an approximate 1000 kernel weight of 300g. S-99TLWQ-HG-AB also performed consistently and had intermediate kernel yield (5.52 t/ha, 7.0±1.5) among the QPM genotypes. Days-to-male and female flowering was 55 days and 58 days, respectively, and it matured in 108 days. The plant type is semi- upright leaves and tall (191 cm), with intermediate EH (91 cm). Kernels are white and weighed 266 g for 1000 kernels. These two genotypes were identified as outstanding genotypes and were released as Loeung Mongkul (COTAXTLA-S0031) and Sar Chey (S-99TLWQ-HG-AB).

Under farmers' field conditions and management, both varieties performed comparably to the farmers' varieties, which were hybrids at most sites. Farmers gave their highest preference to COTAXTLA-S0031 (18 out of 45 farmers) and S-99TLWQ-HG-AB (12 out of 45 farmers), as these had good kernels and are of open pollinated type so seeds can be kept from season to season.

Conclusion

Because year, location, genotype and their interaction effects for kernel yield were significant, plant breeders would need to conduct experiments across years and locations to select the best genotypes for specific or broad adaptation. However, among the tested genotypes, two high yielding and good quality genotypes were broadly adapted across tested environments and under farmers' field management (Table 9). With farmers' management, Loeung Mongkul, a normal maize with white kernel type (COTAXTLA-S0031) yielded 6%, and Sar Chey, a QPM genotype with white kernel type (S-99TLWQ-HG-AB) yielded 5% higher than the farmers' varieties (7.59 t/ha), indicating that both released varieties are comparable to the maize hybrids grown by these farmers.

Acknowledgement

Financial support from the Cambodian Agricultural Research Fund (CARF) and the Rockefeller Foundation (partly for on-farm trial) for implementing this project is highly acknowledged. We also thank CIMMYT for providing maize germplasm for the project.

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Efforts to develop maize technologies and their adoption in the Eastern hills of Nepal: A review

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Abstract. Maize is the staple food for hill farmers and it is grown under a wide range of agro-climatic and ecological conditions of both hills and plains of Nepal under rainfed, complex farming system. Attempts have been made to review maize technologies and adoption/rejection of the developed technologies. Research papers on developed technologies were reviewed, and all District Agriculture Development officers (from ten hill districts), representatives from NGOs, Agrovets, Seed companies and participating farmers were interviewed for persisting problems of maize and its technologies. Suggestions were collected from them and incorporated in this discussion. With financial support from the Hill Maize Research Project (HMRP), a dozen problem based technologies were developed since 2000, and efforts were made to disseminate them through stakeholders and line agencies. Farmers in these hill districts were seen yearning for support, and they never tried to adopt unless facilitated and influenced by line agencies, farmers leaders, etc. Some research on technology generation conducted on research stations was not considered useful by farmers, and hence they were reluctant to adopt these technologies. This paper highlights drawbacks, key areas for consideration before project implementation, rapport building, monitoring methods, etc., and offers suggestions for developing quicker dissemination channels within agrarian communities.

Background

Research and extension are the two pillars of agricultural development. Research findings will be of no use if they are not adopted in farmers' fields. Maize scientists have developed many improved technologies, but many of them are still beyond the reach of the needy farmers. Therefore, there must be a joint effort from government and the private sector to scale up technology adoption. If we consider the history of research and development in Nepal, during the 1970´s and 1980´s the technology development and transfer process was linear and top down. In this linear system, technology flow was one way from research station to extension and then to farmers. Farmers were considered as receivers of technologies, and that is why adoption and diffusion of research generated technologies was slow. In the late 1990s there were some attempts to bring farmers' concerns into technology development and dissemination. Mechanisms were developed for direct involvement of researchers and farmers in the technology development process. Outreach sites were also established to test and verify on-station generated technologies with the participation of farmers. Though there was increased involvement of farmers and good interaction amongst farmers, researchers and extension workers, the technology development and diffusion model was mainly public sector dominated. A partnership and participatory technology development (PTD) approach was adopted later, which mainly focused on PTD with the participation of multiple actors from both public and private sectors. However, agriculture extension mostly focused on the Agriculture Perspective Plan developed by the government of Nepal, pocket package approach, commodity group approach, farmer's field school, etc. In recent years, research and extension models have been developed and all stakeholders are working jointly with the set modalities.

Scenario of maize, its technologies and adoption in the eastern hills of Nepal

Maize is the second staple food crop in Nepal, but in the hills it is the first crop and is grown under a wide range of agro-climatic and ecological conditions of both hills and plains of Nepal under rainfed, complex farming system. Maize is life for hill farmers. In Nepal, maize is grown in two production systems and in three agro zones. In the hills, maize is generally cultivated for food, feed, fodder and fuel. It covers about 80% of the area and there is one planting per year. But in the terai maize is grown mainly for commercial purpose and less importance is given to consumption. It covers only about 20% of the crop area, with 2-3 plantings per year. Agro-ecologically, it is cultivated in three agro zones: Terai and inner terai (below 900m), mid hills (900-1800m) and high hills (above 1800m). The mid hills occupy 70.1% of the area, followed by Terai- Inner

Terai (19.5%) and the high hills (10.4%) (NMRP, 2006/07); the contribution from each of these areas is summarized in Table-1.

At present, the area covered by maize is 870,401 ha, with an average yield of 2.09 t/ha and total production of 1,819,925 tons (MOF, 2007). The productivity trend from 1990 to 2006 is improving, with the current productivity rate of 2.33% as shown in Table-2.

With the initiation of the Hill Maize Research Program (HMRP) in the eastern hills of Nepal in 1999, the area has increased by 0.76%, with increases in production and productivity of 3.66% and 2.89%, respectively. The Eastern Development Region (EDR) alone occupies 27% of the total area and contributes 29% of the total maize production of the country. The productivity is more or less similar with the national average as shown in Table-3. In EDR, hills occupy 79% of the total area but contribute only 75% of the maize production. The productivity in hills of EDR is less than the national average, but in terai it is higher (2.33 t/ha) than the national average (Table-4). HMRP gives major thrust to the productivity and sustainability of maize based systems; both research and development activities were carried out for development of technologies and disseminating the developed maize based technologies through extension and input delivery channels.

Methodology

Some research papers, articles, reports, etc on maize were reviewed. Farmers groups involved in community based seed production were interviewed by preparing semi-structured questionnaires. Nine hill district DADOs were also interviewed with semi-structured questionnaires. Responses from them were analyzed critically and suggestions were also collected for higher production and productivity of maize.

Table-1. Maize production statistics based on agro-ecozones (2007)

Agro-ecozones	Area (ha)	Production (mt)	Yield (kg/ha)
High hills	88,288	163609	1853
Mid hills	613774	1273111	2074
Terai and Inner Terai	168339	383205	2276
National Average	870401	1819925	2091

Source: MOF, 2007

Prevailing maize technologies in the eastern hills of Nepal

Variety: Many maize varieties were developed and introduced according to their suitability for specific agro-climates. Farmers and stakeholders were trained and encouraged to widely verify, disseminate and adopt the varieties. Some of the released varieties for low hills (1100 masl) are Arun-1 and Arun-2. Similarly, for mid hills (1100-1700 mals), released varieties are Manakamana-1, Manakamana-3, Deuti, Shitala, etc., whereas for high hills (1700 masl and above), Ganesh-1 is the only improved variety. However, there are some varieties for hills in the research pipeline, amongst which mention may be made of Population-45, Hill Pool Yellow, Hill Pool White, Arun-4, Arun-1EV, Pool-17, Across P502, etc. Quality protein maize (QPM) has also been introduced in recent years, with S99TLWQ-HG-AB, S01SIWQ-3 and S99TLYO-B among those that have shown promising results.

Soil fertility: Research on soil fertility to elucidate appropriate doses of chemical fertilizers and compost improvement were carried out, and some of the achievements were:

- Fertilizer recommendation of 60:30:30 of N, P_2O_5 and K_2O kg/ha, along with 10 ton farm yard manure (FYM)/ha.
- Improved composting techniques
    - o Compost making in bamboo made structures had better quality (0.96% N content).
    - o Covering the pit with black polythene sheet during the decomposition process not only prevents volatilization losses, but also accelerates decomposition.
    - o Vermicomposting was found attractive and preferred by many farmers.

Table-2: Five yearly growth rates (%) of maize area, production and productivity

Particulars	1970/71-1974/75	1976/77-1980/81	1981/82-1985/86	1986/87-1990/91	1991/92-1995/96	1996/97-2000/01	2001/02-2005/06
Area	0.87	0.24	8.58	4.88	0.92	1.01	0.76
Production	0.54	-2.73	4.33	9.75	2.08	2.95	3.66
Productivity	-0.38	-4.50	-2.08	4.96	1.15	1.94	2.89

Source: MOAC, 2006

Table-3: Area, production and productivity of maize in different development region (2007)

Development Region	Area (ha)	Production (mt)	Yield (kg/ha)
Eastern	228744	452800	1980
Central	208967	461575	2209
Western	221196	503488	2276
Mid Western	157526	304737	1935
Far Western	53968	97325	1803
Nepal	870401	1819925	2091

Source: MOAC, 2007

- Plant protection technologies
  - o Control of white grub using an indigenous entomopathogenic fungus (*Metarrhizium anisopliae)*.
  - o *Management of storage pest weevil (Sitophilus oryzae*) using pea flour and rapeseed cake powder.
- Research on economic aspects of maize production
  - o Intercropping of vegetables, spices, etc. obtained better benefits.
  - o Double planting of maize per hill in intercropping increased income without reducing maize yield.
- Gender friendly technologies
  - o *Kute kodalo*, a triangular shaped weeding tool was found effective for earthing-up; it reduced drudgery of women's work.
- Agro-forestry: Establishment of fodder on maize terrace riser was found beneficial considering all farming aspects of eastern hills.

Seed Production

- Pakhribas Agriculture Research Station is the main seed source centre for Breeder and Foundation seed in the eastern hills of Nepal.
- Nine community based seed production groups are working effectively and producing certified seeds. These groups are producing about 20 tons of certified seeds per year.

Prevailing technology disseminating system

1. Participatory varietal selection (PVS):
   - Mother baby trial
   - Informal research and development
   - Diamond trial
2. Farmer acceptance test

Table-4: Area, production and productivity of maize in different agro-zones (2007)

Agro-zone	Area (ha)	Production (mt)	Yield (kg/ha)
Mountain	39065	69448	1778
Hills	141779	271603	1916
Terai	47900	111750	2333
EDR	228744	452800	1980

Source: MOAC, 2007

3. Community based seed production
4. Distribution of publications (booklets, posters, leaflets, etc.)

The dissemination system followed by government agencies is adequate at policy level. However, practically it is not sufficient because the ultimate users of the technologies are not fully utilizing them.

Disseminating partners of HMRP

- Government Organizations (Research and Extension)
- I/NGOs
- CBOs, Farmer groups (CBSP)

These partners disseminate the technologies as per their own working modalities. One of the prevailing modalities is presented in Figure-1.

Maize production problems faced by farmers on priority basis

- Diseases and pests
- Insufficient high-yielding varieties (HYVs)
- Insufficient chemical fertilizers
- Irrigation facilitis lacking
- Fertilizer and manure management
- Weed and storage losses

Problems faced by Community based seed production groups

- Difficulty to raise awareness in all group members
- Lack of knowledge about disease management
- Marketing difficulties
- Difficulties in seed grading and selection
- Unfavorable climate for seed drying
- Lack of technical skill for seed storage
- Forced to use seed for consumption purpose
- Problem of white grub and other pests

- Lack of knowledge about seed production
- Poor aeration of soil after rainfall
- Poor germination
- Late maturity of varieties

Major issues and problems

- Seed replacement rate by farmers is low
- Low risk bearing capacity of the farmers
- Slow variety release process
- Slow diffusion process
- Budgetary constraints
- Insufficient quality seed (time, quality and quantity)

Adoption situation of maize technologies in eastern hills

- 60% of the project intervention area in the eastern hills is covered by improved maize

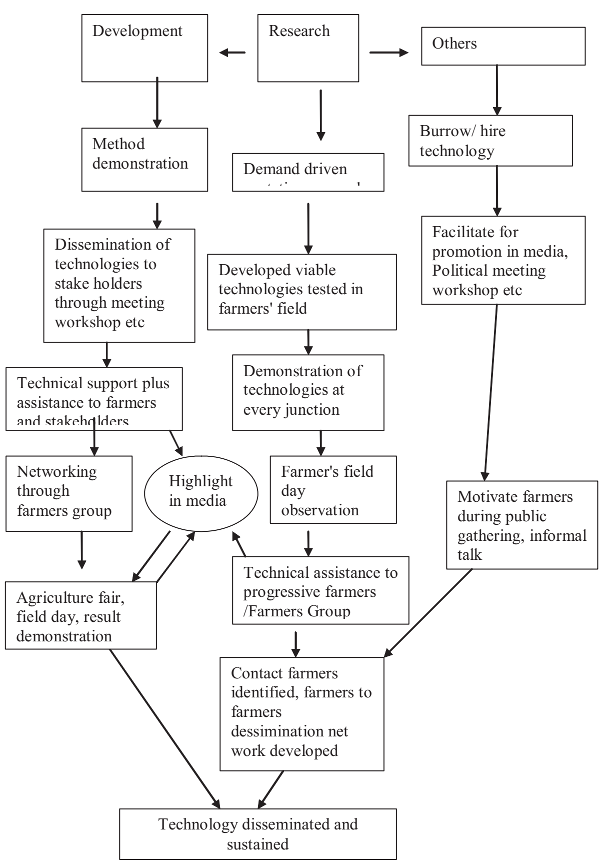

Fig.1: A model of technology development and dissemination.

- Other maize based technologies are also adopted along with maize varieties
- Technology coverage in the area is mainly concentrated along road corridors
- Farmers in remote areas still practice traditional cultivation practices

Conclusions and the way forward

- Though there are plenty of maize technologies (both recommended and in the research pipelines), as well as dissemination models, the delivery process is slow.
- Quick delivery channels need to be identified and adopted.
- Effective monitoring should be done in time.
- There must be a good collaboration between and within public and private sector for effective delivery of the identified technologies. It is also recommended to strengthen the interrelationships among these institutions.
- Potential dissemination systems, like cyber extension, radio broadcasting (mass media), farmer to farmer approach, etc., need to be encouraged for quicker dissemination of the technologies.

Acknowledgement

The authors highly acknowledge HMRP for providing the opportunity to work with them as a partner in their maize projects. Funding from SDC during the project implementation period is also highly acknowledged. We are grateful to CIMMYT for funding us to present this paper in the 10th ARMW at Makassar. We thank Dr. Ferrera and Dr. Tiwari for their day to day support, inspiration, suggestions and constructive criticism.

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Evaluation of Farmer Interst to Maize Variety (A Case Study in East Nusa Tenggara)

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Abstract. The evaluation of farmer interst to maize variety was conducted in February 2007 at three zones of maize development in East Nusa Tenggara Province (NTT). The data collected in survey were analyzed by using chi square (X^2). The research findings indicated was ecosystem different, production use and marketing system on each zona influence farmer interest in selecting a variety, especially in Zona III, but zona I with use of Pioneer variety and in zona II with use of Lamuru variety. The different uses of these varieties were caused by different use systems. In Zona I, the farmers harvested maize in the form of still green stem because they wanted varieties with early maturity and sweet taste. Zona II had opened it's channel with Community Social Institution (LSM), the farmers wanted maize with producte of bigger seeds, where as zona III generally consumed maize as basic, that the farmers liked delicious and soft varieties. The different strengthen with statistically (X^2), factors to influence farmer interest at zona I was small size (<6 mm), early maturity (85-90 days) and crop performance. Zona II was big size (<10 mm), high vigor (>90%), and resistant pets/disease and zona III was medium size (<8 mm), grain colour, harvested (early, middle, late) and performance of maize.

Key words: different, interest, maize varieties , zona

Introduction

Maize (Zea Mays L) was season crop which grow in dry farming with wet dry climate and dry climate on rice field by irrigation and wet ricefield depend to rain, tolerance to competition at pattern plant the intercropping, according to for the agriculture of subsisten, commercial agriculture of small scale, middle till very big (Subandi *et al*, 1988).

Maize request mount from year to year in line with the increasing of resident amount and industrial, despitefully the rare of in raw material of oil from fossil push various state look for the energy alternative from vegetation fuel (bio fuel), among other things maize to be made by bio etanol as premium subsidy. This matter cause the got difficult maize and costly of its price because biggest exported maize of world of like United States, lessening its exporting because its home affairs requirement progressively mount, among other things to be industrial of bio etanol.

Change of Pattern of maize request, also push the technological adoption of seed start early year 1990 an. Industrial of seed hibrida rapidly grow followed by technological adoption speed of maize hibrida. Estimated wide of areal of maize hibrida come near 40% from totalizeing areal pertanaman maize in Indonesia with the productivity reach 6-8 t / ha. estimated local Maize spreading less than 25% with the productivity only 1,5 t / ha - 2 t / ha, what majority planted in Madiun, Nusa of South-East of East and South Sulawesi, local Use variety. Difference for example caused was research to local varieties not yet got the priority especially in relevant crop glorifying area with the effort of make-up of local ability genetic varieties (Kasryno *et al*, 2007).

Important three things go together the seed quality: (1) quality genetic that is seed quality determined by pursuant to identity genetik which have been specified by breeder and mount the perity from yielded varieties, such seed identity not was only determined by seed appearance, but also phenotype crop; (2) quality grow and resilience keep the seed, and (3) physical quality, determined by hygiene storey: level, unform seed from facet of size measure and also wight, kontaminasi from other dissimilar crop seed or seed of weed and rate irrigate (Sania S, *et al*, 2007). This research aim to evaluate the farmer interest to maize variety.

Methodology

Location

This Research was executed in February 2007. Location determined by pursuant to secondary data collected by through report from various related institution: ICERI, Agriculture Extantion Service, CBS and AIAT in Province of NTT and also interview with the prominent society with consideration: as regional bases of development of hybrid maize and or have free gist;sari of dry land, rainfall very less with the spreading not flatten the, different land;ground factor and also mount the life masayarakatnya of below chosen poorness line so that province NTT. Here in after pursuant to typelogi farm in general start from farm level off that is farm of wet rice field depend to rain until hilly farm which was divided into 3 zona that are (a). Zona I that is Countryside Naibonat, Subdistrict of East Kupang with the characteristic of wet ricefield dependant to rain farm, tropical climate, type of alluvial land, (b). Zona II that is Countryside Nun Kurus, Subdistrict of East Kupang with the farm characteristic level off the vegetasi predominated by the tree of gewang and lontar, tropical climate, type of transition farm of between alluvial and Bobonaro Clay, (c). Zona III that is: Countryside Camplong, Subdistrict Fatuleu with the hilly farm characteristic, inclination 5-40%, vegetasi of primary forest (gewang, kapok, sandalwood, teak and crop of cashew), land ground type was raddle rock.

Data collecting

Data collecting of primary obtained by interview to 60 responder farmer taken at random modestly (Simple Random From other side). Data collected go together a) seed characteristic feeled a desire for by the farmer of like: size measure seed (small, medium, big), colour and vigor, b) characteristic of crop performance: high plant, number of cobs, cob size, leaf position and resistance to pest / disease and also characteristics of maturity] like early, middle, late, production cost and revenue of on farm.

Analysis Data

Data gathered later; then in tabulation and is here in after analysed by using analyse chi square (X^2) of pursuant to wanted storey; level characteristics of maize farmer given by the value with score: 0= not reply, 1 = not wanted, 2= less wanted, 3 = wanted, 4 = more wanted and 5 = most wanted, where maize of Lamuru variety used as a comparator. Analysis R / C ratio used to know the efficiency,. As for formula analyse the X^2 and R / C ratio shall be as follows (Anonim, 1988 and Sujana, 1989)

I. Analysis of X^2 :

$$X^2 = fo \left(\frac{fi - ft}{ft} \right)^2$$

Where:
X2 = Chi Square
fo = result of perception
ft = expected perception

II. Analysis of R / C Ratio

$$R/C = \frac{\sum_{i=1}^{k} Yi \,.\, Pyi}{\sum_{i=1}^{n} Xi.\, Pxi}$$

Where:
Σ = sigma
R = Revenue
C = Cost
Y = Production of area
Py = Price of product
X = Kinds of input in set of physical
Pxi = Price of set of used input

Result and Discussion

Area Generality

Farmer generally labour the maize crop of farm of wet ricefield depend to rain and non irigated dry field was very varying from 0, 12 ha - 1,15 ha. For the sharpness of visible at Tables 1.

Table 1. Area and ownership at farm of maize at 3 zona in Province NTT. 2007

Type of Farm	Rainfeed lowland		Dry Land		Total Farm of maize (ha)
	Area (ha)	farm Ownership (%)	Area (ha)	farm Ownership (%)	
Zona I	0.59	71.43	0	0	0.59
Zona II	0.51	42.10	0.64	89.47	1.15
Zona III	0.12	100.00	1.15	100.00	1.27
Mean	0.40	71.18	0.26	63.17	0.66

Source: Primary Data, 2007

Table 1 was seen by area and ownership of maize farmer in Province NTT 0.66 ha where area of zona III own the broader dry farming from farm of wet rice field dependant to rain in area of zona I own the broader wet ricefield dependant to rain farm. With the status of is ownership of and this different situation agroecology, influencing decision making of farmer in used varieties

Type and Seed Source

Seed represent the object live, for that have to be defended was till used by consumer at the correct or season plant to come, but from third research zona, in the reality use the different seed source, for example from seed shop, For the sharpness of, visible at Table 2.

Table 2, indicated that zona I, seed used only varietas hibrida Pioneer which was generally bought in Shop (72%) medium of zona II predominated by the composite varietas of Lamuru (57%), medium in zona III predominated by the Local varietas was which not yet regeneration so that result obtained very low. Used different, varieties, yielding different income of farm, visible at Table 3.

Table 3 seen that farmer to 3 zona of Province NTT, not yet efficient in farm of maize, this was seen from value R / C ratio < 1 (0.61-0.91). Highest acceptance at zona II with the production 3.00 t/ha, was lower the than national productivity 3.4 t/ha (BPS Indonesia, 2006), medium potency of result of Lamuru variety 6 t / ha (Syuryawati *et al*, 2007). Lowering production obtained, besides because dryness, rare of substance burn, also because local use

Table 2. Typologi of farm, type of varieties, and seed source at 3 zona in Province NTT. 2007

Typelogi of Farm	Type of Varietas	Seed Source	
		Place of Purchasing	Persentarse Purchasing (%)
Zona I	Pioneer	Shop	72
		AIAT of NTT	14
		Generation (F2)	14
Zona II	Lamuru	ICERI	10
		Farmer Group	10
		AES	32
		Generation (F2)	5
	N35	AES	10
		Shop	10
	Local	Generation (F...)	23
Zona III	Local	Generation (F...)	57
	Kalingga	Generation (F...)	29
	Harapan	Generation (F...)	22
	Lamuru	Farmer Group	3

Source: Primary Data after processed, 2007
Note F = hereditary was planted variety without there was regenerasi

Table 3. Mean production, revenue, cost and income at farm of maize at 3 zona in Province NTT, 2008.

Description	Zona of Research			Zona of Mean Research
	Zona I	Zona II	Zona III	
Produce				
• Seed (t / ha	2.50	3.00	2.00	2.50
• Young maize (cob / ha)	991.00	-	-	991.00
Revenue	3,997,750	4,560,000	4,180,000	4,163,333
• Seed (t / ha	3,750,000	-	-	247,750
• Young maize (cob / ha)	247,750	4,560,000	4,180,000	4,411,083
cost of farm (Rp/ha)	2,482,538	2,386,250	2,387,700	2,418,829
income of farm (Rp/ha)	1,575,221	2,173,750	1,792,300	1,827,090
R/C ratio	0.61	0.91	0.75	0.77

Source: Primary Data, 2007
Note: Maize Price at Zona I = Rp 1,500/kg; Zona II = Rp 1,520/kg; Zona III = Rp 2,090/kg . Young Maize price= Rp 2,500/cob

Tables 4. Seed performance interested by the farmer in 3 zona at Province NTT. 2007

Performance score	Zona I (%)						Zona II (%)						Zona III (%)					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Zise measur seed																		
Small	43	14	14	28	0	0	0	100	0	0	0	0	0	100	0	0	0	0
Medium	43	14	28	0	0	0	0	47	0	32	10	10	0	82	0	0	7	11
Big	28	0	0	14	14	43	0	0	16	10	0	74	0	7	0	25	0	68
Colour	43	0	0	0	14	43	5	0	0	32	0	63	0	0	0	25	4	71
Vigor	43	0	0	0	14	43	10	0	0	16	16	58	0	0	0	19	0	16

Source: Primary Data, 2007
Note: big (≥10 mm), medium (≥ 8 mm), small (≥ 6 mm)

varietas and pre-eminent varietas was which not yet diregenarasi of like Kalingga vaiety and Harapann at zona II. Margaretha *et al.* (1998) opening that expanding assorted use of varieties at one particular area of because farmer of opinion the varieties can give the result which high enough, but there was also farmer perforced plant the certain maize hibrida because don't have other; dissimilar choice was which sometime cause the result obtained inappropriate of expectation of because lack of farmer knowledge of about way of budidaya and sometime energy grow the downhill seed

Seed Performance

Used composite varieties by Kalingga expectation and of Litbang year 1986 and 1964, showing this varietas own the excess compared to by varietas of other; dissimilar which have been released by litbang and also private sector. Since year 2000, Litbang have released the composite varietas of Lamuru and have distributed the province in Indonesia the inclusive of Province NTT, with forming of seed grower of variety Lamuru. According to Educating Harnowo (1994), seed have to fulfill 6 precisely that is: (1) precisely sum up, namely was available in appropriate amount of requirement, (2) precisely varietas that is varietas matching with desire of farmer and condition of agro ekosistem of where seed will be planted, (3) on schedule that is made available at the time of required, (4) precisely location that is made available in place requiring, (5) precisely quality of gilt edged namely, and (6) precisely price namely its price was reached by farmer / seed consumer. How type and performance of varietas taken a fancy to by the farmer, visible at description hereunder . Seed performance In percentage, size measure of seed not yet influenced the farmer desire, but at colour of seed and vigor have score 4 and 5 that is more feeling a desire for until most feeling a desire for. Visible sharpness at Table 4.

Table 4, seen by that farmer at zona I, most (71%) taking a fancy to king sized seed (score 3, 4 and 5). Farmer of zona II and III of besides taking a fancy to king sized seed (84% and 93%) by score 3 and 5, also pay attention to the colour and vigor (57%-96%) by score 3, 4, and 5 like Lamuru variety as its comparator. This matter in line with Rahmawati *et al* (2005) that seed of the size minimize shall not be made by seed of because yielding dry weight of lower sprout (0.13-0.19g / sprout), medium size medium seed (0.16-0.23 g / sprout) and big size measure seed can reach 0.19-0.27 g / sprout, that way also Hussaini *et al* (1984) opening that size measure of seed of maize of larger ones after experiencing of flagellation, still has the ability germinate by vigor was compared to by higher level of smaller seed.

Performance of Maize

Component of result of maize represent a[n maize characteristic influenced by performance of crop, like: high plant, number of cob, cob size, leaf position and resistance to pest and disease. visible at Tables 5.

From Table 5 above, seen by that crop appearance also represent one of factor feeled a desire for by farmer, this was seen to by start from high plant, number of cob, cob size, leaf position and resistance of pest and disease obtain; get the high percentage at to 3 research zona that is 57 - 100% with score 3, 4 and 5 meaning farmer in Province NTT take a fancy to the maize by characteristics of maize like varietas Lamuru, its comparator.

Maize of Maturity

Difference of marketing system of result, in the reality not influence the age harvest. This matter was seen at Tables 5 where farmer at zona I, II and III remain to very take a fancy to the maize which early maturity (85-90 day). Visible at Table 6.

Tables 5. Performance of maize by the farmer in 3 zona of Province NTT. 2007

Performance score	Zona I (%)						Zona II (%)						Zona III (%)					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
High Plant	28	0	0	0	14	57	10	10	0	42	5	31	0	7	0	4	0	78
Number of cob	28	0	0	0	14	57	10	0	0	26	16	47	0	0	0	18	7	75
Cob size	28	0	0	0	14	57	5	0	0	63	0	31	0	0	0	14	11	75
Leaf position	28	0	0	0	14	57	16	16	0	21	16	31	0	18	0	21	18	42
Resistance H/P	28	0	14	0	0	57	16	0	0	16	5	63	0	0	0	14	0	86

Source: Primary Data, 2007

Tabel 6. Interested of maturity of maize at3 zona in Province NTT

Performance score	Zona I (%)						Zona II (%)						Zona III (%)					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Early (85-90)	28	14	0	0	14	43	5	0	0	21	0	74	0	4	4	14	4	74
Midle (95-100)	43	43	0	14	0	0	21	16	0	47	10	5	0	74	0	4	14	4
Late 105-110)	43	57	0	0	0	0	32	63	5	0	0	0	0	82	7	4	4	4

Sumber: Data primer, 2007

Tables 6 showing that 57% farmer in zona I wish the maize which old age the early, that way also farmer in zona of II and III, 74% farmer wish the maize with the age early. This matter is not quit of exploiting of ground water of rest of paddy plant in the rains and also marketing system of result of 4. Farmer Perception to seed characteristics.

Statistically, seed appearance influence the farmer interest to varieties maize, Farmer in zona I, taking a fancy to varietas owning pocket edition seed, farmer of zona II, size measure seed, colour of seed and vigor become the election factor of an varietas, farmer in zona of III of size measure of seed and seed colour becoming factor determining farmer interest to varieties. Other; Dissimilar factors, visible at Tables 7

Tables 7, also seen by that any maize performance: (high plant, number of cob, size cob, leaf position and resistance of pest and disease) representing determinant of interest by farmer in zona I, farmer in zona II only at resilience factor to pest and disease was zona III take a fancy to the varietas owning high plant, number of cob and size cob of like its comparator (Lamuru Variety).

Statistically, maturity of maize not give the influence to farmer enthusiasm of at zona I, in chosening / taking a fancy to an varieties, but on the contrary farmer in zona of II and III age harvest to influence the farmer interest in selecting a fancy to varieties.

Table 7. Result of chi square (X^2) analysis at 3 zona research in Province NTT. 2007

No. Variable	X^2 Count at Reaseach Area		
	Zona I	Zona II	Zona III
I. Seed Measure			
Small	2.00*	0^{ns}	0^{ns}
Medium	1.57^{ns}	7.32^{ns}	30.07*
Big	1.57^{ns}	14.00*	9.93^{ns}
Colour	1.14^{ns}	9.58*	20.21*
Vigor	1.14^{ns}	11.10*	11.57^{ns}
II. Performance of plant			
High of plant	2.00*	7.58^{ns}	26.00*
Number of cob	2.00*	1.84^{ns}	22.36*
Size of cob	2.00*	6.42^{ns}	21.93*
Leaf Position	2.00*	2.32^{ns}	4.86^{ns}
Resistance of H/P	2.00*	15.32*	14.29^{ns}
III. Maturiry of plant			
Early	1.57^{ns}	14.63*	54.14*
Midle	1.14^{ns}	10.21*	38.00*
Late	0.14^{ns}	9.58*	67.71*

Source: Primary Data, 2007
Note: Lamuru variety (Comparator)
ns = Not Significant of level X2 Naibonat (0.95) = 1.64
ns = Not Significant of level X2 Nun Kurus (0.95) = 9.39
ns = Not Significant of level of X2 Camplong (0.95) = 16.15

Conclusion

1. Difference ecosystem at each zona own the separate character so that influence the good use at]election of varieties maize used by and also orient the market

2. Zona I generally use the pre-eminent varieties newly hybrid (Pioneer), harvested young to sold so that wish the pre-eminent varieties were which early with the hight production and feel sweet
3. Zona II predominated by the pre-eminent varieties newly OPV (Varieties Lamuru) with the high result potency and open the marketing network cooperatedly by LSM-LSM [
4. Zona III still use the local varieties and OPV pre-eminent varietas is which not yet regeneration. On Farm orientation still have the character of the subsisten
5. Varieties Lamuru represent one of varieties which released ICERI can fulfill the criterion interested by farmer, because tolerance to drought, also have the high result potency and also good performance.
6. Forming of seed grower of Variety Lamuru, can promotion varieties and distribution fulfilling 6 precisely (sum up the, varietas, time, location, quality of price draught

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Farmers and Local Government Responses in Demonstration Technology of High Yield Varietes of Open Pollinated Maize in Prima Tani of Boyolali District-Central Java

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Abstract. Prima Tani in Kembang Village, Ampel Sub District, Boyolali District started in 2007. The main objective of Prima Tani (Program of Initiation and Acceleration for Agricultural Technologies Innovation Delivery) is to develop agribusiness laboratory both to demonstrate promissing agriculture technologies and as a media to link all stake holders and beneficiaries to increase farmers welfare. The agroecosystem of Kembang village is dry upper land which locates in 700 – 900 meters above sea level. In this village, maize as a dominant food crops, is planted twice in a year. Maize contributes as much as 40% of farmers annual income even though most of farmers do not practice recommended technologies, such as local varieties, 100-200 kg per hectare of urea dossage, and practice irregular cropping distance. However in 2007 dry season (May-August) maize demonstration plot was introduced in Kembang Village. The technologies consisted of two high yield open pollinated varieties of maize, namely *Sukmaraga* and *Srikandi Kuning*, balance fertilizer dossages (300 kilograms of urea and 300 kilograms of 15:15:15 - NPK fertilizers), crops planting distance 75 x 40 cm, two seeds per hole, and physiological maturity harvesting system. The demonstration was carried out in 5000 m^2 of five farmers land. Farmers and researchers were participatively involved along the whole activities. In average yield of *Sukmaraga* was 4 tonnes per hectare, while yield of *Srikandi Kuning* was 4.5 tonnes per hectare. Farmers processed most of both new varieties yield as a seed to be planted in the following planting season. Farmers enthusiastically respond the new technologies since the yield was double compare to their practices (2-2.5 tones per hectare). In addition, as much as 80 farmers either from Kembang Village or from surrounding villages bought those maize seed (in average of 4 kilograms of seed per farmer) for the following 2007 wet planting season. The positive respond was also showed by the Boyolali Local Government inwhich subsidized further to the development of high yield open pollinated maize varieties in 20 hectares of Kembang Village for the next planting season.

Key words: open pollinated maize, newly high yield varieties, demonstration technology, Prima Tani, Boyolali

Background

Maize is considered as one main source of carbohydrate of food and feed industries. Share of feed industries demand for maize in Indonesia is about 30%. In 2005, estimation of national maize demand for feed industries was as much as 4.9 million tones and it was estimated that in 2010 the demand will be amounted as much as 6.6 million tones (Ditjen Tanaman Pangan, 2006). Unfortunately, maize national production was below the demand, for example in 2010 maize production nationally was estimated 3.67 million tones or only around 55% of feed industries demand. Infact, availability of promising technologies in research stations make it possible to increase maize production, especially in Java Island, up to 4.0 – 9.0 tones per hectare.

The wide yield gap between farmers and researches achievement rooted on the slow process of dissemination and adoption rates, in which being affected by government policies in supporting maize agribusiness (M.Akil *et.al,* 2007). In addition, implementation of introduced technologies by farmers varied and depended on farmers production orientation, soil fertility, farmers risk aversion, and their inputs' accessibility (Zubactirodin, *et al*. 2007). To increase maize production, however, internal and external factors of production inputs should be taken into account, such as varieties, and optimization of crops intensities and resources management. Those was well known as integrated crops management (ICM) (*Pengelolaan Tanaman - Sumberdaya secara Terpadu/ PTT)* (Purwanto, S., *et al*. 2007)

Program of Initiation and Acceleration for Agricultural Technologies Innovation Delivery (*Prima Tani*), as one of Indonesian Agency for Agricultural Research and Development (AARD) program, was carried out in 200 districts of 25 provinces in 2007. The main objective of Prima Tani is to develop agribusiness laboratory both demonstrating promising agriculture technologies and media to link all of stakeholders and beneficiaries to increase farmers' welfare. In Central Java, there are 19 sites of Pima Tani which are spread on 19 districts and one of which is in Boyolali District. Boyolali District covered 101,510.2 hectares of land and 77.4% out of its areas are dry land (78,563.6 hectares) while the remaining (22,964.7 hectares) are irrigated land. In further the productive dry land of Boyolali are around 39% (30,616 hectares). In that case, maize dominates productive dry land in Boyolali, for example in 2005 maize covers 29,234 hectares (95.5%) of dry land (BPS Boyolali, 2006).

In Boyolali District, Prima Tani locates in Kembang village, Ampel sub-district in which characterized by dry upper land (700-900 meter above sea level) and productive upland covers around 73.15% (411.9 hectares) of its area. In Kembang Village maize, as dominant food crops, is planted twice a year and contributes as much as 40% of farmers' income. Agroecosystem characteristics in Kembang village are suitable for maize commodity, such as annual rainfall averages 2500 mm, pH 5-6, soil texture silty lump (SL) – silty clay lump (SCL). Farmers existing technologies for maize are local varieties usage, irregular plant spacing, unbalance fertilizer application (only nitrogen fertilizer with dosage 100-500 kg/hectare), and without any organic fertilizers. However, it is reasonable to carried out maize demonstration technology. The objective of demonstration technology is to promote newly maize technology implementation which inturn make it possible to increase farmers income.

Methods

Demonstration technology was carried out in dry season of 2007 (May – August) and used Maize Integrated Crop Management (ICM) approach. The component of Maize ICM are the following:

1. Introducing of two new open pollinated maize varieties (*Srikandi kuning* and *Sukmaraga* varieties)
2. Optimal population regular plant spacing (40 x 70 centimetres), two seeds per hole
3. Organic matter application (2,000 kilograms per hectare)
4. Specific location of balance fertilizer recommendation: nitrogen (urea) 300 kilograms per hectare (leaf color chart/LCC using), 300 kilograms per hectare compound fertilizer (NPK 15-15-15)
5. Timing management of fertilizer applications - three times for urea: 1st: 7-14 days after plantation/DAP, 2nd: 30-35 DAP, 3th: 50-55 DAP and all dosage of NPK at the first time of fertilizer application.
6. Physiological maturity harvesting system

The demonstration involved actively 5 farmers who each farmer applied maize ICM on 1,000 m^2 of their land or totally covered 5,000 m^2 of land.

Procedures of demonstration technology are the following:

1. Farmers and location identifications
2. Program designing and technology introducing
3. Technologies implementation
4. Periodical meeting which involved farmers' group, field extension agents, local government staffs, and researchers
5. Participative supervision and intensive discussion among farmers, field extension agents and researchers
6. Post harvest management – seed processing manage by farmers group under researchers' supervisions
7. Data analysis (descriptive analysis) and evaluation

Results

ICM implementation of technology demonstration in Kembang Village has increased maize yield significantly. As farmers' practices yielding was 2.5 tones per hectare of maize grain yield, yield of ICM for *Sukmaraga* and *Srikandi Kuning* Varieties were 4.0 and 4.5 tones per hectare respectively. Accordingly, cash input-output analysis (Table 1) showed that new technologies practices benefited farmers. Benefit-cost analysis of new technology were 5.63 and 5.82 for *Sukmaraga* and *Srikandi Kuning* varieties respectively compare to 7.14 for farmers' practices. In further, net cash income of farmers from maize increased significantly (more than 400%) in which for every 1000 meters square their income increased from Rp.430,000 to Rp. 1,809,400 for *Sukmaraga* variety and Rp. 2,049,400 for *Srikandi Kuning* variety.

Farmers responded positively maize ICM, especially for new maize composite varieties. Farmers interested on their better vigor performances compare to the local varieties and planned to plant further in their own field. The positive responds of surrounding farmers encouraged participant farmers' group to process new varieties of maize yield for a seed purposes. Seeds processing were supervised by researchers, extension agents, and Food crops Services of Boyolali District

Boyolali District Government responded further by designing subsidy programs which contain new composite maize varieties and other inputs needed in the following planting season (rainy planting season of 2007).. Subsidy programs were targeted to five farmers' groups in Kembang

Table 1. maize cash performances of input – output of farmers' practice and demonstration technology

No	Description	Farmers' practice			Technology demonstration			
			local variety		Sukmaraga		Srikandi Kuning	
		per ha	volume	Price (Rp)	volume	Price (Rp)	volume	Price (Rp)
1	Number of farmer	N	4		2		3	
2	Inputs (a+b+c+d+e)			700,000		3,906,000		4,256,000
	a Seed	Kg	30	60,000	20	140,000	20	140,000
	b Seed treatment	g	-	-	10	9,000	10	9,000
	b Organic matter	Kg	-	-	2000	-	2000	-
	c N fertilizer (urea)	Kg	400	640,000	300	360,000	300	360,000
	d NPK fertilizer	Kg	-	-	300	525,000	300	525,000
	e Insecticide	Kg	-	-	8	72,000	8	72,000
3	Grain Yield (average)	kg	2500		4000		4500	
4	Maize price (consumption)	Rp		2,000		2,000		2,000
5	Revenue from consumption yield	Rp		5,000,000	1200	2,400,000	1350	2,700,000
6	Maize price (seed)	Rp		-		7,000		7,000
7	Cost for seed processing	Rp	-	-		2,800,000		3,150,000
8	Revenue from seed production	Rp	-	-	2800	19,600,000	3150	22,050,000
9	Total revenue (TR)	Rp		5,000,000		22,000,000		24,750,000
10	Benefit (9-2)	Rp		4,300,000		18,094,000		20,494,000
11	TR/TC			7.14		5.63		5.82
12	Benefit per 1000 m2	Rp		430,000		1,809,400		2,049,400

Table 2. New varieties of maize seeds buyers and volume

No	Buyers'	Srikandi Kuning		Sukmaraga	
		Volume (kg)	(%)	Volume (kg)	(%)
1	Farmers from Kembang village	105	34.2	121	46.5
2	Farmers from outside of Kembang village	62	20.2	59	22.7
3	Boyolali district government	140	45.6	80	30.8
	Total	307	100.0	260	100.0

Village. In that case, farmers were required to implement maize ICM approach. The new varieties of maize seed were produced by Kembang Village farmers. Table 2 presented new varieties of maize seeds buyers and their volume.

Based on seed recommendation as much as 20 kilograms per hectares, Srikandi Kuning varieties in Kembang Village were adopted and planted in 12.25 hectares and 5.25 hectares out of which were self budgeted by farmers. Moreover, Sukmaraga new varieties were planted in 12.05 hectares and 6.05 hectares out of which were funded by farmers. It was interesting that farmers from surrounding villages also started to adopt new varieties. It was recorded that Srikandi kuning and Sukmaraga were planted in 3.1 and 2.96 hectares respectively Farmers' group of Kembang Village planned to process maize yield under Boyolali District subsidy program for seed production. Hopefully it will be a maize seed agribusiness community bases in Kembang Village, Ampel Sub District, Boyolali District.

Conclucion

1. Farmers and local government enthusiastically responded the new maize ICM technologies since the yield were almost double compare to farmers' practices
2. Local Government supported the dissemination of new technologies by subsidizing 20 hectares of open pollinated high yield varieties of maize for the following planting season in Kembang Village and encourage farmers group to be a seed producer of high yield maize varieties.

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Section 2

Maize Breeding

Inheritance of Grain Yield of Maize with a Diallel Design

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Abstract. Maize yields are very low under the prevailing environmental and management conditions in Sri Lanka. To enhance productivity, varieties that can exploit the limited resources available under Sri Lankan conditions have to be developed. Diallel design is one of the most widely used mating designs for maize breeding. Seven elite maize lines were crossed in a diallel scheme as per the Griffing model 2, and the crosses were assessed at the Field Crops Research and Develoment Institute (FCRDI), Maha-Illuppallamma, during the Yala 2004 and Maha 2004/05 seasons. The experiments were set up in a randomized complete block design (RCBD) with three replications. Local commercial hybrids were sown as control. The plots consisted of two 5-m long rows, spaced at 0.6 × 0.3 m. The grain yield was obtained from all plants in both rows of each plot. General and specific combining (GCA and SCA) effects were determined by the Griffing model. The results showed that GCA and SCA effects were significant for both seasons. The relative contribution of SCA effects to general variability was higher than that of GCA effects since the percentages of the SCA sum of squares were 73% and 91% for Yala 2004 and Maha 2004/05 seasons, respectively, which indicated that dominance effects predominated for the grain yield increment, and crosses CML 161 × CML 171, CML 168 × CML 194, CML 194 × CML 189, CML 171 × CML 193, CML 168 × CML 193, CML 161 × CML 168 and CML 164 × CML 168 are more suitable for use in future maize breeding programs.

Key words: Maize, diallel, CIMMYT Maize Line, general combining ability, specific combining ability, combining ability

Introduction

Maize (*Zea mays* Linn.) is the most widely used cereal crop with uses as food and feed and in industry. Land for maize production in Sri Lanka has averaged about 25 000 ha per year in the last three or four decades. From the 1990s, there has been much demand for maize in the local markets to meet the needs of the poultry industry. However, as local production fell short of both quantity and quality requirements, imports of the commodity increased sharply within a short period of time to over 200 000 t at present. This has led to public and private sector companies launching programs to produce more quality maize grain locally. These organizations provide improved hybrid seed, fertilizer, loans, improved production technologies and marketing facilities, etc to help farmers enhance productivity and production across the country.

As a result, maize acreage and productivity in certain districts has risen several fold, generating high incomes for maize farmers. Consequently, seed supply has had to be increased. Demand for seed is now solely met by import of hybrid seeds to the extent of about 400 t per year. To reduce dependence on imports and lower import expenditure, the Department of Agriculture developed a local maize hybrid using CIMMYT (International Maize and Wheat Improvement Center) lines in 2004 and commenced its seed production.

Along with this normal corn hybridization program, a quality protein maize (QPM) hybrid development program was initiated with inbred lines obtained from CIMMYT. Quality protein maize hybrids are suitable for both food and feed purposes and have high yield.

Hybrid development and seed production are novel experiences to Sri Lanka. Several breeding procedures have been established to increase the grain yield of maize populations and their hybrids. In order to choose the best hybrid combinations, a large number of subjectively chosen inbred lines have been crossed. It would be a considerable advantage to be able to estimate the combining ability of parents and gene effects before making crosses among inbred lines. Diallel crossing programs have been initiated to achieve this goal by providing a systematic approach for the detection of suitable parents and crosses for select characters, especially grain yield of maize. In addition, diallel analysis provides plant breeders the opportunity to choose the most efficient selection method by allowing them to estimate genetic parameters (Verhalen and Murray 1967).

Combining ability describes the breeding values of parental lines to produce hybrids. Sprague and Tatum (1942) used the term general combining ability (GCA) to designate the average performance of a line in hybrid combinations, and the term of specific combining ability (SCA) to define those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved. In many studies, GCA effects for parents and SCA effects for crosses have been estimated in maize (Dehghanpour *et al.* 1996; Chaudary *et al.* 1998; Araujo and Miranda 2001).

The objectives of this research were to estimate the genetic parameters and to determine suitable parents and promising crosses for grain yield in a 7 × 7 diallel experiment conducted under Sri Lankan conditions with maize inbred lines obtained from CIMMYT.

Materials and Methods

Seven QPM inbred lines, CML 161, CML 164, CML 168, CML 171, CML 189, CML 193 and CML 194 (CML: CIMMYT Maize Line), were selected from the gene bank of CIMMYT in Mexico, and were sown at the Field Crops Research and Development Institute, Maha-Illuppallama, Sri Lanka for seed multiplication purposes.

The diallel crosses made among the seven QPM lines (21 crosses and seven self lines) were thereafter tested using three replications for promising hybrids in terms of grain yield during the *Yala* 2004 and *Maha* 2004/2005 seasons. During *Yala* 2004, only the crosses were used for yield evaluation due to inadequate seed availability of one inbred. Apart from the inbred lines and crosses, a widely cultivated local hybrid Sampath and a commercial hybrid Pacific 984 were included in the *Maha* 2004/2005 experiment for comparison purposes. The plots consisted of 2 rows 5.0 m long and spaced 0.6 m apart and plants spaced 0.3 m apart within each row. Diazinon with 3% granules (12-15 kg ha^{-1}) was added to the whorl of 3-weeks-old plants to control stalk borer damage. Fertilizer was added at the rates recommended by the Department of Agriculture for *Yala* and *Maha* crops. Grain yields of individual genotypes were obtained from the plots and expressed in t ha^{-1}.

The Hayman (1954) Griffing (1956) and Cockerham (1963) model I was used to test the significance of combining abilities. Thereafter, GCA for each line and SCA for each cross were obtained as described by Hallauer and Miranda (1988). Genetic parameters were estimated using the SAS (2002) diallel analysis program developed by Suriyagoda and Peiris (2008). Analysis was performed separately for the two seasons.

Results and Discussion

Preliminary analysis of variance, including GCA and SCA effects, indicated that the genotypes are significantly different in terms of grain yield. Observations were similar for both seasons (Table 1). The average grain yields of pure lines and off-diagonal crosses and the GCA values for each parent are shown separately for both seasons in Table 2. CML 189 had the highest GCA value for both seasons, and CML 193 and CML 168 the smallest GCA values, representing the 6th and 7th ranks respectively. Thus, the GCA effect of parental lines for the two experimental seasons was fairly consistent. The average yield of off-diagonal cross CML 189 was the highest and the yield of CML 168 the lowest in *Yala* 2004 among the CML lines, and the difference was significant at ($p \leq 0.05$). However, the local hybrid Sampath had a similar yield as the best CML line, CML 189. Contrastingly, the grain yield of Sampath was similar to that of the CML lines in *Maha* 2004/2005 while the standard hybrid Pacific 984 showed significantly higher yields. Also, the yields of CML 189, CML 161, CML 194 and CML 164 were similar to that of Sampath in *Yala* 2004.

Table 1. Preliminary ANOVA providing the significance of GCA and SCA effects.

Source	*Yala* 2004			*Maha* 2004/2005		
	Df	SS	P	Df	SS	P
Replicate	2	11.55	0.01	2	0.04	0.97
Crosses	20	65.96	0	27	199.9	0
GCA	6	17.71	0.02	6	17.91	0
SCA	14	48.25	0	21	181.99	0
Residual	40	39.34		54	32.66	
Total	62	116.85		83	232.6	

Table 2. Average grain yield (t ha^{-1}) of pure lines and off-diagonal crosses and GCA effects of each parental line for two seasons.

Parent	*Yala* 2004			*Maha* 2004/2005		
	Yield of pure lines	Yield[1] of off-diagonals	GCA	Yield of pure lines	Yield of off-diagonals	GCA
CML 161	3.38	5.28ab	0.451(2)	2.47	4.42a	- 0.179 (5)
CML 164	2.09	4.88ab	-0.025 (4)	2.86	4.62a	0.021 (4)
CML 168	NA[2]	4.41a	-0.587 (7)	1.49	4.18a	- 0.470 (7)
CML 171	NA 2.94	4.65a	-0.301 (5)	3.53	4.62a	0.094 (3)
CML 189	1.86	5.43b	0.634 (1)	2.39	5.18a	0.408 (1)
CML 193	2.59	4.62a	-0.336 (6)	1.76	4.39a	- 0.273 (6)
CML 194	6.22	5.04ab	0.163 (3)	3.09	5.07a	0.400 (2)
Sampath	NA	6.22b		5.52	5.52ab	
Pacific 984		NA		5.89	5.89b	

[1.] Different letters following yield values indicate significant difference at ($p \leq 0.05$).
[2.] NA = Not available.

Table 3. SCA effects and average grain yield of the test crosses. Above-diagonal and below-diagonal values indicate the SCA effects for *Yala* 2004 and the corresponding average grain yield (t ha^{-1}) respectively.

Parents	SCA values and grain yield of crosses						
	CML 161	CML 164	CML 168	CML 171	CML 189	CML 193	CML 194
CML 161		-0.84	0.45	1.03	-0.05	-0.25	-0.33
CML 164	4.48		0.98	0.77	-0.75	-0.29	0.12
CML 168	5.21	5.27		-3.01	0.41	0.39	0.78
CML 171	6.08	5.34	1		0.1	0.9	0.22
CML 189	5.93	4.76	5.36	5.33		0.17	0.12
CML 193	4.77	4.25	4.36	5.16	5.37		-0.92
CML 194	5.19	5.16	5.26	4.98	5.82	3.81	

Table 4. SCA effects and average grain yield of the test crosses. Above-diagonal and below-diagonal values indicate the SCA effects for *Maha* 2004/2005 and the corresponding average grain yield (t ha^{-1}), respectively.

Parents	SCA values and grain yield of crosses						
	CML 161	CML 164	CML 168	CML 171	CML 189	CML 193	CML 194
CML 161		-0.19	1.6	0.36	0.3	0.49	0.53
CML 164	4.02		1.09	0.7	0.09	1.09	0.34
CML 168	5.33	5.02		-2.82	0.61	1.43	1.97
CML 171	4.65	5.19	1.18		1.3	1.66	0.87
CML 189	4.90	4.10	4.93	6.17		1.16	2.13
CML 193	4.41	5.21	5.06	5.85	5.67		-1.69
CML 194	5.13	5.13	6.27	5.74	7.31	2.81	

The SCA effects and average grain yield for the test crosses are given in Table 3 and Table 4 separately for the two seasons. The crosses that showed the highest SCA values were CML 171 × CML 161 in *Yala* 2004 and CML 189 × CML 194 in *Maha* 2004/2005. Furthermore, CML 168 × CML 194, CML 171 × CML 193, CML 161 × CML 168, CML 168 × CML 193 and CML 164 × CML 168 showed higher positive SCA values in both seasons. Contrastingly, the SCA values of crosses CML 161 × CML 164, CML 168 × CML 171 and CML 193 × CML 194 were the lowest in both seasons. In addition, the SCA values of the all-self progeny were negative in *Maha* 2004/2005.

The parents in the diallel cross were selected from CIMMYT since they had been tested in environments similar to Sri Lankan conditions. The GCA and SCA effects were highly significant ($p \leq 0.05$) for both seasons, indicating the significant variability of genetic potential for grain yield (Table 1). Even though the yield responses of the CML lines in the *Yala* season were significantly different, no such yield difference was observed for *Maha* among the CML lines. This might be due to significant interaction between season and cross effects. Further, the better performing lines in terms of grain yield had higher positive GCA values and CML parents with lower grain yield showed negative GCA effects. The GCA effects of parents were also lower than the SCA estimates for most of the lines. Further, the low GCA estimates of most parents also indicate that transfer of quantitative traits may require more time and money in maize breeding, such as more selection cycles or multiple crosses to accumulate yield-additive GCA effects. In both seasons the major part of genetic variability was due to the SCA. The percentage of the SCA sum of squares was approximately 73% and 91% for *Yala* 2004 and *Maha* 2004/2005 respectively. This suggests that there is sufficient dominant gene action to support a breeding program for maize grain yield.

Grain yields from the crosses as well as their SCA values revealed that the genotypic background contributes considerably to grain yield. In this experiment, higher positive SCA effects occurred more frequently in the higher-yielding lines, and negative SCA effects happened more in populations with lower yield potential. Several crosses had means that surpassed even the highest-yielding parent, resulting in improved yield response. The higher yield performance of some populations provides the possibility of developing lines even better than the best parents. This knowledge of the quantitative yield advantage of parents may be of significance in planning genetic combinations.

Conclusions

Since individual plant data were not available for each line, information on heritability was not obtained. Considering the SCA estimates of both seasons, crosses (CML 161 × CML 171), (CML 168 × CML 194), (CML 194 × CML 189), (CML 171 × CML 193), (CML 168 × CML 193), CML 161 × CML 168) and (CML 164 × CML 168) may be suitable for use in future breeding programs for higher grain yield. Further, the resistance of these lines to pest and disease conditions and to diverse environments and different locations needs further investigation.

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Performance of New QPM Inbred Lines Developed by Anther Culture and Conventional Conversion from Normal Maize Lines in North Vietnam

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Abstract. Double-haploid (DH) QPM lines developed by anther culture, followed by four seasons of selection using SSR primers phi057 and phi112, revealed that 30 out of 90 QPM inbred lines, developed from 8 normal maize lines (C 2, C 4, C 153, C 154, C 164, C 172, T 2, T 5), and four normal hybrids (SC 1614, SC 18161, SC 7114, C 919) and one QPM hybrid HQ 2000 showed high general combining ability (GCA) when crossed with callus, embryo and plant regeneration-responsive line AC 24 and CML 161 (QPM). Eight new QPM inbred lines were developed from two normal inbred lines (TCH 1 and DF 7) having high yield and GCA by backcrossing the 8 lines with CML 161 and CML 165 lines (QPM) followed by selection under a fluorescence lamp.

Forty-four QPM lines (38 newly developed and 6 imported from CIMMYT) and 1 normal line (control) were evaluated for yield and agronomic performance in the winter of 2007 and the spring of 2008 at Dan Phuong, Hanoi, Vietnam. Through evaluation and by applying the selection index (SI), 6 DH QPM lines [V 62, V 64, V 66, V 68, V 72 and C 141 (SI =14.34–17.52)], 7 QPM conventional-conversion lines: Q 1, Q 5, Q 11, Q 16, Q 18, Q 21, KQ 1 (SI = 15.0 – 17.85), CML 161 and CML 161/422 were selected. These lines had good tolerance for ear rot (8.3-33.7%), longer stay green leaves, good plant and ear characters (scores of 2-3), high grain yield ranging from 25.48 q ha^{-1} to 46.89 q ha^{-1} compared to normal control (DF 2) which had grain yield of 24.4 q ha^{-1}, ear rot percentage of 34.6% and plant and ear character scores of 3. These lines are under further development for promising hybrids.

Key words: QPM lines, anther culture, conventional conversion, evaluation, selection index.

Introduction

Quality protein maize (QPM) cultivars enhance the quality of animal feed and serve to address the food problem in mountainous regions. Therefore, developing QPM hybrids is becoming an emerging strategy for CIMMYT, some African countries and Vietnam where land is limited and demand for maize is increasing. However, QPM lines still suffer from some major agronomic drawbacks such as susceptibility to insect pests and fungal and bacterial diseases (Le and Tran 2002).

To solve these deficiencies of QPM lines, two kinds of conversion of normal into QPM lines, conventional backcrossing and anther culture, have been undertaken in Vietnam since 2003. In this study, we evaluated these lines in the winter of 2007 and the spring of 2008 at the National Maize Research Institute of Vietnam.

Materials and Method

Forty-four QPM lines (8 lines obtained by conventional conversion: QL 1 to QL 7 and QL 41; 4 imported from CIMMYT: QL 9 to QL 12; 30 lines obtained by DH anther culture: QL 8, QL 13 to QL 40 and QL 42; 2 QPM controls (CML 161 and CML 165, parents of QPM hybrid HQ 2000 in Vietnam) and 1 normal control (DF 2) were used in this study.

Conversion of normal lines into QPM lines

I. Double-haploid lines developed by anther culture (Bui *et al.* 2006) from 8 normal lines (C 2, C 4, C 153, C 154, C 164, C 172, T 2 and T 5), 4 normal hybrids (SC 1614, SC 18161, SC 7114, C 919) and QPM hybrid HQ 2000 were crossed with DH-responsive AC 24, and then crossed with CML 161 (CIMMYT line with o_2o_2). Immature anthers from these crosses were consecutively cultured with embryo-initiation YP media, plant regeneration media (N6 + 2 ppm Kinetin), complete plant regeneration media (MS + 2 ppm áNAA), and glasshouse plant media (1/10 MS + rice straw ash). The crossing scheme is presented in Figure 1.

II. The other method of conversion of normal into QPM lines that we followed was conventional (Gevers 2002; Vasal 2002). Fig. 2 shows the procedure of crossing normal TCH 1 (parent of LCH 9 single-cross) and DF 7 (parent of LVN 4 single-cross) with QPM lines from CIMMYT (CML 161 and CML 165).

At harvest, if segregation of opaque-2 on the ear was observed, then we discarded about 50% of the normal ears, and selected 50% of the seeds from the remaining ears with modified QPM under a fluorescence lamp, then sowed well-modified seeds as the S_1 generation, and continued backcrossing until stable o_2o_2 kernels were obtained. The kernels were then analyzed for protein and amino acid content of the converted QPM lines. After 5 backcrossing generations, combined with QPM selection under the fluorescence lamp, seven QPM lines were developed.

Season 1 (Year 1): Normal × AC 24 (callus/embryo initiation-responsive)

Season 2 (Year 1): F_1 × QPM (CML 161)

Season 3 (Year 2): Anther culture and plant regeneration

Season 4 (Year 2): Field evaluation

Figure 1. DH QPM conversion by anther culture.

Experimental design and data analysis

Forty-five elite lines were evaluated in the field (Table 1) in an alpha lattice design with 2 replications, the plot size being 5 m long. Standard procedures for national variety testing (10 TCN-312-2006, Vietnamese Agricultural Standards 2006) were followed. Data was analyzed by Alpha program (CIMMYT 1999).

Results and Discussion

Tolerance to biotic and abiotic stresses

We evaluated 45 QPM lines during the two cropping seasons (winter 2007 and spring 2008). However, the results presented here are mostly based on 7 QPM lines (Q 11, Q

Season 1. Materials QPM × Normal
Locus (o_2o_2)(NN)

Season 2. Materials F_1 × Normal
Locus (N o_2) (NN)

Season 3. Materials BC1
Locus (50% NN and 50% N o_2)

Season 4: Selfing BC1

Figure 2. Conventional conversion of normal into QPM lines.

Table 1. QPM inbred lines evaluated in winter 2007 and spring 2008 at Dan Phuong, Hanoi, Vietnam.

Entry	Code	Inbred line	Origin	Entry	Code	Inbred line	Origin
1	QL 1	Q 1	Conventional -VN	23	QL 23	V 71	DH – VN
2	QL 2	Q 5	Conventional -VN	24	QL 24	V 72	DH – VN
3	QL 3	Q 10	Conventional -VN	25	QL 25	V 73	DH – VN
4	QL 4	Q 11	Conventional -VN	26	QL 26	V 74	DH – VN
5	QL 5	Q 16	Conventional -VN	27	QL 27	V 78	DH – VN
6	QL 6	Q 18	Conventional -VN	28	QL 28	V 80	DH – VN
7	QL 7	Q 21	Conventional -VN	29	QL 29	V 94	DH – VN
8	QL 8	C 141	Conventional -VN	30	QL 30	V 95	DH – VN
9	QL 9	CML 161/422	CIMMYT	31	QL 31	V 120	DH – VN
10	QL 10	CDVA 16	CIMMYT	32	QL 32	V 121	DH – VN
11	QL 11	CDVA 82	CIMMYT	33	QL 33	V 122	DH – VN
12	QL 12	CDVA 119	CIMMYT	34	QL 34	V 123	DH – VN
13	QL 13	V 57	DH - VN	35	QL 35	V 139	DH – VN
14	QL 14	V 59	DH - VN	36	QL 36	V 170	DH – VN
15	QL 15	V 61	DH - VN	37	QL 37	V 173	DH – VN
16	QL 16	V 62	DH - VN	38	QL 38	V 182	DH – VN
17	QL 17	V 63	DH - VN	39	QL 39	V 289	DH – VN
18	QL 18	V 64	DH - VN	40	QL 40	V 334	DH – VN
19	QL 19	V 66	DH - VN	41	QL 41	KQ 1	Conventional -VN
20	QL 20	V 68	DH - VN	42	QL 42	KQ 7	Conventional -VN
21	QL 21	V 69	DH - VN	43	QL 43	CML 161 (QPM control 1)	CIMMYT
22	QL 22	V 70	DH - VN	44	QL 44	CML 165 (QPM control 2)	CIMMYT
				45	QL 45	DF 2 (normal control)	Vietnam (normal)

Table 2. Tolerance of abiotic and biotic stresses in selected QPM lines, Dan Phuong, Hanoi, Vietnam, 2007-2008.

Entry	Line	Ear rot (%)		Stem borer (%)		BLSB (%)		Root lodging (%)	
		Winter 2007	Spring 2008	Winter 2007	Spring 2008	Winter 2007	Spring 2008	Winter 2007	Spring 2008
1	Q 11	13.6	11.6	33	27.5	13	1.8	9.9	4.5
2	V 62	21.2	12.3	20	26	21.5	1.8	10.0	1.8
3	V 64	13.8	8.3	28.5	21.3	4.0	1.5	2.5	5.8
4	V 68	8.8	14.2	39.5	24.3	19.0	1.3	4.0	7.4
5	Q 18	2.5	9.0	39.5	19.6	0.0	1.0	3.0	11.0
6	CML 161/422	10.9	12.9	20	25.6	3.5	2.0	26.7	12.7
7	V 72	13.7	19.4	30	23.5	20	2.5	13.3	3.3
8	CML 165 (QPM control 2)	18.2	16.3	23	22.3	9	1.0	6.7	2.9
9	CML 161 (QPM control 1)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	6.7
10	DF 2 (control 3 - normal)		24.6		29.4		2.5		9.5

Table 3. Yield components of selected QPM lines in winter 2007 and spring 2008 in Hanoi, Vietnam.

Ent	Line	Ear length (cm)	Ear tip length (cm)	Ear diameter (cm)	Kernel rows	Kernel row^{-1}	Ear $plant^{-1}$	Shell percentage (%)	1000-seed weight (g)	Grain yield (q ha^{-1})
1	Q 11	12.9	0.6	4.1	14.8	19.2	1.4	0.7	227	37.2
2	V 62	12.1	0.6	3.6	14.2	18.0	1.3	0.7	221	37.2
3	V 64	12.7	0.6	3.7	12.3	16.9	1.3	0.7	249	26.3
4	V 68	12.2	0.9	3.2	12.8	18.0	1.2	0.7	259	26.3
5	Q 18	13.0	1.6	3.7	13.2	19.3	1.4	0.7	267	31.9
6	CML 161/422	12.7	0.3	3.6	16.2	22.7	1.5	0.7	235	37.6
7	V 72	12.5	1.3	3.6	15.0	18.8	1.3	0.6	255	23.7
8	CML 165 (control 2 - QPM)	11.5	0.8	3.0	11.4	13.5	0.8	0.6	185	6.7
9	CML 161 (control 1 – QPM	12.0	1.5	3.9	13.3	17.7	1.4	0.7	280	32.3
Spring 2008										
1	Q 11	12.2	0.2	4.1	13.4	24.6	1.1	64.9	273.5	44.6
2	V 62	12.4	0.9	3.7	12.9	23.6	1.1	66.5	278.8	38.4
3	V 64	12.5	0.4	4.0	13.5	24.1	1.1	65.3	296.2	46.5
4	V 68	14.1	0.3	3.7	12.6	27.1	1.1	62.2	311.7	44.5
5	Q 18	14.8	0.7	3.6	12.4	24.7	1.1	62.4	307.7	38.6
6	CML 161/422	13.3	0.2	4.1	14.7	23.9	1.0	58.8	260.6	44.6
6	V 66	12.4	0.9	4.2	16.7	25.3	1.1	65.2	245.9	46.9
7	V 72	14.2	1.2	3.8	12.7	22.6	1.0	65.9	324.3	38.7
8	CML 161 (control – QPM)	12.5	2.0	4.2	13.7	21.9	1.0	62.6	337.7	40.3
9	CML 165 (control 2 - QPM)	11.7	0.4	3.5	13.2	20.1	1.0	52.5	202.9	12.9
10	DF2 (control 3 -normal)	13.8	0.5	3.6	12.6	18.7	1.0	60.3	336.6	24.4
	LSD (0.05)									3.32

18, V 62, V 64, V 68, V 72, CML 161/422) which were chosen by way of the selection index. Ear rot fungi contamination in these lines ranged from 2.5% (winter 2007) to 19.4% (spring 2008); stem borer ranged from 8.3% (spring 2008) to 39.5% (winter 2007); and root lodging ranged from 1.8% (spring 2008) to 26.7% (winter 2007). Stem borer damage and ear rot were also found in the other lines tested, but the infection rates were quite different. V 62, V 64 and CML 161/422 were more tolerant to abiotic and biotic stresses than the two QPM controls (Table 2).

Yield potentials of new QPM inbred lines

Grain yield of the 45 lines varied a lot from 6.7 q ha^{-1} to 37.6 q ha^{-1} in winter 2007 and from 7.64 q ha^{-1} to 46.9 q ha^{-1} in spring 2008. In winter 2007 (Table 3), CML 165 (QPM control 2) had the lowest yield (6.7 q ha^{-1}) while CML 161/422 had the highest (37.6 q ha^{-1}). In spring 2008, V 70 had the lowest yield (7.74 q ha^{-1}), and V 66 the highest (46.9 q ha^{-1}, higher than the 3 controls). Across the two seasons, 28.8% of the total number of lines and 60% of the selected lines (Q 11, CML 161/422, V 64, V 66, V 68, Q 5) had yields over 40 q ha^{-1}, significantly higher than the yields (3.3 q

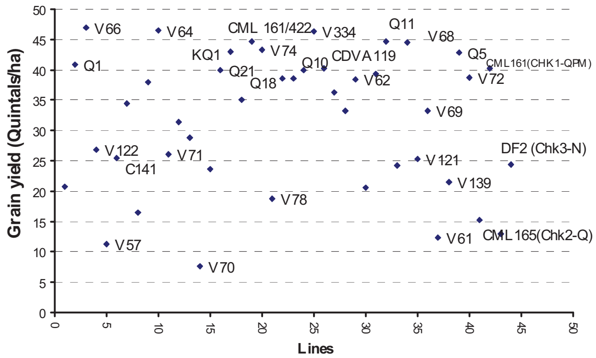

Figure 3. Grain yields of 18 selected QPM lines evaluated in winter 2007.

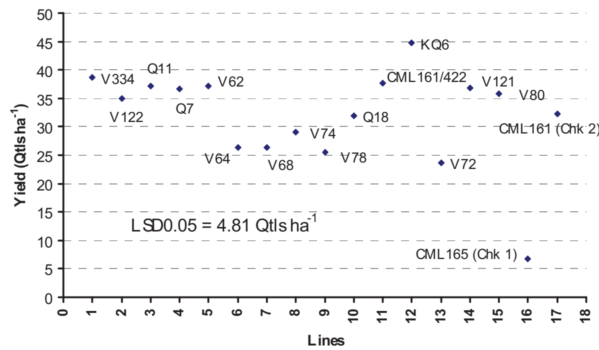

Figure 4. Grain yields of 45 QPM lines selected by SI, spring 2008.

Table 4. Correlation between grain yield and agronomic traits of selected QPM lines, spring 2008.

Criteria	Ear rot	Stay green	Ear tip length	Ear length	Ear diameter	Kernel rows	Kernels row^{-1}	Ear $plant^{-1}$	Kernel weight
Stay green	0.101	1							
Ear tip length	0.145	0.331	1						
Ear length	0.05	0.384	0.869	1					
Ear diameter	-0.349	0.095	0.119	0.081	1				
Kernel rows	-0.297	-0.005	-0.206	-0.129	0.635	1			
Kernels row^{-1}	-0.25	0.189	0.425	0.592	0.387	0.253	1		
Ear $plant^{-1}$	-0.398	0.107	0.074	0.249	0.266	0.215	0.482	1	
Kernel weight	-0.093	0.135	0.417	0.321	0.293	-0.276	0.235	0.16	1
Grain yield	-0.536	0.041	0.077	0.239	0.564	0.490	0.585	0.603	0.264

ha^{-1}) of the 3 controls at LSD 0.05 (Fig. 3; Fig. 4). Yield components of the selected QPM lines were comparable to normal lines in terms of the number of kernel rows, diameter of ears and ears per plant. In short, across the two seasons, Q 11 and CML 161/422 produced higher yields than the QPM check CML 161 at 95% significant level.

Correlation between yield and other traits

During spring 2008, grain yield was positively correlated (Table 4) with ear diameter (r = 0.564). Ear rot was negatively related to ear diameter, kernel rows, kernels per row, kernel weight, number of ears per plant, suggesting that the *opaque-2* gene in the cobs and kernels might affect the level of ear rot infection while improving yield components. However, compared to the QPM controls, ear rot in some DH lines (V 62, V 64) and conventional QPM lines (Q 11, Q 18) was found not to be as serious as in the normal control.

Conclusions

QPM inbred lines produced by DH anther culture are as promising as conventional QPM lines. However, DH QPM conversion is 2-3 seasons faster. In spring 2008, several QPM lines including DH (V 66, V 64) and conventional lines (Q 1, Q 11), gave higher yields and higher tolerance to abiotic stress than the normal control.

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Heterosis for Morphophysiological Traits Associated with Drought Tolerance in Maize

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Abstract. Drought is one of the important universal abiotic stress conditions, particularly in maize which is prone to terminal moisture stress during the flowering/grain-filling stages. In India, maize is vulnerable to the vagaries of the monsoon. Hence, there is an urgent need to develop superior cultivars as an affordable alternative for drought-prone rainfed areas. Therefore, an attempt was made to study the role of different morphophysiological traits in withstanding terminal drought stress and to obtain productive derivatives from heterosis breeding under varied environments. The present investigation comprised of 87 strains (60 hybrids, 23 parents and 4 controls) derived by line × tester mating design (20×3). They were evaluated under two different moisture regimes: (i) optimum moisture at critical stages (E_1); and (ii) terminal moisture stress at the flowering stage (E_2). Observations were recorded on 16 morphophysiological traits: days to 50% tasseling, days to 50% silking, anthesis-silking interval (ASI), days to 50% brown husk, plant height, ear height, leaf senescence, (1-5), leaf rolling (1-5), tassel score (1-5), number of cobs per plant, 100-seed weight, grain yield per plant, harvest index (%), chlorophyll stability index (CSI), desiccation injury (%) and drought susceptibility index (DSI). Data were computed for heterosis, heterobeltiosis and economic heterosis as per the usual procedure. Heterosis breeding is primarily based on the identification of parents and their cross combinations capable of producing the highest level of transgressive segregants. Parents EI-519, EI-522, EI-517, EI-509, EI-527 and EI-518 had, respectively, 51.8%, 51.3%, 38.8%, 49.7%, 46.9%, 14.6% and 5.6%, reduction in yield under moisture stress conditions. Tester EI-472 exhibited 26% reduction in grain yield in the stress environment coupled with the least CSI, leaf rolling and DSI in both normal and moisture stress conditions. Among hybrids, (EI-517 × EI-472) recorded the highest grain yield closely followed by (EI-509 × EI-472), (EI-518 × EI-472) and (EI-519 × EI-499) with 38.7%, 29.5%, 48.7% and 48.3% yield reduction, respectively, in the stress environment. The highest magnitude of economic heterosis, heterobeltiosis and heterosis was also observed in these hybrids in both moisture regimes. Thus it can be concluded that parent EI-518 revealed the least reduction (14.61%) in grain yield in a terminal moisture stress environment, possessed fairly good yield, 100-seed weight, least CSI, leaf senescence, desiccation injury %, ASI and DSI in both environments and was found quite suitable as a parent for use in drought-tolerance breeding programs. Hybrid (EI-517 × EI-472) turned out to be promising as it possessed the highest grain yield and favorable drought-adaptive traits along with significant heterotic expression.

Key words: Drought, maize, morphophysiological traits, heterosis

Introduction

Maize has worldwide significance in terms of potential productivity and has great demand for human and animal consumption and for a larger number of industrial uses. The diversity of environments under which it is grown is unmatched by any other crop. One of the most important factors affecting maize yield under rainfed conditions is drought during the crop season. Drought is a multidimensional universal abiotic stress condition which limits maize production. Productivity in marginal environments has to be increased in order to meet the increasing demand for maize. This largely depends on the moisture stress tolerance of maize genotypes. Therefore an attempt was made to study the role of different morphophysiological traits in withstanding drought stress and to obtain productive derivatives from heterosis breeding under varied environments.

Materials and Methods

Twenty early-maturing, white-seeded inbred lines were crossed with three testers in a line tester design to generate 60 hybrids. These hybrids along with the 23 parents and 4 controls were planted in a randomized block design with three replications in 6 m single-row plots with 60 cm × 25 cm crop geometry under two moisture regimes: (i) optimum moisture at critical stages (E_1); and (ii) terminal moisture stress during the flowering stage (E_2). Recommended agronomic practices were adopted to raise a healthy crop. The material were evaluated for grain yield (g) per plant, 100- seed weight (g), harvest index (%), chlorophyll stability index (CSI), desiccation injury (%) and drought susceptibility index (DSI). The desiccation injury percentage, harvest index, CSI and DSI were computed as per procedures given by Donald and Hamblin (1976), Murthy and Majumdar (1962), Sullivan (1972) and Fischer and Maurer (1978) respectively. Heterosis, heterobeltiosis and economic heterosis were calculated as per standard procedures.

Results and Discussion

Heterosis is a complex genetic phenomenon. Its extent depends on the magnitude of nonadditive gene action and genetic diversity among parents. Heterotic response has often been expressed as an F_1 deviation from either the mid-parent or the better-parent value. Heterosis from the better parent may be useful in identifying the heterotic cross combination but these crosses can be of practical utility only if they show superiority over the standard/ best cultivar. In the present investigation, heterosis over the standard variety and mid-parent were estimated in optimum as well as stress environments.

A perusal of the means presented in Table 1 for grain yield shows that parental line EI-519 gave the maximum yield of 69.17 g, closely followed by EI-517 (68.33 g) and EI-509 (68.33 g) in optimum environment E_1 whereas the highest yield of 48.00 g was recorded by parental line EI-522 followed by EI-518 (47.67 g) and EI-517 (41.87 g) in moisture stress environment E_2. Among the parental lines, EI-522 exhibited the least reduction in yield (5.56%) in the stress environment.

Among the testers, EI-472 exhibited grain yields per plant of 67.67 g and 50.00 g in E_1 and E_2, respectively, with 26.00% yield loss in E_2. It also possessed the least value for chlorophyll stability index and drought susceptibility index in both normal and moisture-stress environments. Parents EI-518 and EI-522 revealed the least reduction in grain yield in stress conditions, possessed fairly good yield, 100-seed weight, least CSI, desiccation injury and DSI in both environments and were found quite suitable as parents for use in drought-tolerance breeding programs.

Similarly, out of the 60 hybrids tested, EI-517 × EI-472 manifested the maximum grain yield followed by EI-519 × EI-499, EI-509 × EI-472, EI-518 × EI-472 in E_1. Hybrid EI-509 × EI-472 showed the least reduction (26.00%) in yield in the stress environment (Table 2). Estimates of economic heterosis for yield and yield-contributing traits revealed that EI-517 × EI-572 exhibited significant positive economic heterosis for grain yield per plant with 36.08% and 18.08% superiority in E_1 and E_2 environments, respectively, over the best control (Table 3). This hybrid also had significant economic heterosis for 100-seed weight and harvest index (Table 4; Table 5). These results are in conformity with the findings of Betran *et al.* (2003) and Tollenaar *et al.* (2004).

The stability of chlorophyll pigments has been described as an indicator of drought tolerance, and direct correlation between low chlorophyll stability index and

Table 1. Mean performance of lines and tester for grain yield per plant in optimum (E_1) and moisture stress (E_2) environments.

Genotype	Grain yield per plant (g)		% reduction in yield in E_2 environment
	E_1	E_2	
Lines			
L13 (EI-519)	69.17	33.33	51.81
L11 (EI-517)	68.33	41.87	38.78
L16 (EI-522)	50.83	48.00	5.56
L20 (EI-527)	61.50	32.67	46.87
L12 (EI-518)	55.83	47.67	14.60
L4 (EI-509)	68.33	34.33	49.75
Tester			
T3 (EI-472)	67.67	50.00	26.00

Table 2. Hybrids identified on the basis of per se performance for grain yield per plant in optimum (E_1) and moisture stress (E_2) environments

Genotype	Grain yield per plant (g)		% reduction in yield in E_2 environment
	E_1	E_2	
L11× T3 (EI-517 × EI-472)	115.68	70.83	38.77
L4 × T3 (EI-509 × EI-472)	99.33	70.00	29.50
L12× T3 (EI-518 × EI-472)	99.17	50.83	48.70
L13 × T2 (EI-519 × EI-499)	96.67	50.00	48.30
PHM 1 (control)	85.00	57.67	32.15
PEHM 2 (control)	80.83	60.00	25.77

drought tolerance has been reported by Gautam *et al.* (1998) and Kumar *et al.* (2004). Negative heterosis and heterobeltiosis for CSI was observed in EI-517 × EI-472 and EI-522 × EI-499, revealing the stability of these two hybrids under moisture stress conditions (Table 6). These crosses also manifested a similar trend of negative heterosis for desiccation injury (Table 7). Thus confirms their stability in drought environments.

Table 3. Heterosis, heterobeltiosis and economic heterosis for grain yield per plant of selected crosses.

Cross	Heterosis		Heterobeltiosis		Economic heterosis	
	E_1	E_2	E_1	E_2	E_1	E_2
EI-517 × EI-472	70.10**	54.26**	69.27**	41.67**	36.08*	18.06*
EI-509 × EI-472	66.01**	46.08**	45.37**	40.00**	16.86**	10.86*
EI-518 × EI-472	60.59**	-	46.55**	-	16.67*	-
EI-519 × EI-499	48.72**	36.36**	39.76**	25.00*	13.73*	-
EI-522 × EI-499	59.09**	29.85**	45.83**	19.18*	16.67*	-

*Significant at 5% level. **Significant at 1% level.

Table 4. Heterosis, heterobeltiosis and economic heterosis for 100-seed weight of selected crosses.

Cross	Heterosis		Heterobeltiosis		Economic heterosis	
	E_1	E_2	E_1	E_2	E_1	E_2
EI-517 × EI-472	40.31**	38.95**	34.35**	16.61**	9.87*	2.42*
EI-509 × EI-472	29.85**	20.43**	26.76**	4.66**	8.85**	-
EI-518 × EI-472	17.08**	11.61*	10.63**	3.29	-	-
EI-519 × EI-499	55.81**	40.31**	41.11**	37.68*	9.18*	-
EI-522 × EI-499	21.16**	17.70**	14.31**	13.24*	-	-

*Significant at 5% level. **Significant at 1% level.

Table 5. Heterosis, heterobeltiosis and economic heterosis for harvest index of selected crosses.

Cross	Heterosis		Heterobeltiosis		Economic heterosis	
	E_1	E_2	E_1	E_2	E_1	E_2
EI-517 × EI-472	41.18**	27.41**	32.96**	15.43**	16.51*	13.29*
EI-509 × EI-472	23.17**	21.26**	14.33**	12.72**	12.21**	1.59
EI-518 × EI-472	26.61**	-	15.06**	-	5.22	-
EI-519 × EI-499	26.76**	5.29**	25.07**	-	-	-
EI-522 × EI-499	38.06**	22.35**	36.52**	22.13*	-	-

*Significant at 5% level. **Significant at 1% level.

Table 6. Heterosis, heterobeltiosis and economic heterosis for chlorophyll stability index of selected crosses.

Cross	Heterosis		Heterobeltiosis		Economic heterosis	
	E_1	E_2	E_1	E_2	E_1	E_2
EI-517 × EI-472	-44.05	10.05	-24.19	-	-9.62	-
EI-509 × EI-472	158.23**	18.75	-	-	-	-
EI-518 × EI-472	78.38	41.06	-	-	-	-
EI-519 × EI-499	2.13	-74.97**	-	-63.36**	-	-
EI-522 × EI-499	-75.14**	50.09	-47.73	-	-11.54	-

*Significant at 5% level. **Significant at 1% level.

Table 7. Heterosis, heterobeltiosis and economic heterosis for desiccation injury of selected crosses.

Cross	Heterosis		Heterobeltiosis		Economic heterosis	
	E_1	E_2	E_1	E_2	E_1	E_2
EI-517 × EI-472	-73.30**	10.05**	-71.96**	-	-	-
EI-509 × EI-472	-38.54*	25.81	-23.96	-	-	-
EI-518 × EI-472	-0.55	0.39	-	-	-	-
EI-519 × EI-499	-60.72	13.54	-7.31	-	-	-
EI-522 × EI-499	2.20	-47.54**	-	-25.15	-	-

*Significant at 5% level. **Significant at 1% level.

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Identification of Parents and Experimental Hybrids in Maize

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Abstract. Genetic analysis of 72 experimental single-cross hybrids involving 4 testers and 18 newly developed inbred lines was conducted on 10 quantitative traits. The estimates of variance component revealed that SCA variance was greater in magnitude than GCA, indicating the preponderance of nonadditive gene action for all the traits. The inbreds CML-32-⊗ , M9-⊗-S$_6$-2-2 and SIDS-8445-S$_7$-# were identified as good general combiners based on GCA effects. The hybrids SIDS-8445-S$_7$-# × PKMS-49-S$_6$-⊗, CML-32-⊗ × PKMS-49-S$_6$-⊗, CML-32-⊗ × MS$_1$DR-S$_6$⊗, SIDS-8445-57# × MS$_1$DR-S$_6$⊗ and P501-C$_2$-280-2-1-5-S$_6$-⊗ × PKMS-49-S$_6$Ä exhibited high SCA effects and heterosis. It is proposed that inbreds CML-32-⊗, M9 ⊗-S$_{6-}$2-2 and SIDS-8445-S$_7$# might be utilized to exploit additive and additive × additive type of gene action, whereas the hybrids that manifested significantly high SCA effects coupled with excellent heterosis and per se performance could be more rewarding in a hybrid breeding program after intensive investigation at different locations.

Objective

Hybrid development is an evolutionary process emphasizing development and identification of simple hybrid types as a short-term breeding objective with a gradual shift toward producing more diversified types to cater to various uses. In recent years, single-cross maize hybrids have become popular among Indian farmers due to their high yield potential and excellent uniformity. Success depends on the availability of diverse and productive inbred lines to develop hybrids. (Ahloowalia and Dhawan 1963; Moll *et al.* 1965; Vasal *et al.* 1992). Combining ability analysis is a powerful tool in identifying the best combiners which may be used to exploit heterosis and help breeders to select parents for breeding programs. It provides valuable information on the cross combinations that can be exploited commercially. The present investigation was undertaken to study the combining ability of parents and hybrids as well as to evaluate the nature and magnitude of heterosis in line × tester analysis in maize.

Materials and Methods

Eighteen diverse inbred lines (P-502-C$_2$-24-S$_6$⊗, CML-345#, CML 32-⊗, P-501-C$_2$-280-215-S$_6$⊗, P-501-C$_2$-53-1-2-S$_6$⊗, M$_9$⊗-S$_6$-2-2#, CM 601-S$_6$-2-2#, AB(W)-S$_6$-3-2#, Pop(49)-C$_4$-S$_7$⊗, SPESINIFTICOELS-S$_6$⊗, CML-78#, 89G 32/ DMR, STEW-S6⊗, SIDS-8445-S$_7$⊗ Varied VS-L-S$_6$⊗, Tuxpeno Sequio C$_6$-S$_6$-⊗, AB(w)-4-1S$_6$⊗, Pop 412 EISEWGPO S$_6$⊗ and Jogia-S$_{6-}$⊗) were planted in the *kharif* (rainy) season of 2003 in a line × tester mating design. A complete set of experimental materials comprising 18 lines, 4 testers and 72 F$_1$s with two control hybrids were evaluated in a randomized block design with three replications during the *rabi* (postrainy) season of 2004/05. Each entry was planted in a two-row plot of 5 m length with a spacing of 75 × 25 cm. Observations were recorded on 10 randomly chosen competitive plants from each entry in each replication for the days to tassel (50%), days to silking (50%), plant height (cm), days to maturity, ear length (cm), ear girth (cm), kernel rows per ear, number of kernels per row and grain yield at 15% moisture (q ha^{-1}). The data were subjected to combining analysis as per the model of Kempthorne (1957).

Results

The analysis of variance for combining ability revealed that differences among the treatments were highly significant, indicating inherent genetic differences among them. Partitioning of variances into parents, crosses and parents vs crosses showed that variance due to parents and crosses was highly significant for all the traits. The GCA estimates for the inbreds and tester were found to be quite variable for the different traits studied. The parents CML-32-⊗, M$_{9-}$⊗-S$_6$-2-2 and SIDS-8445-S7# were judged as good general combiners for a majority of the traits with high GCA effect for yield per plant and positive and significant GCA effect for many of the yield traits. The five

Table 1. Top five experimental single-cross maize hybrids selected separately on the basis of GCA effect of the parents' per se performance, SCA effects and heterosis over the control hybrid for grain yield and some other yield-attributing traits.

Top five experimental single-cross hybrids	*Per se* performance (q ha^{-1})	SCA effect	Heterosis (%)	GCA effect of the parents	Other yield attributing traits
SIDS-8445-S7# × PKMS-49-S_6⊗	96.10	12.67^{**}	39.57^{**}	High × low	Plant height, days to maturity, kernels per row
CML-32-⊗ × PKMS-49-S_6⊗	91.87	11.13^{**}	33.43^{**}	High × low	Plant height, days to maturity, ear diameter
CML-32-⊗ × MS_1DR-S_6⊗	90.77	8.29^{**}	31.83^{**}	High × low	Plant height, ear diameter, kernels per row
SIDS-8445-S7# × MS_1DR-S_6⊗	88.77	3.61	28.93^{**}	High × low	Plant height, ear height, kernels per row
P501-C_2-280-2-1-5-S_6-⊗ × PKMS-49-S_6⊗	88.78	10.59^{**}	24.58^{**}	High × low	Days to maturity, kernels per row

**Significant at 1% level.

most promising cross combinations (SIDS-8445-S7# × PK MS-49-S6⊗, CML-32-⊗ × PKMS-49-S_6⊗, CML-32-⊗ × MS_1DR-S_6⊗, SIDS-8445-S7# × MS1DR-S6⊗ and P501-C_2-280-2-1-5-S_6-⊗ × PKMS-49-S_6⊗) were selected separately on the basis of *per se* performance, GCA effect, heterosis and GCA effect of the parents (Table 1). This revealed that high × low GCA combination gave high SCA effects coupled with excellent heterosis. The genetic system controlling these traits showed the major role of additive × dominance type gene action. Heterosis for yield was generally accompanied by heterosis for yield components

Conclusions

The GCA estimates of inbred lines and testers were found to be quite variable for different characters. The inbreds CML-32-⊗, M_{9-}⊗-S_6-2-2 and SIDS-8445-S7# were promising parents giving high heterosis for yield and a majority of the yield-contributing traits, which may prove useful for enhancement of yield and other attributes. The superior cross combinations manifested high *per se* performance, heterosis and SCA effects for many of the attributes including grain yield, indicating that these experimental hybrids could be commercially exploited in maize hybrid breeding programs.

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Starch Characterization of Several Maize Varieties for Industrial Use in Indonesia

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Abstract. Demand for starch in Indonesia is relatively high, particularly as substitution material in food and other uses. Maize starch has been used for food and other purposes in the pharmaceutical, cosmetic and chemical industries. Utilization of maize starch is determined by some specific characteristics such as starch yield, physical characteristics, proximate properties, physicochemistry, amylograph properties, and the form and size of granules. These characteristics of maize starch vary from variety to variety as they have different physical properties and starch utilization potential. However, little is known about the specific characteristics of starch from different varieties of maize. In ths study, two quality protein maize (QPM) lines from CIMMYT that were released in 2004 (Yellow Srikandi-1 (SK-1) and White Srikandi-1 (SP-1)) and four other varieties were used as materials for starch functional characterization. The parameters observed were starch granule size and dimension, proximate properties, physicochemistry and amylograph properties. Our results indicated that maize kernel contained an average of 30-45% starch with polygonal granules and roughly rounded and 5.3-17.8 µm in size. For comparison, the amilograph photograph of wheat was also presented (Fig. 2). As seen in Figure 2, wheat and maize granules have several similarities such as smaller rounded dimensions and less void space. This results indicated that maize has potential to be a substitute for wheat at a certain level.

Key words: Characterization, corn starch, industry materials

Introduction

Maize development would get a boost if we increased its utilization and product diversity. The use of maize starch in industry is a promising alternative that can enhance the economic value of maize. In Indonesia, maize starch has found uses in the food, pharmaceutical, cosmetic and chemical industries (Suarni *et al*. 2007). In the food industry, it is used as an emulsifier and a filler. Starch contains two types of glucose polymers: amylose and amylopectin (Bello-Perez *et al*. 1996; Campbell *et al*. 1999). The former consists of long, unbranched chains of D-glucose residues, connected by an $\alpha(1 \rightarrow 4)$ linkage. Such chains vary in molecular weight from a few thousand to more than a million. Amylopectin also has a high molecular weight but unlike amylose is highly branched. The glicosidic linkages joining successive glucose residues in amylopectin chains are $\alpha(1 \rightarrow 4)$, and the branch points are $\alpha(1 \rightarrow 6)$ linkages (Dziedzic and Kearsley 1995; Klucinec and Thompson 1999). Starch is an important component in food due to the functional properties of this molecule. Food processing generally requires stability in ingredient properties such as pH, viscosity, emulsion, integrity and texture. Those properties can be obtained from the correct selection of starch among others (Biotec 2003; Kays and Kays 1998). The larger the variety of food products, the larger the variety of functional properties required. In this paper we report the physicochemical and functional properties of starch from several maize varieties. The data obtained is expected to provide some useful information for users in the food industry.

Materials and Methods

Starch characterization was carried out *in vitro* on wet-processed starch from six varieties of maize: Anoman-1, Srikandi Putih-1 (SP-1), Srikandi Kuning-1 (SK-1), Sukmaraga, local nonwaxy variety Jeneponto and local waxy variety Jeneponto. The local varieties acted as control to the maize produced by Balitsereal Maros. Unhulled maize seed were refluxed in water for 36 hours. During this process, starch is released from the protein matrix. The seed were then ground to produce maize porridge which was then filtered to separate the fiber in a small plastic wash basin. After centrifugation, wet starch was obtained. This starch was dried in the open air at 40-50ºC for 16 hours (modified from Singh *et al*. 1997; Johnson and May 2003). Maize flour was prepared by shattering the starch and filtered (70-80 mesh filter). The dry starch was packed in a plastic bag for further analysis.

The parameters analyzed in our study were water content (oven 105°C), ash content (Tanur 550°C), lipid content (Soxhlet extraction with petroleum ether), protein content (micro-Kjeldahl), reduction sugar (spectrophotometry), starch content (Anthrone method), amylose content (Sun-Hun and Matheson 1999), amylograph properties (*Visco Amylographer Brabender*), granule size and dimension (scanning electron microscope EDX type JEOL), refinement degree (80 mesh), and whitening degree (Kromameter, colour standard $BaSO_4$).

Results and Discussion

The proximate content of maize starch from six varieties showed that the extract contained additional material in this case and was still nonpurified. This was caused by the imperfect hull release by the instrument utilized.

Water content ranged from 10.73% (Local Jeneponto) to 13.98% (Srikandi Putih). The difference in water absorption in Srikandi Putih (White Srikandi) may be caused by the different protein content. According to Ghiasi *et al.* (1985), water can be trapped inside the protein molecule as in dough where water is trapped inside the gluten. The difference in water absorption may also be due to the granule structure. Our previous research has showed that the difference in water absorption was caused by amylose concentration and protein concentration (Afdi 1989; Suarni *et al.*, 2007).

The oil absorption rate was in the range of 0.89-1.02 g g^{-1}, With Srikandi Putih having the highest and local Jeneponto the lowest (Table 1). Lipid content did not influence the oil absorption capacity, which is influenced by the presence of protein on the surface of the starch granule. The protein forms a complex compound with starch, which provides space for bonding the oil (Seguchi and Matsuki 1984). These findings are supported by previous research which showed that modification of maize starch with a cross bond affects oil absorption due to a decrease in protein concentration (Afdi 1989). However, the mechanism of the protein effect on oil absorption capacity has not been studied and understood completely. Modification of maize flour by a-amylase showed that the increase in protein content was followed by a decrease in the oil absorption capacity (Suarni *et al.* 2007).

The range of reducing sugar content for all varieties was 0.12-0.189%, with the lowest content observed in Sukmaraga and the highest in Anoman-1. The amylose content of maize starch was low in all varieties because other additional material content such as protein and lipids was high. The highest amylose content was found in Local Jeneponto and the lowest in waxy Jeneponto with a concentration range of 6.96-27.75%. Maize with 50-70% amylose is classified as high-amylose maize (amilomaize). Based on this classification, all the six varieties should be classified as medium-amylose maize except waxy Jeneponto which would be a low-amylase maize (6.96%). The range of starch content in the six varieties was 90.25-97.15%, the lowest being in Sukmaraga and the highest in waxy Jeneponto.

The lowest starch product was found in Sukmaraga and the highest in Local Jenepento within a range of 22.14-

Table 1. Chemical composition, physicochemical and functional characterization of starch from six maize varieties.

Chemical composition	Varieties					
	Anoman-1	SK-1[1] QPM	SP-1[2] QPM	Sukmaraga	Waxy Jeneponto	Local Jeneponto
Moisture (%)	9.89	9.24	8.97	9.29	9.97	10.02
Ash (%)	0.20	0.20	0.24	0.32	0.25	0.22
Fiber (%)	0.08	0.06	0.07	0.09	0.08	0.07
Lipid (%)	0.81	0.72	0.89	0.45	0.64	0.71
Protein (%)	0.88	1.08	1.22	0.97	0.90	0.82
Amylose (%)	23.26	25.85	24.60	27.75	6.96	26.18
Reducing sugar (%)	0.18	0.13	0.17	0.16	0.12	0.16
Physicochemical properties						
pH	8.12	8.45	8.09	9.61	9.12	8.95
Water absorbtion capacity (g g^{-1})	1.16	1.08	1.12	1.05	0.85	0.92
Oil absorption capacity (g g^{-1}) and	1.02	0.91	0.89	0.72	0.83	0.87
Size granules (μm)	7.3-14.2	5,3-15,6	6.3-17.8	5.3-17.1	8.3-14.0	7.3-14.7

[1.] SK-1: Yellow Srikandi; [2.] SP-1: White Srikandi.

Table 2. Amylograph properties of starch.

Varieties	Gelatinization T (ºC)	Optimum Viscosity (BU)	Final Viscosity (BU)	Breakdown Viscosity (BU)	Setback Viscosity (BU)
Anoman-1	72	600	1240	140	850
SP-1[1] QPM	74	640	1580	200	890
SK-1[2] QPM	76	680	1360	110	1010
Sukmaraga	76.5	720	1320	180	890
Waxy Jeneponto	70	410	1020	70	720
Local Jeneponto	73.5	720	1460	270	1150

[1.] SK-1: Yellow Srikandi; [2.] SP-1: White Srikandi.

35.22%. This is supported by data reported by Afdi (1989), but a bit on the lower side compared to Jegenheimer (1970). The wet-milling procedure gives a starch product of around 55%. The low product may have been caused by hull separation which was manually carried out so that some starch was released in the hull. In addition, fiber separation with cloth filter might reduce the starch product. Extraction carried out over a long time might also reduce the starch product due to the solubility of starch in water.

The initial gelatinization temperature in amylograph properties was in the range of 70-76.5ºC, peak viscosity 410-720 BU, and viscosity at 50ºC set 20´ was 720-1150 BU (Table 2). The gelatinization temperature is affected by several factors including starch granule size, amylose content, protein content and lipid content. The low gelatinization temperature for Waxy Jeneponto might be caused by its large starch granule size and low amylose content. According to Khutson *et al.* (1982) and Stone and Lorent (1984), gelatinization temperature increases with a decrease in granule size. Peak viscosity of starch is also affected by amylose content, protein content, lipid content and granule size (French 1994). The amylose molecule tends to combine with other amylose molecules by hydrogen bonding formation. The higher the amylose content of starch, the stronger the hydrogen bond thus formed.

Based on SEM (s*canning electron microscope*) JEOL JSM-6360LA observations, the shape of the starch granules of all varieties was polygonal, irregular spheric with a smooth surface. The size of the starch granules of Srikandi Kuning (enlargement 1000x) was 5.3-15.6 µm and that of Waxy Jeneponto 8.3-14.0 (Figure 1). The latter was the highest size observed.

Semiquantitative analysis by EDX showed that all the samples analyzed had the same dominant element, ie, carbon (C) as a principal element. Trace elements were not detected due to the small amount. Semiquantitative analysis was only focused on the Anoman-1 variety.

Conclusions

Based on the proximate content of all varieties, it is evident that starch extract is still not pure and contains some additional materials. Five varieties (Sukmaraga, Anoman, Srikandi Kuning, Srikandi Putih and Local Jeneponto) were classified as medium-amylose maize (23.26-27.75%), while Waxy Jeneponto was found to be a low-amylose variety (6.96%). The range of initial gelatinization temperature and peak viscosity of all the varieties was 70-76.5ºC and 410-480.0 BU, respectively. Viscosity at 50ºC was 70-270 BU, and at 50ºC 202 it was 720-1150 BU.

The shape of the starch granules of all the varieties was polygonal, irregular spheric with a smooth surface. The size of the starch granule ranged 5.3-17.1 µm, with an average of 6.3-17.8 µm. These functional properties indicate that maize starch can be used in the food industry. These results indicated that maize had potential to be a substitute for wheat at certain level. Compared to other varieties, Anoman-1, SP-1 (QPM) and Local Jeneponto as white dent corn are recommended for used as raw material in the food industry.

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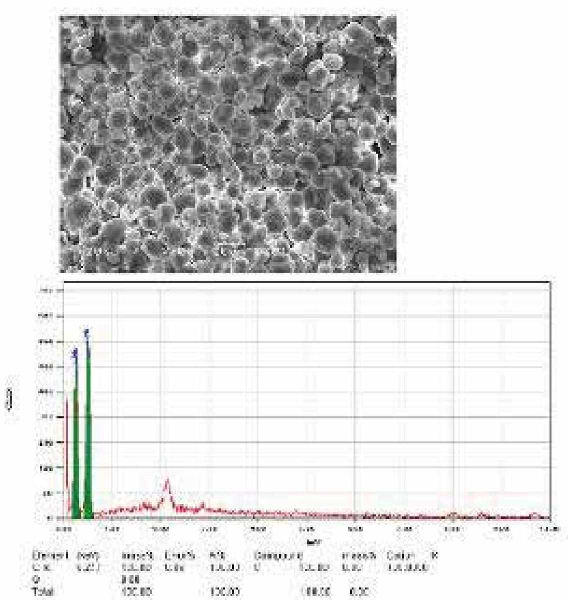

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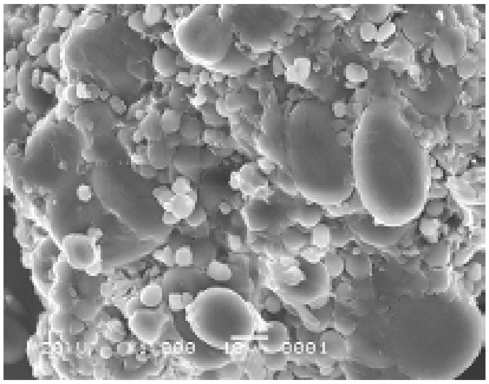


WHEAT

Wheat granule (1000x)

Figure 1. Starch granules and element of maize variety Anoman-1 (scanning electron microscope ED type JEOL, 600x)

[1]**Corn kernel**

·SK-1: Yellow Srikandi; [2]SP-1: White Srikandi.

Mixering

Milling

Dissolved

Germ out

Drying

Centrifugation

Wet starch

Washing and drying

Dry starch

Filtering

SO2 0,1-0,5%

Alcohol

ANOMAN-1

W H I T E

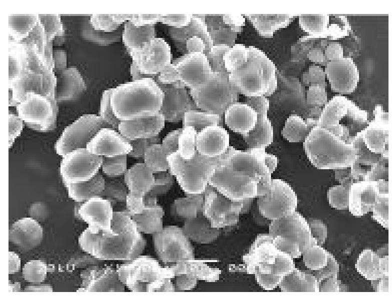
SRIKANDI-1

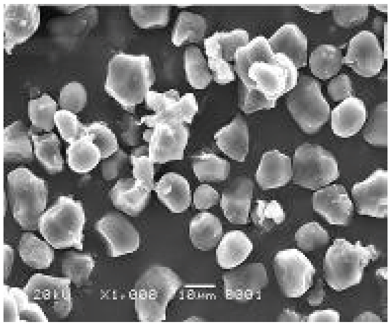
Germ out

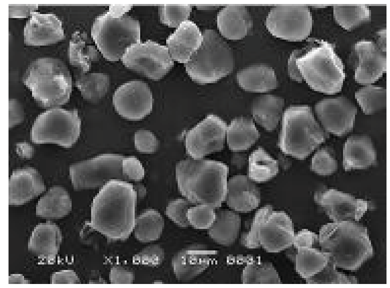
SUKMARAGA

Y E L L O W

SRIKANDI-1







W A X Y

JENEPONTO

LOCAL JENEPONTO

Figure 1. Starch granules of six maize varieties and wheat with 1000x enlargement (scale 10 micrometre).

Figure 1. Starch processing by wet-milling (modified from Singh *et al.* 1997; Johnson and May 2003).

Effect of Drying Temperature on Nutritional Quality of Protein Maize

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Abstract. Drying is an important part of the postharvest process in maize. It reduces the moisture content prior to shelling and storage. The purpose of our research was to examine the effect of the drying temperature on the quality of corn. The experimental design was a completely random design with a two-factor treatment. The first factor was drying corn with a flat-bed dryer at three temperature levels: 40°C, 50°C and 60°C. The second factor was maize varieties, consisting of two varieties: Yellow Srikandi and White Srikandi. The study was done in the yield physiology and chemistry laboratory as well as the Central Laboratory in Bogor, Indonesia. The result of this research indicated that starch content, protein content and the weight of maize varied with the drying temperature. At drying temperatures of 40°C, 50°C and 60°C as well as in sun drying, the lysine content was 0.38%, 0.32%, 0.21%, and 0.32%, respectively; tryptophan content was 0.14%, 0.13%, 0.09% and 0.12%, respectively; and b-carotene content was 0.09 mg 100 g^{-1}, 0.63 mg 100 g^{-1} and 0.76 mg 100 g^{-1}, respectively. From these results we concluded that in order to inhibit the decrease of starch, protein and amino acids, maize should be dried at 40-50°C.

Key words: Maize, drying, temperature, nutritional quality

Introduction

The low nutritional quality of maize is due to the low content of lysine and tryptophan. Their concentrations generally are 0.05% and 0.225%, respectively, of the total seed protein. This is less than half of the recommended concentration by the World Health Organization and the Food and Agriculture Organization (FAO 1985; Schmidl and Labuza 2000). To utilize maize as human and animal food, increased levels of these two amino acids is necessary (Vasal 2001; Widowati *et al.* 2006).

Quality Protein Maize (QPM) varieties have higher lysine and tryptophan concentration compared to other varieties. The Indonesian Cereal Research Institute has released two QPM varieties for cultivation: Srikandi Putih and Srikandi Kuning.

Drying is an important part of the postharvest operations in maize. It reduces the moisture content to a certain level prior to shelling and storage. However, drying has been found to affect the lipid and amino acid content, especially lysine and tryptophan and b-carotene, in the grain. Winarno and Aman (1981) stated that change in seed colour can be an indicator of the quality of fresh seed. At room temperature, seed experience high respiration to accumulate metabolite products which accelerate the aging of seed cells. As a consequence, there is a deterioration of seed immunity. Seed storage at low temperature retards the respiration rate so that accumulation of metabolite products is inhibited .

The purpose of this research was to examine the effect of drying temperature on the quality of maize seed protein.

Materials and Methods

Maize cultivation for this study was carried out at the experimental farm of ICERI, Maros. Nutritional analysis was done in the yield physiology and chemistry laboratory as well as the Central Laboratory in Bogor. The experimental design was a completely random design with a two-factor treatment. The first factor was drying temperature consisting of three levels of temperature during flat-bed drying: 40°C, 50°C and 60°C. The second factor was maize variety, consisting of two varieties: Yellow Srikandi and White Srikandi.

Sampling was carried out at physiological ripening, which is indicated by the presence of a black layer at the bottom of the seed attached to the maize stem (90% from the amount of seed at the center of the stem). The harvest age was based on the varietal description issued by ICERI.

Drying was done at the following temperatures: ±40 ^{0}C , 50 ^{0}C , 60 ^{0}C and sun drying. Drying of maize is meant to reduce the moisture content to a certain level (± 17 %) prior to shelling. The shelled seed is then dried until the water content is reduced to ±14%.

The samples were prepared for analysis of the following types of content: lipids (Soxhlet extraction with petroleum ether); crude fiber (aqueous base and acid hydrolysis); starch (Anthrone method); amylose (Sun-Hun and Matheson 1999); protein (micro-Kjeldahl), amino acids (HPLC), essential fatty acids (HPLC) and â-carotene (provitamin A) (HPLC).

Results and Discussion

The proximate content of the maize seed of the two experimental varieties was analyzed for water, ash, protein, lipid, crude fiber and carbohydrate content. The water content considered was the water content of the sample analyzed, not of the harvested seed.

Amino acid analysis was carried out by the acid extraction method, and then measured with HPLC and compared to a standard amino acid. The results of the amino acid content of Srikandi Putih and Srikandi Kuning are given in Table 1. The change in amino acid composition at different drying temperatures (40 ^{0}C , 50 ^{0}C , 60 ^{0}C and sun drying) was not significant. For example, the change in the glutamic acid content of Srikandi Putih was 1.56%, 1.39% and 1.35% at the four temperature levels, respectively. The corresponding change in Srikandi Kuning was 1.87%, 1.70% and 1.65%, respectively.

We found that protein content decreased with increase in drying temperature. This was probably caused by the Kjeldahl method used for protein analysis in which nonprotein nitrogen is also detected. At 60°C drying temperature, some nitrogen compounds dissolve. A drastic change was found in the fatty acid content, especially nonsaturated fatty acids (Table 2). The protein molecule is chemically more stable than the lipid molecule. The presence of many nonsaturated bonds in lipid molecules will provide the structure change of this molecule with the increase of temperature, and finally the amount of that molecules will decrease (Winarno and Aman 1981; Winarno 2002).

β-carotene analysis was carried out by the extraction method and measured with HPLC, while amylose content was analyzed by the spectroscopic method. b-carotene content decreased in Srikandi Kuning with an increase in the drying temperature (Table 2). It was 0.84 mg 100g^{-1}, 0.69 mg 100g^{-1} and 0.63 mg 100g^{-1} at 40ºC, 50ºC and 60ºC , respectively. It has been suggested by Mayne (1996) that the change in drying temperature changes the molecular structure so that the final concentration would be low. The change was not detected in Srikandi Putih because the seed is colourless. It means no that b-carotene will be found. Amylose content in both varieties under every drying temperature experienced a relatively small decrease. The reason might be that the amylose molecule is not

Table 1. Amino acid content (%) of two quality protein maize (QPM) varieties[1] under machine drying at three different temperatures and sunshine drying.

Drying temperature	40°C		50°C		60°C		Sunshine drying	
AminoAcid content	QPM 1	QPM 2	QPM 1	QPM 2	QPM 1	QPM 2	QPM 1	QPM 2
Protein	9.22	9.50	8.98	9.04	7.99	8.21	9.01	9.09
Aspartat	0.55	0.69	0.52	0.68	0.45	0.60	0.55	0.54
Glutamic acid	1.87	1.56	1.70	1.39	1.65	1.35	1.72	1.68
Serine	1.35	0.34	0.32	0.31	0.30	0.29	0.32	0.28
Hystidine	0.25	0.28	0.22	0.25	0.22	0.25	0.23	0.22
Glisine	0.33	0.40	0.27	0.34	0.27	0.34	0.29	0.31
Threonine	0.28	0.29	0.25	0.26	0.22	0.25	0.25	0.24
Arginine	0.57	0.61	0.42	0.48	0.39	0.51	0.39	0.41
Alanine	0.57	0.49	0.52	0.45	0.49	0.43	0.54	0.45
Tyrosine	0.29	0.24	0.24	0.19	0.20	0.23	0.26	0.20
Methionine	0.10	0.07	0.06	0.04	0.06	0.04	0.06	0.04
Valine	0.46	0.45	0.41	0.40	0.42	0.43	0.43	0.42
Fenilalanine	0.34	0.32	0.32	0.30	0.31	0.29	0.35	0.31
I-Leusine	0.27	0.27	0.25	0.25	0.25	0.25	0.28	0.27
Leusine	0.85	0.65	0.81	0.61	0.78	0.61	0.88	0.66
Lysine	0.38	0.40	0.32	0.39	0.21	0.29	0.32	0.40
Tryptophan	0.14	0.15	0.13	0.12	0.09	0.11	0.12	0.12

[1.]QPM 1: Srikandi Kuning; QPM 2: Srikandi Putih.

Table 2. ß-carotene, fatty acid and protein content of two QPM varieties[1] of maize under machine drying at three different temperatures and sunshine drying.

Maize seed content	40°C		50°C		60°C		Sunshine drying	
	QPM 1 (%)	QPM 2 (%)	QPM 1 (%)	QPM 2 (%)	QPM 1 (%)	QPM 2 (%)	QPM 1 (%)	QPM 2
ß-carotene (mg 100 g^{-1})	0.84	-	0.69	-	0.63	-	0.76	-
Saturated fatty acids	1.54	1.33	1.6	1.08	0.10	0.21	1.61	1.10
Nonsaturated fattty acids	3.93	3.12	3.39	2.28	0.61	0.33	5.05	2.31
Amylose	28.42	26.88	28.33	26.12	27.98	25.78	28.38	26.18

[1.]QPM 1: Srikandi Kuning. QPM 2: Srikandi Putih.

sensitive to temperature change compared to β-carotene. Drying temperature change may not affect the amount of amylose and amylopectin in the maize sample (Klucinec and Thompson 1999; Winarno 2002).

The concentration of saturated and nonsaturated fatty acids analyzed by the GLC method showed that there was a decrease with an increase in drying temperature. The change in saturated fatty acid concentration of Srikandi Kuning was 1.54%, 1.16% and 0.10% at 40ºC, 50ºC and 60ºC, respectively. In Srikandi Putih, the change was 1.33%, 1.08% and 0.21% at the three temperatures, respectively.

Conclusions

The β–carotene content of Srikandi Kuning when dried at 40ºC, 50ºC and 60ºC was 0.84 mg 100g^{-1}, 0.69 mg 100g^{-1} and 0.63 mg 100g^{-1}, respectively. The concentration of saturated and nonsaturated fatty acids at these drying temperatures was 1.54% and 1.16%, 0.10% and 3.93%, and 3.39% and 0.61%, respectively. For Srikandi Putih b-carotene was not detected; saturated and nonsaturated fatty acid content at the three drying temperatures was, respectively, 1.33% and 1.08%, 0.21% and 3.12%, and 2.28% and 0.33%; starch composition did not show a significant change.

Nutritional composition of maize can be kept constant at a drying temperature of 40-50ºC. If the weather is good, it is better to dry in the open (drying floor) because it keeps nutritional composition at the same levels as that attained by the drying machine at 50ºC. The advantage of using a drying machine is that the seed quality will be better/cleaner because contamination can be avoided.

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Comparison of S_2 Progeny and Testcross Performance in Suwan 5 Maize Varieties

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Abstract. The relative performance of selfed progenies (S_1, S_2 and S_3) vs testcrosses is of interest to maize breeders. The objective of our research was to compare the S_2 progeny (S_2) method and the S_2 testcross (TC) method for population improvement and inbred and hybrid development. For this we used two subpopulations of Suwan 5(S)C3(F) (A) and Suwan 5(S)C3(SF) (B) and two inbred testers (Ki 45 for A and Ki 46 for B). A hundred progenies of the two subpopulations by each method were evaluated in the early rainy season of 2000 in Suwan. The 10 highest-yielding entries selected from both subpopulations by each method were grouped using their S_4 seed to form 4 synthetics. Fourteen topcrosses of the 4 synthetics and 3 original populations crossed with the 2 testers, 21 population crosses of the 7 populations, and the 7 populations themselves were evaluated in the late rainy season of 2001. Ten S_6 lines from the highest-yielding selection by each method of AC0 and BC0 crossed with the 2 testers were evaluated. Ten S_7 lines of both subpopulations by the two methods were also evaluated. Genetic variability among the progenies evaluated over both subpopulations showed that the S_2 method was 1.84 times greater than the TC method for all 15 agronomic characters measured, and 4.35 times for grain yield. The correlation coefficient between the two methods for grain yield was 0.069. Two S_2 lines were common to the two methods for the 10 highest- and 10 lowest-yielding lines of both subpopulations. The S_2 method was more efficient in improving the yields of the populations per se. The TC method was more efficient in improving the yields of related population crosses and populations topcrossed with the two inbred testers. The two methods were not different in selecting high-yielding inbreds and hybrids.

Key words: S_2 progeny, testcross, maize, improvement, inbred, hybrid

Introduction

Maize (*Zea mays* L.) breeders have always tended to choose the more productive lines but have hesitated to discard those with mediocre yields because of the possibility that some of them might possess superior combining ability (Horner *et al*. 1977). Therefore, an effective method is needed for breeders to identify good lines which have both high seed yield and high combining ability with other lines. Selection for performance of selfed progenies (S_1, S_2 and S_3) and testcrosses has been widely used for that purpose.

The selfed-progeny method is used by maize breeders where stable and high-yielding female lines are needed. It is expected to be more efficient due to the lack of a genetic contribution by the tester. It is also expected to be more efficient in utilizing additive genetic variance than the testcross method when selecting for combining ability (Comstock 1964). These methods for improving the yields of populations *per se* as well as the combining ability of improved populations and resulting inbred lines needs to be further investigated in related populations.

The objective of this research was to compare the S_2 progeny (S_2) method and the S_2 testcross (TC) method for population improvement and inbred and hybrid development in two subpopulations of Suwan 5(S)C4(F) (A) and Suwan 5(S)C4(SF) (B).

Materials and Methods

Two subpopulations of Suwan 5(S)C4(F) (A) and Suwan 5(S)C4(SF) (B) and 2 inbred testers (Ki 45 for subpopulation A and Ki 46 for subpopulation B were used in this study. A hundred progenies of both subpopulations derived by each method were evaluated in the early rainy season of 2000 at the National Corn and Sorghum Research Center (Suwan Farm) in Nakhon Ratchasima, Thailand. The

10 highest-yielding entries selected from each method and each subpopulation were grouped using their S_4 seeds to form 4 synthetics. Fourteen topcrosses of these 4 synthetics and 3 original populations crossed with Ki 45 and Ki 46 (topcrosses), 21 population crosses of the 7 populations, and the 7 populations themselves were evaluated in the late rainy season of 2001. Ten S_6 lines from the highest-yielding selection of each method of AC0 and BC0 crossed with Ki 45 and Ki 46, respectively, were evaluated in the same season. Their 10 S_7 lines from the 2 methods of both subpopulations were also evaluated for grain yield and agronomic traits.

Results and Discussion

Genetic variability among the progenies evaluated over both subpopulations showed that the S_2 method was 1.84 times greater than the TC method for all 15 agronomic characters measured, and 4.35 times for grain yield (Table 1). The S_2 method gave greater genetic variability among the S_2 lines *per se* than was found in the S_2 testcrosses by the TC method because the S_2 lines did not have the interference of the tester's genetic contribution. The greater genetic variability achieved by the S_2 method agreed with the theoretical study of Comstock (1964) and the comparative studies of S_1 and testcross (half-sib) performance by Genter and Alexander (1962); Lonnquist and Lindsey (1964); Duclos and Crane (1968); Carangal *et al*. (1971); Genter (1973); Lamkey and Hallauer (1987); Aekatasanawan (2002).

The coefficients of correlation between the two methods were 0.069 for grain yield and 0.182 for 15 agronomic traits. The correlation between line *per se* performance and testcross performance was less than 0.5, which is in good agreement with the theoretical study of Smith (1986) and the comparative studies of Lonnquist and Lindsey (1964), Hallauer and Lopez-Perez (1979), Jensen *et al*. (1983), Aekatasanawan (2002) and Aekatasanawan *et al.* (2007), due to the masking effects of the favorable dominant alleles in the tester. The number of common S_2 lines in the 10 highest- and 10 lowest-yielding groups over both subpopulations was 1 line in each group, respectively. Among the S_2 lines, the S_2 method gave a higher mean yield (by 74.67%) within the 10 highest-yielding group than the TC method, but also had the lower mean yield (by 45.20%) within the 10 lowest-yielding group than the TC method (Table 2). As testcrosses, the TC method gave higher and lower mean yields than the S_2 method by 13.01% and 11.95%, respectively. The S_2 method gave higher efficiency in selection for high and low yields than the TC method. These findings are supported by the results of S_3 vs TC methods (Aekatasanawan *et al.* 2007).

Over both subpopulations, improved populations by the S_2 and TC methods gave average grain yields of 3.60 t ha^{-1} and 3.24 t ha^{-1}, higher than ABC0 by 6.20% and -4.43%, respectively. Several reports indicated that the selfed (S_1, S_2, and S_3) method was more effective than the TC method for improving population yields (Genter and Alexander

Table 1. Genotypic coefficients of variation (GCV, %) between the S_2 and testcross methods for grain yield and other agronomic characters of 100 S_2 lines of Suwan 5(S)C3(F) and Suwan 5(S)C3(SF) subpopulations, tested at Suwan, Thailand, early rainy season, 2000.

Agronomic character	Suwan 5(S)C3(F)		Suwan 5(S)C3(SF)	
	S_2 line *per se* (S_2)	S_2 line × Ki 45 (TC)	S_2 line *per se* (S_2)	S_2 line × Ki 46 (TC)
Grain yield (t ha^{-1})	32.09	8.15	27.30	5.50
Days to 50% anthesis (d)	3.89	1.64	4.70	1.18
Days to 50% silking (d)	3.92	2.10	4.78	1.20
Plant height (cm)	4.82	2.49	3.61	1.98
Ear height (cm)	6.25	3.10	8.54	4.40
Root lodging (1-5)[1]	37.82	16.59	28.80	18.30
Stalk lodging (%)	60.04	27.81	106.74	70.79
Foliar diseases (1-5)[1]	14.37	8.78	16.92	1.48
Husk cover (1-5)[1]	0.00	7.91	0.00	5.99
Plant aspect (1-5)[1]	21.70	14.43	26.43	8.39
Ear aspect (1-5)[1]	20.42	17.62	20.01	10.99
Rotten ears (%)	64.31	71.63	127.61	33.09
Ears per plant (%)	13.22	6.94	18.91	1.50
Grain moisture (%)	5.48	2.63	6.66	3.82
Grain shelling (%)	5.47	1.86	2.42	2.05
Mean	19.59	12.91	25.17	11.38

[1.] Scored on a 1-5 rating scale in which 1 = best, 5 = poorest.

Table 2. Mean grain yield (t ha^{-1}) of eight selected groups of lines (S_2) and testcrosses (TC) derived by the S_2 and testcross methods in two maize subpopulations, tested at Suwan, Thailand, early rainy season, 2000.

Mean of 10 Selections[1]	Selection method: S_2 High (t ha^{-1}	S_2 Low (t ha^{-1})	S_2 Low in % of high (%)	TC High (t ha^{-1})	TC Low (t ha^{-1})	TC Low in % of high (%)
Suwan 5(S)C3(F)						
As S_2 lines	5.66	1.74	30.74	2.80	2.62	93.40
As testcrosses	7.50	7.10	94.67	8.49	5.67	66.78
Suwan 5(S)C3(SF)						
As S_2 lines	4.81	1.34	27.86	3.19	2.99	93.73
As testcrosses	9.56	8.30	86.82	10.78	7.89	73.19
Mean of 2 subpopulations						
As S_2 lines	5.24	1.54	29.39	3.00	2.81	93.67
As testcrosses	8.53	7.70	90.27	9.64	6.78	70.33

[1.] Underlined figures are means within the selected group for the indicated method and paired figures in the same column are averages of the same lines for the other testing method.

Table 3. Response to selection for grain yield of populations *per se* by the S_2 and testcross (TC) methods in two maize subpopulations evaluated at Suwan, Thailand, late rainy season, 2001.

Population[1]	Method	Cycle	Mean yield (t ha^{-1})	Relative to C0 (%): AB	Relative to C0 (%): A or B
AB	-	0	3.36	0	0
A	-	0	3.5	4.17	0
A	S_2	1	3.24	-3.57	-7.43
A	TC	1	3.17	-5.66	-9.43
B	-	0	3.3	-1.79	0
B	S_2	1	3.96	17.86	20
B	TC	1	3.3	-1.79	0
Mean					
AB, A, B	-	0	3.39	0	
A, B	S_2	1	3.6	6.2	
A, B	TC	1	3.24	-4.43	

[1.] AB = Suwan 5(S)C3; A = Suwan 5(S)C3(F); B = Suwan 5(S)C3(SF).
LSD (0.05) = 0.62 t ha^{-1}; LSD (0.01) = 0.82 t ha^{-1}.

1966; Burton *et al.* 1971; Carangal *et al.* 1971; Aekatasanawan *et al.* 2007). Theoretical comparisons of different methods of recurrent selection indicated that in the absence of overdominance the S_1 or S_2 method is expected to be appreciably more effective than the TC method for changing population gene frequencies (Comstock 1964; Wright 1980).

Average grain yields (3.46 t ha^{-1}) of improved populations crossed with other populations by the TC method were higher than that of ABC0 and the average of AC0 and BC0 by 2.83% and -0.72%, respectively. However, the S_2 method gave higher average yield of population crosses (3.55 t ha^{-1}) by 5.21% and 1.58%, respectively (Table 3). From the results of a diallel cross of 7 original and improved populations, the midparent heterosis means were ranked as B(TC)C1 (5.07%) > A(S_2)C1 (4.36%) > BC0 (2.43%) > AC0 (2.41%) > A(TC)C1 (2.19%) > B(S_2)C1 (-1.21%) > ABC0 (-3.30%) (Table 4). However, for improved populations crossed with the 2 inbred testers, the S_2 and TC methods gave average grain yields of 3.87 and 3.97 t ha^{-1}, higher than average grain yield of AC0 and BC0 (3.93 t ha^{-1}) by -1.53 and 1.02%, respectively (Table 5). The S_2 method was more efficient in improving yields of

Table 4. Mean grain yields (t ha^{-1}, above diagonal) and midparent heterosis (%, below diagonal) for 21 population crosses and mean grain yield (t ha^{-1}, diagonal) for 7 populations per se, evaluated at Suwan, Thailand, late rainy season, 2001.

Population[1]	ABC0	AC0	$A(S_2)C1$	A(TC)C1	BC0	$B(S_2)C1$	B(TC)C1
ABC0	**3.36**	3.35	3.07	2.91	3.41	3.42	3.48
AC0	-2.33	**3.50**	3.50	3.72	3.69	3.43	3.43
$A(S_2)C1$	-6.97	3.86	**3.24**	3.63	3.76	3.68	3.23
A(TC)C1	-10.87	11.54	13.26	**3.17**	2.79	3.57	3.65
BC0	2.40	8.53	14.98	-13.76	**3.30**	3.52	3.48
$B(S_2)C1$	-6.58	-8.04	2.22	0.14	-3.03	**3.96**	3.92
B(TC)C1	4.50	0.88	-1.22	12.83	5.45	7.99	**3.30**
Heterosis mean	-3.30	2.41	4.36	2.19	2.43	-1.21	5.07
Cross mean	3.27	3.52	3.48	3.38	3.44	3.59	3.53

[1.] AB = Suwan 5(S)C3; A = Suwan 5(S)C3(F); B = Suwan 5(S)C3(SF).
LSD (0.05) = 0.62 t ha^{-1}; LSD (0.01) = 0.82 t ha^{-1}.

Table 5. Mean grain yields for 7 populations testcrossed with inbred testers Ki 45 and Ki 46, evaluated at Suwan, Thailand, late rainy season, 2000.

Population[1]	Inbred tester(t ha^{-1})		Mean yield (t ha^{-1})	Relative to original population (%)	
	Ki 45	Ki 46		ABC0	AC0, BC0
ABC0	3.97	4.58	4.28	100.00	-
AC0	3.65	4.00	3.83	-10.51	-
$A(S_2)C1$	3.72	3.89	3.81	-10.98	-0.52
A(TC)C1	3.28	4.43	3.86	-9.81	0.78
BC0	3.51	4.55	4.03	-5.84	-
$B(S_2)C1$	3.79	4.07	3.93	-8.18	-2.48
B(TC)C1	3.38	4.76	4.07	-4.91	0.99
Mean of 2 populations					
AC0, BC0	3.58	4.28	3.93	-8.18	0
$A(S_2)C1$, $B(S_2)C1$	3.76	3.98	3.87	-9.58	-1.53
A(TC), B(TC)	3.33	4.6	3.97	-7.24	1.02

[1.] AB = Suwan 5(S)C3; A = Suwan 5(S)C3(F); B = Suwan 5(S)C3(SF).
LSD (0.05) = 0.62 (t ha^{-1}); LSD (0.01) = 0.82 t ha^{-1}.

populations *per se* but the TC method was more efficient in improving yields of related population crosses and populations topcrossed with the 2 inbred testers. These findings are in good agreement with the results of S_3 and TC methods used by Aekatasanawan *et al.* (2007) only the high efficiency of the selfed progeny methods for improving yields of populations *per se*.

In the inbred yield trial, the S_2 method gave a significantly higher ($P<0.05$) average grain yield in the 10 highest-yielding group than the TC method in the A subpopulation (2.57 t ha^{-1} vs 2.33 t ha^{-1}) but nonsignificantly higher yield in the B subpopulation (2.66 t ha^{-1} vs 2.37 t ha^{-1}) (Table 5). The results of inbreds crossed with the inbred testers in the 10 highest-yielding group showed that the TC method gave nonsignificant average grain yield than the S_2 method in the A (8.54 t ha^{-1} vs 8.74 t ha^{-1}) and B (8.98 t ha^{-1} vs 9.18 t ha^{-1}) subpopulations (data not shown). Both methods were not different in selecting high-yielding inbreds and hybrids. These findings were supported by the results of Aekatasanawan *et al.* (2007).

In conclusion, the S_2 method was more efficient in improving the yields of populations *per se*. However, the TC method was more efficient in improving the yields of related population crosses and populations topcrossed with the two inbred testers. Both methods were not different in selecting high-yielding inbreds and hybrids.

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Utilization of Genetic Components and Heritability of Physiological Traits in Female Inbred Parents in Developing Hybrid and Synthetic Maize Varieties

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Abstract. Conventionally, parental inbred selection has been based on the combining ability of male parents, utilizing diallel cross design, Comstock and Robinson's Design I or Design II. Those designs in maize require methodical crossing of a number of male and female parents and 3-4 plantings before one is enabled to identify males with good combining ability in the diallel design, or tester males in Design II. On the other hand, selecting for females with good combining ability is rather more straightforward since one actually measures the advantages in terms of the physiological traits shown in the crossed progenies as compared to those of their female parents. This study was done in 2005 and 2006 to lay a strong foundation for a breeding program utilizing selected inbred females to develop superior hybrid and synthetic (open-pollinated) progenies. In 2005, our objectives included (1) developing an appropriate unbiased method of statistical analysis in selecting females with good combining ability; (2) testing 10 female inbred populations for their combining abilities through a bulked-pedigree method; (3) deriving 10 single-cross F_1 and 10 reciprocal hybrid progenies; and (4) developing 5 synthetic progenies by intermating the 5 selected good-combining females. All hybrid and synthetic progenies were tested in 2006. The study resulted in (1) modified diallel analysis fit for testing good-combining females; the tests of inbreds, diallel crosses and synthetic progenies indicated a significant relationship between general combining ability and cross population means, and the synthetic progenies may be useful in predicting superior inbreds suitable for good-combining females; (2) the inbred populations showed high interpopulation variability which may simplify selection for certain desirable traits; and (3) the values for ó²M (maternal) and ó²NM (nonmaternal) measured a similar importance at >1 se and 2 se; ó²M being indicative of the presence of a maternal effect. The maternal effect for productivity was not proved.

Key words: Maize, hybrid, synthetic, good-combining female

Introduction

Selection of parental inbred lines is the most important part of a plant breeding program aimed at identifying superior hybrids and synthetic progenies (Fehr 1987). Parental inbred selection has conventionally been based on the combining ability of males, utilizing diallel cross design and Comstock and Robinson's Design I or Design II. Those designs in maize involve methodically crossing a number of male and female parents and 3-4 plantings before one is enabled to identify good-combining males in the diallel design, or tester males in Design II (Hallauer and Miranda 1981). On the other hand, selecting for females with good combining ability is rather more straightforward since one actually measures advantages in terms of physiological traits in the crossed progenies as compared to those of their female parents (Hikam 2003). Previous studies employing diallel-cross design have indicated nonsignificancies between F_1 cross progenies and their reciprocals when maternal effects were not assumed (Sudrajat 2002). However, Supandi (2005) reported significant ó^{2}A and ó^{2}D, and concurrently, maternal effect. A problem arose in developing appropriate an analysis of variance and the concurrent expected mean-squares which should take the parental inbreds and progenies, ie, F_1 hybrids, reciprocals and open-pollinated (synthetics) into consideration.

Our study, therefore, intended to (1) develop an appropriate unbiased statistical analytical method for selecting good-combining females; (2) test 10 female inbred populations for their combining abilities in a bulked-pedigree method; (3) derive 10 single-cross F_1 and 10 reciprocal hybrid progenies; and (4) develop five synthetic progenies by intermating the five selected good-combining females. This was accomplished in 2005. In 2006, we tested all the hybrid and synthetic progenies developed.

Materials and Methods

Plant materials

The study was done in 2005 and 2006 in the red-yellow podzolic at the State Polytechnic of Lampung in Bandar Lampung. In 2005, five S_6 inbreds developed by Hikam (2003), UL1.04, UL1.06, UL2.03, UL3.01 and UL4.01, were mated following a diallel cross design (Griffing 1956). The cross resulted in three populations: the five inbreds, ten F_1 crosses and their ten reciprocals. A fourth population was developed by intermating the inbreds which resulted in synthetics. In 2006, all the developed populations were tested for their vegetative and productive performance.

Field design and measurement

The plots were preplant treated with glyphosate and 2,4D herbicides at 2 L ha^{-1} 7 days prior to tilling. Planting density was about 71 400 plants ha^{-1} (spacing 0.7 m between rows and 0.2 m within a row). In 2005, the plots were set up to accommodate diallel-cross and open-pollinated matings. The progeny populations that were obtained are presented in Table 1. The seeds were harvested, and bagged and tagged accordingly. In 2006, the plots were prepared in a randomized complete-block design (Steel and Torrie 1981) with three replications. Open-pollinated seeds were bulked with 500 seeds of each to form the synthetics population prior to planting. The plots were fertilized with urea, SP 36 and KCl at the rate of 300 kg ha^{-1}, 150 kg ha^{-1} and 150 kg ha^{-1}, respectively. The urea was split-applied with 100 kg ha^{-1} at day 7 and 200 kg ha^{-1} at day 30; whereas SP 36 and KCl were applied all at day 7.

On anthesis, ten sample plants from each plot were self-pollinated. The self-pollination was so prepared as to completely protect the ear from cross contamination (Hikam 2003). Paper bags were used to cover the tassels and ears of the sample plants; the tassels were covered 24 hours before pollination and the ears were were covered before silk emerged. The tassel bags were used to harvest pollen which were then pollinated to the ear of the same plant. The 2-3 cm top husk was cut 24 hours before pollination to permit the silk to grow at the same length. The ear was covered again after pollination with the tassel bag.

The parameters measured were: (1) plant height (cm); (2) leaf number; (3) ear length (cm); (4) ear diameter (cm); (5) ear weight (g per ear); (6) number of kernel rows per ear; and (8) productivity (kg of dried kernels m^{-1} at 15.5 % moisture).

Data analysis

The proposed model of analysis of variance for diallel-cross design which takes open-pollinated progeny populations into consideration is presented in Table 1. Variance components and narrow-sense heritabilities, and their respective standard errors were calculated following Hallauer and Miranda (1981; not shown). The values of variances and heritabilities were tested for significance at 95% and 99% confidence levels.

Results and Discussion

Mean-squared analyses

The mean-squared values derived from the analyses of variance (Table 2) indicated differences at Pd"0.01 as well as Pd"0.05 for various sources of variation although leaf number and above-ear leaves indicated differences at smaller numbers of sources compared to the rest. Interested readers may look at Table 2 and Table 3 simutaneously.

Parental inbreds, open-pollinated, cross and reciprocal

The differences ($P \leq 0.01$ or ≤ 0.05) for parental inbreds indicated that at least one inbred differed from the other four. The differences among inbreds would assure better hybrid and synthetic performances when recombined due to heterosis (Fehr 1987). This finding is rather consistent with the differences in open-pollinated, cross and reciprocal sources, except ear position and ear number in open-pollinated populations, and ear diameter in reciprocated populations.

Parents vs cross and open-pollinated vs cross

Differences ($P \leq 0.01$ or ≤ 0.05) for parent vs cross which indicated realized heterosis existed for plant height and kernel row. Differences ($P \leq 0.01$ or ≤ 0.05) for open-pollinated vs crossed progeny existed for plant height, leaf number, ear length, and kernel row.

Combining ability, maternal and nonmaternal effect

General combining ability (GCA, additive effect is prominent) was significant ($P \leq 0.01$ or ≤ 0.05) for plant height, ear length, ear diameter and kernel row. Specific combining ability (SCA, dominant and epistatic effects are prominent) was significant ($P \leq 0.01$ or ≤ 0.05) for plant height, ear length, kernel row, ear weight and productivity. Coexistence of GCA and SCA for plant height and ear length were of similar magnitude, except for kernel row where GCA > SCA

Analyses of genetic variances, heritabilities and combining abilities

Data in Table 3 indicate that ó^{2}D was more important than ó^{2}A, suggesting that some particular crosses were expected to perform better than average. This result confirmed the fact that the inbreds used in our study were derived from three different pedigrees (Hikam 2003). The values for ó^{2}M and ó^{2}NM measured similar importance at >1 se and 2 se, ó^{2}M being indicative of the presence of a maternal effect. Unfortunately, we did not prove maternal effect for productivity.

Table 1. Proposed model of analysis of variance for diallel-cross design, taking open-pollinated progeny population into consideration.

Source of variation	Df	Mean-squared	Expected mean-squared
Replicate	(r-1)	MS_3	
Entry	$(n^2 + p)$-1	MS_2	
Parental inbred	(n-1)	MS_{21}	
Open-pollinated	(p-1)	MS_{22}	
Parent vs cross	1	MS_{23}	
OP vs vross	1	MS_{24}	
Cross	n(n-1)-1	MS_{25}	
GCA	(n-1)	MS_{251}	
SCA	n(n-3)/2	MS_{252}	
Reciprocal	n(n-1)/2	MS_{253}	
Maternal	(n-1)	MS_{2531}	

Table 2. Mean-squared values of analyses of variance of measured variables.

Source of variation	df	Plant height	Leaf number	Ear length	Ear diameter	Kernel row	Ear weight	Productivity
Replicate	2	374.34*	1.12	4.07	0.02	0.84	292.8	34989**
Entry	29	1612.03**	7.03	12.53**	0.46**	5.39**	1262.51**	8539
Parental inbred	4	6033.63**	1.26	31.98**	0.79**	10.68**	4772.14**	36813**
Open-pollinated (OP)	1	33 907.15**	13.67	157.04**	3.84**	15.61**	25 039.95**	730133**
Parent vs cross	1	422.8*	0.05	6.29	0.00005	7.59**	455.76	2545
OP vs cross	4	646.02**	25.31*	9.11**	0.105	4.58**	282.79	8243
Cross	19	1054.23**	5.15	10.47**	0.515**	5.01**	862.80**	25157**
GCA	4	1281.18**	0.57	19.11**	0.69*	13.62**	518.13	15108
SCA	5	2484.41**	9.07	9.98**	0.48	2.78*	1769.69**	51597**
Reciprocal	10	248.36*	5.02	7.26**	0.46	2.69**	547.21*	15957*
Maternal	4	249.67*	4.56	4.74	0.52	2.84*	243.34	7097
Nonmaternal	6	247.49*	5.32	8.94**	0.42	2.58*	754.79**	21864
Error	58	91.21	7.93	1.94	0.19	0.82	228.79	6671

* and ** indicate significant differences at $P \leq 0.05$ and ≤ 0.01, respectively.

Table 3. Values of genetic variances (ó^2), narrow-sense heritabilites (h^2_{NS}), combining abilities, and genetic coefficients of variation (CV_g).

Measurement	Plant height	Leaf number	Ear length	Ear diameter	Kernel row	Productivity
Xbar	185.69	13.59	16.44	3.80	13.11	471.21
ó^{2}e	91.21	7.93	1.94	0.19	0.82	6671.32
ó^{2}P	1980.81*	-2.22	10.01*	0.20*	3.29*	10 047.40*
Se[1](ó^{2}P)	1161.19	0.54	6.16	0.15	2.06	7096.39
ó^{2}OP	11 271.98**	1.91	51.70*	1.22*	4.93*	241 154.00*
se(ó^{2}OP)	9228.36	3.75	42.74	1.05	4.25	198 717.54
ó^{2}C	321.01**	-0.93	2.84**	0.11**	1.40**	6162.18**
se(ó^{2}C)	108.59	0.72	1.08	0.05	0.52	2619.61
ó^{2}GCA	132.22	-0.82	1.91	0.06	1.42	937.46
se(ó^{2}GCA)	246.63	0.49	3.68	0.13	2.62	2935.84
ó^{2}SCA	797.73*	0.38	2.68*	0.10*	0.65*	14 975.47*
se(ó^{2}SCA)	442.69	1.69	1.78	0.09	0.50	9202.34
ó^{2}R	78.58*	-1.46	2.66*	0.14*	0.94*	4643.17*
se(ó^{2}R)	51.38	1.25	1.49	0.10	0.55	3313.78
ó^{2}M	15.85*	-0.34	0.28*	0.03*	0.20*	42.61
se(ó^{2}M)	14.51	0.30	0.28	0.03	0.16	427.49
ó^{2}NM	26.05*	-0.44	1.17**	0.04*	0.29*	2532.19*
Se(ó^{2}NM)	15.61	0.38	0.56	0.03	0.16	1374.98
Ó^{2}A	528.88	-3.27	7.63	0.22	5.69	3749.85
Se(ó^{2}A)	986.50	1.98	14.72	0.53	10.49	11 743.36
ó^{2}D	3190.93*	1.52	10.72*	0.39*	2.61*	59 901.88*
se(ó^{2}D)	1770.77	6.75	7.13	0.35	1.99	36 809.37
h^2NS	13.88	-52.94	37.61	27.82	62.36	5.33
se(h^2NS)	25.89	32.04	72.54	66.74	114.96	16.70
ó^{2}Ph	3811.02*	6.18	20.29	0.80	9.12	70 323.05*
se(ó^{2}Ph)	2773.93	10.17	22.20	0.91	12.63	49 770.74
CVg(%)	5.14	20.72	8.47	11.47	6.91	17.33

[1.] se = Standard error.

* and ** indicate that ó^{2}g and h^2_{NS} were different from zero at >1 and 2 se, respectively.

Conclusions

1. The modified diallel analysis was found suitable for testing good-combining females. The tests of inbred, diallel-cross and synthetic progenies indicated a significant relationship between the general combining ability and the cross-population means, and the synthetic progenies may be useful to predict superior inbreds suitable for good-combining females.
2. The inbred populations showed a high interpopulation variability, which may simplify selection for certain desirable traits.
3. The values for ó^{2}M and ó^{2}NM measured similar importance at >1 se and 2 se, ó^{2}M being indicative for the presence of maternal effect. Maternal effect for productivity was not proved.

Acknowledgement

We thank the Directorate General of Higher Education, Department of National Education of the Government of Indonesia and the University of Lampung for providing us the necessary funds from the Competitive Research Grant Project 2005 and 2006 for this study.

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Genotype × Environment Interaction in Hybrid Maize Using the AMMI Model

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Abstract. Genotype-environment interaction (GEI) has a decisive influence on cultivar recommendation when a genotype is assessed in different environments. One of the main objectives of breeders is to obtain hybrids with a high mean yield and good adaptability to different environments. Our study assessed GEI and stability of grain yield, days to silking, plant height and ear height of 16 genotypes across four locations in Bangladesh during 2006-07. The AMMI (additive main effects and multiplicative interaction) model was used to assess the additive and multiplicative effects of interaction. Significant variations of genotype (G), environment (E) and GEI were found for all the characters studied. In terms of the stability parameter, the genotypes CML 486 × CML 487, IPB 911-22 × E -32 and BHM 3 showed small sum of squares for deviations from regression (S^2di) and a large coefficient of determination (r^2) indicating that the hybrid were stable across environments for days to silking. The hybrids BARNALI × IPB 911-18 and IPB 911-22 × E-32 appeared to be more or less stable for moderate plant height; the hybrids IPB 911-2 × E-32, BM7 × IPB 911-47, BM7 × IPB 911-16 and BHM 5 were found stable for ear height; the hybrids BARNALI × IPB 911-18, BM 7 × IPB 911-47 and BHM 5 were more stable for grain yield with near unit regression value with S^2di~0 as well as a large coefficient of determination (r^2). Based on the results from this study, four hybrids were found promising across locations and will be released for future cultivation.

Introduction

One of the greatest challenges for a maize breeder is to obtain a hybrid with high yield potential and the widest possible adaptation to diverse environments. Such a hybrid could be produced on a large scale with lower production costs and wide accessibility to producers. In initial assessments, maize hybrids are usually tested in relatively few environments, which allows genotype-envrionment interaction to interfere in their performance. This may lead to errors in selection, and promising materials may be discarded for lack of a more careful analysis of the data. The relative performance of genotypes can alter with the environment. Such differential responses are due to genotype-environment interaction (GEI). Several statistical analytical procedures have been used to interpret GEI, ie, to analyze the performance of genetypes in diverse environments and ascertain their genotype stability. The most widely used methods for genotype stability analysis are based on regression analyses (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Silva and Barreto 1985; Cruz *et al.* 1989; Razil 1990). Agronomic zoning is used to stratify environments in subregions within which GEI is not significant (Brasil 1990; Duarte and Zimmermann 1991). These methods are dependent on the genotypes and the environments under study, and may not be informative if the linearity fails (Crossa 1990). The additive nature of the common analysis of variance (ANOVA) allows for an adequate description of the main effects (genotypic and environmental effects). However, GE interaction (residue after fitting an additive model for these effects) may not be additive, and other techniques may be required to identify the existing relationships. The principal components analysis is a statistical procedure that gives a multiplicative model with the ability to diagnose and analyze the interaction though it too can be faulty in identifying the main significant effects (Shafii and Price 1998). In this sense, the AMMI model (Crossa 1990) is a method that combines in a single model estimation of the main effects and multiplicative components for the GEI effects. More precise GEI estimates can be obtained with the AMMI model which makes it easier to interpret the results (Duarte and Vencovsky 1999). The objective of this study was to use the AMMI analysis model to assess the stability of some maize hybrids.

Materials and Methods

The experiment was conducted at four locations – Gazipur, Jamalpur, Ishurdi and Hathazari in Bangldesh – during *rabi* (postrainy season) 2006-2007. Thirteen locally developed hybrids (selected from diallel cross and topcross evaluation during 2004-05) and three local controls, ie, BARI Hybrid Maize (BHM) 2, BHM 3 and BHM 5 were evaluated in this trial. Seeds were sown during 20-24 Nov at Joydebpur, Jamalpur, Ishurdi and Hathazari in an Alpha lattice design with 3 replications. The unit plot size was 5.0 × 1.5 m with 75 × 20 cm spacing between rows and hills, respectively. One healthy seedling per hill was left after proper thinning. Fertilizers were applied at the rate of 250 kg ha^{-1} N, 120 kg ha^{-1} P_2O_5, 120 kg ha^{-1} K_2O, 40 kg ha^{-1} S and 5 kg ha^{-1} Zn. Standard agronomic practices were followed (Quayyum 1993) and plant protection measures as required. Two border rows were used for minimizing the border effect. Data on silking was recorded on a whole plot basis. Ten randomly selected plants were used for recording observations on plant and ear height. All the plants in two rows were considered for plot yield. Grain yield (t ha^{-1}) data was assessed and adjusted to 12% moisture in seeds.

Analysis of variance (ANOVA) was used and GEI was estimated by the AMMI model (Zobel *et al.* 1988; Duarte and Vencovsky 1999). Thus, the mean response of the genotype *i* in environment *j* (***Yij***) is modeled by: *Yij* = ì + *gi* + *aj* + Ó*ëkãikájk* + ñ*ij* + ?*eij*, where ì is a common constant to the responses (normally the general mean); ***gi*** is the fixed effect of genotype *i* (*i* = 1, 2, ..., g); ***aj*** is the fixed effects of environment *j* (*j* = 1, 2, ..., a); Ó*ëkãikájk* is the fixed significant effect or pattern of the specific interaction of the genotype *i* with environment *j* (***gaij***), where ë*k* is the k-th singular value (scalar), ã*ik* and á*jk* are the corresponding elements, associated with ë*k*, of the singular vectors (rows vector and column vector) of the matrix of interaction estimated by ANOVA. For the same matrix, ñ?***ij*** is the nonsignificant effect or noise of (***ga***)***ij***, which is an additional residue, and ***eij*** is the pooled experimental error, assumed independent and *eij* ~ N(0, ó2). In this procedure, the contribution of each genotype and each environment to GEI is assessed by use of the biplot graph display in which yield means are plotted against the scores of the first principal component of the interaction (IPCA1). The computational program for AMMI analyses is supplied by Duarte and Vencovsky (1999). The stability parameters, regression coefficient (bi) and deviation from regression (S^2di) were estimated according to Eberhart and Russell (1966). Significance of differences among bi values and unity was tested by t-test, and between S^2di and zero by F-test.

Results and Discussion

The mean squares for the genotypes and environments in the study were highly significant, which revealed the presence of genetic variability in the material under investigation for all the characters studied (Table 1). The genotype × environment interactions for all the characters were significant when tested against pooled error, suggesting the data might be extended for stability analysis. The significant environment + (genotype × environment) component indicated that the genotypes reacted differently in different environments. The highly significant mean squares due to environments (linear) indicated the difference between the environments. Both the linear as well as nonlinear (pooled deviation) components of GEI interaction were significant for all the traits except days to 50% silking and plant height which indicated that the genotypes responded well with environmental fluctuations (bi) and to their stability (S^2di). Eberhart and Russell (1966) emphasized the need for considering both the linear (bi) and nonlinear (S^2di) components of GEI in judging the phenotypic stability of a genotype. In this model, the regression coefficient (bi) is considered as the parameter of response, and deviation

Table 1. Full joint analysis of variance including apportioning of genotype × environment interaction in maize.

Source of variation	df	Mean sum of squares			
		Days to silking	Plant height (cm)	Ear height (cm)	Yield (t ha^{-1})
Genotypes (G)	15	4.13**	744.28^{**++}	217.39^{**++}	2.61^{**++}
Environment (E)	3	997.45^{**++}	3907.46^{**++}	1748.95^{**++}	30.04^{**++}
G × E interaction (GEI)	45	4.72^{**++}	195.32*	74.01**	1.79**
AMMI component 1	17	4.11**	280.31**	131.67**	1.40**
AMMI component 2	15	2.48^{ns}	93.20^{ns}	42.96**	0.53**
AMMI component 3	13	1.19^{ns}	80.83^{ns}	34.44**	0.30^{ns}
G × E (linear)	15	2.98^{ns}	122.80^{ns}	100.57**	1.27**
Pool deviation	30	2.59^{ns}	179.07^{ns}	60.73**	0.53**
Pooled error	120	1.85	130.03	12.61	0.21

*P<0.05 and **P<0.01 (tested against pooled error); $^{+}$P<0.05 and $^{++}$P<0.01; ^{ns}Not significant (tested against pooled deviation).

Table 2. Regressions of yield for each variety on means of yield at each site.

Variety	Days to silking				Plant height (cm)			
	Mean	bi	S^2di	r^2	Mean	Bi	S^2di	r^2
Barnali × IPB 911-18	98.58	0.964	2.2	0.05	187.79	0.743	17.73	0.58
BM 7 × IPB 911-47	98.83	0.969	3.22	0.03	192.30	0.845	25.55	0.26
CML 486 × CML 487	96.67	0.675	5.51	0.64	185.33	1.287	123.31	0.20
IPB 911-2 × E –32	99.5	0.865	2.35	0.42	194.42	1.41	103.68	0.37
BIL 43 × BIL 29	98.33	1.072	2.8	0.15	181.53	0.335	207.03	0.44
CML 480 × CML 486	96.75	0.848	3.27	0.40	195.38	0.807	119.08	0.10
IPB 911-47 × BM 7	98.33	1.005	0.23	0.01	184.53	1.438	195.51	0.27
BM 7 × IPB 911-16	99.67	1.075	2.08	0.20	199.71	0.869	104.37	0.06
IPB 911-22 × E –32	99.92	1.091	0.34	0.69	188.53	0.488	53.12	0.65
IPB 911-22 × Barnali	99.42	0.986	3.93	0.00	194.31	1.234	467.45	0.04
IPB 911-36 × Barnali	98.17	1.035	0.16	0.42	177.50	0.757	18.35	0.54
CML 480 × CML 481	98.67	0.961	1.05	0.12	216.78	0.882	38.61	0.12
IPB 911-50 × E –32	98.33	1.084	3.47	0.16	181.19	0.899	251.3	0.01
BHM 2	98.42	1.072	2.27	0.18	219.59	0.77	452.17	0.04
BHM 3	99.92	1.222	2.77	0.63	217.59	1.051	448.51	0.00
BHM 5	100	1.077	3.24	0.15	208.54	2.008	60.29	0.86

Variety	Ear height (cm)				Yield (t ha^{-1})			
	Mean	bi	S^2di	R^2	Mean	Bi	S^2di	r^2
Barnali × IPB 911-18	87.81	0.832	30.37	0.13	9.99	0.837*	0.01	0.93
BM 7 × IPB 911-47	89.84	0.534	38.29	0.48	9.82	0.574*	0.01	0.98
CML 486 × CML 487	84.45	1.683	64.72	0.54	10.31	1.232	1.54	0.09
IPB 911-2 × E –32	95.65	1.396	23.81	0.52	10	1.014	1.7	0.00
BIL 43 × BIL 29	80.59	0.704	24.07	0.37	8.94	1.036	0.29	0.01
CML 480 × CML 486	89.07	0.512	38.36	0.50	9.92	0.792	0.22	0.36
IPB 911-47 × BM 7	91.12	1.847	48.12	0.71	9.7	1.056	1.07	0.01
BM 7 × IPB 911-16	91.95	0.329	22.92	0.76	10.52	0.732	3.12	0.06
IPB 911-22 × E –32	88	0.701	31.89	0.31	8.56	0.894	0.81	0.04
IPB 911-22 × Barnali	92.87	1.72	98	0.46	9.54	0.773	1	0.13
IPB 911-36 × Barnali	83.99	0.804	48.36	0.12	9.29	1.039	0.42	0.01
CML 480 × CML 481	98.6	0.298	106.28	0.43	11.14	1.267	1.33	0.13
IPB 911-50 × E -32	83.72	1.515	86.91	0.33	9.3	1.315	0.82	0.25
BHM 2	99.86	0.386	138.21	0.31	11.21	1.089	0.79	0.30
BHM 3	107.91	1.069	93.54	0.01	11.31	1.067	2.73	0.00
BHM 5	100.8	1.673	17.07	0.81	10.62	1.281*	0	0.99

bi = slopes of regressions of variety means on site index.
* indicates slopes significantly different from the slope for overall regression, which is 1.00, r.
**2 - squared correlation between residuals from the main effects model and the site index.

from regression (S^2di) as the parameter of stability. A relatively lower value of b, say around 1, indicates a less responsive genotype to environmental change and, therefore, more adaptive. If however, b is negative, the genotype may be grown only in poor environments. Deviation from regression (S^2di), if significantly different from zero, will invalidate the linear prediction. If S^2di is nonsignificant, the performance of a genotype in a given environment may be predicted. Therefore, a genotype whose performance in a given environment can be predicted, ie, $S^2di \sim 0$, is said to be a stable genotype.

Results of stability and response of the genotypes in different environments according to Eberhart and Russell are discussed character-wise as follows: the stability parameters, ie, regression coefficient (bi) and deviation from regression (S^2di) for plant height of individual genotypes, are presented in Table 2. The environmental mean and genotypic mean ranged from 176.37 cm to 208.62 cm and 166.85 to 223.96 cm, respectively. All the hybrids showed a negative phenotypic index (Pi) except the control variety and hybrids BM 7 × IPB 911-16, IPB 911-22 × Barnali, and thus seemed desirable for plant height. The genotypes BARNALI × IPB 911-18, CML 480 × CML 486, BM 7 × IPB 911-16 and IPB 911-22 × BARNALI appeared to be more or less stable for plant height.

The regression coefficient (bi) values of these genotypes ranged from –1.98 to 4.72. These differences in bi values indicated that all the genotypes responded differently to different environments. Considering the three parameters, it was evident that all the genotypes showed different adaptability responses under different environmental conditions. Among the genotypes, BM 7 × IPB 911-16, CML 480 × CML 481, BHM 2, BHM 3 and BHM 5 exhibited higher grain yield. Genotypes BARNALI × IPB 911-18, BM 7 × IPB 911-47, CML 486 × CML 487, IPB 911-2 × E -32, CML 480 × CML 486 and IPB 911-47 × BM 7 showed medium yield over environments.

Considering the yield potentiality and stability parameter, four hybrids were found promising across locations and will be released for future cultivation.

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Line × Tester Analysis of Early Generation Maize Inbred Lines

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Abstract. Line × tester analysis was conducted for grain yield and its contributing characters in maize involving 12 S_4 lines and 3 testers to determine the general combining ability and specific combining ability effects of inbred lines (S_4). Highly significant genotypic differences were observed, revealing a wide range of variability among the genotypes. Significant GCA and SCA variances for yield per plant, number of kernels per row and 100-kernel weight (g) were observed, which indicated the importance of additive as well as nonadditive gene action. The ratios of SCA and GCA variances were high for all the characters studied, suggesting a preponderance of nonadditive gene action over additive gene action. The parents IPB 911-16, IPB 911-12, IPB 911-2 and IPB 911-47 showed significant positive GCA effects and simultaneously possessed a high mean value, indicating that the per se performance of the parents could prove to be a useful index of combining ability. Twelve crosses exhibited significant positive SCA effects for grain yield. These crosses involved high × high, high × low, low × high, average × low and low × low general combining parents. This information on the nature of gene action with regard to variety and characters might be useful depending on the breeding objectives.

Key words: Maize, line, tester, combining ability, GCA, SCA

Introduction

Maize plays a significant role in human and livestock nutrition worldwide (Bantte and Prasanna 2004). In Bangladesh, maize acreage, production and yield decreased by 2.9%, 3.59% and 0.69%, respectively, from 1967-68 to 1986-87 due to utilization of traditional varieties (Mohiuddin 2003). However, introduction of hybrid varieties and appropriate management practices increased acreage, production and yield by 19.83%, 34.40% and 14.56%, respectively, from 1987-88 to 2003-04 (Moniruzzaman *et al.* 2007). Now maize has become an important cereal in terms of yield (maize: 5.36 t ha^{-1}; wheat: 2.21 t ha^{-1}; rice: 2.15 t ha^{-1}; Government of Bangladesh 2003). It could be a good source of nutrition for undernourished and malnourished sections of the population. It is now widely used in poultry farms, fisheries and for animal as well as human consumption.

Combining ability is a prerequisite for developing a good hybrid maize variety. It is a powerful tool in identifying the best combiner to be used in crosses either to exploit heterosis or accumulate fixable genes. The present study involving line × tester analysis was aimed at determining the general combining ability (GCA) and specific combining ability (SCA) of crosses for different traits and to explore heterotic hybrid combinations.

Materials and Methods

Twelve locally developed, advanced-stage inbred lines and 3 testers of maize were selected for the study and crossed in a line × tester fashion to generate 36 cross combinations during rabi (postrainy season) of 2003 at the Bangladesh Agricultural Research Institute (BARI), Joydebpur. Seeds of all the parents, their F_1 hybrids and one control variety, BARI Hybrid Maize 3 (BHM 3), were sown in the same farm in a randomized complete block design with 3 replications during the postrainy season of 2004. The unit plot size was 5.0 × 0.75 m with plant spacing of 75 × 20 cm between rows and hills, respectively. One healthy seedling per hill was left after proper thinning. Fertilizers were applied at the rate of 250 kg ha^{-1} N, 120 kg ha^{-1} P_20_5, 120 kg ha^{-1} K_20, 40 kg ha^{-1} S and 5 kg ha^{-1} Zn. Standard agronomic practices were followed (Quayyum 1993) and plant protection measures taken as required. The combining ability analysis was carried out as per the method suggested by Kempthorne (1957).

Results and Discussion

Analysis of variance showed significant variation among the genotypes for yield and yield-contributing characters (Table 1). Sofi and Rather (2006) found similar results for ear length (cm), ear diameter (cm), kernel rows per ear, 100-seed weight (g) and grain yield per plot (g). Highly significant differences were observed for parents, hybrids and parent × hybrid interaction for all the traits studied except 100-kernel weight, suggesting a wide range of variability. Significant differences were found between the lines for all the traits, indicating substantial variability in lines for these traits. Significant differences were observed between the testers for yield per plant, number of grains per row and number of rows per ear. Highly significant differences also were observed due to line × tester interaction for all traits except 100-kernel weight, indicating a wide range of variability among these traits. Similar results have been reported by Venkatesh *et al*. (2001), Narro *et al*. (2003) and Sofi and Rather (2006). The analysis of variance for combining ability (Table 1) revealed significant GCA and SCA for yield per plant, number of kernels per row and 100-kernel weight, which indicated the importance of additive as well as nonadditive gene actions. The ratios of SCA and GCA variance were high for all the characters studied, revealing the preponderance of nonadditive gene action. Sanghi *et al*. (1983), Debnath *et al*. (1988), Das and Islam (1994), Roy *et al*. (1998) and Uddin *et al*. (2006) also reported predominance of non additive gene action in maize.

General combining ability

The GCA effects and *per se* performance of the parents revealed that none of the parents was a good general combiner for all the characters studied (Table 2). The lines IPB 911-16, IPB 911-18, IPB 911-12, IPB 911-2 and IPB 911-47 showed a significant positive GCA effect and simultaneously possessed high mean values, indicating that the *per se* performance of the parents could prove an useful index for combining ability. Roy *et al*. (1998) Hussain *et al*. (2003) Uddin *et al*. (2006) observed a similar phenomenon. So, these four parents could be used extensively in hybrid breeding programs aimed at increasing maize grain yields.

In the case of number of kernels per row, IPB 911-50 was the best general combiner followed by IPB 911-18, IPB 911-2, IPB 911-22, IPB 911-4 and IPB 911-31. For number of rows per ear, IPB 911-1, IPB 911-22, IPB 911-31 and IPB 911-47 were good general combiners. For ear length, the good general combiners were IPB 911-4, IPB 911-2, IPB 911-31 and IPB 911-50, and for ear diameter IPB 9 11-1, IPB 911-2 and IPB 911-31. Among the testers, BARI Maize-7 (BM-7) was a good general combiner for yield per plant, number of kernels per row, ear diameter and 100-kernel weight; E-32 was a good combiner for ear length only. None of the parents except BM-7 exhibited a significant GCA effect for 100-kernel weight. Sofi and Rather (2006) observed similar good general combiner testers for grain yield.

Significant positive correlation between *per se* performance and GCA effect was found only for grain yield per plant. For number of kernels per row, number of rows per ear, ear length, ear diameter and 100-kernel weight, the correlation between *per se* performance and GCA was positive but not statistically significant. Das and Islam (1994) observed similar results.

The high, significant and positive GCA effects for different desired characters could be helpful in identifying outstanding parents with favorable alleles for yield and other desirable components.

Table 1. Analysis of variance for line × tester analysis including parents in maize.

Source of variation	df	Mean sum of squares and components of variance					
		Yield per plant (g)	No. of kernels per row	No. of rows per ear	Ear length (cm)	Ear diameter (cm)	100-kernel weight (g)
Replication	2	130.12	2.14	0.07	1.39	1.50	22.25
Genotypes	50	2102.46**	49.78**	3.26**	3.30**	2.42**	24.32**
Parents	14	2342.64**	73.34**	5.98**	5.30**	2.83**	47.94**
Parent × hybrid	1	33 720.83**	358.46**	21.66**	25.80**	24.92**	66.56**
Hybrid	35	1103.00**	31.54**	1.65**	1.86**	1.61**	13.66
Line	11	1673.14**	68.01*	4.81**	5.35*	3.26*	59.11**
Tester	2	820.81**	125.83**	11.67**	1.14	0.85	6.78
Line × tester	22	1250.16**	36.88**	1.52**	1.33**	1.58**	6.30
Error	100	44.83	0.65	0.36	0.33	0.51	13.10
Σ^2_{GCA}	14	1.87*	2.45*	0.24	0.06	0.15	1.86*
Σ^2_{SCA}	35	387.17**	9.92**	0.53	0.51	0.34	7.93**
$\Sigma^2_{SCA:}\ \sigma^2_{GCA}$		207.04	4.05	2.21	8.50	2.27	4.26

*P = 0.05; **P = 0.01.

Table 2. GCA effect and mean performance (in parentheses) of parents for different yield-contributing traits in maize.

Parents	Yield per plant (g)	No. of kernels per row	No. of rows per ear	Ear length (cm)	Ear diameter (cm)	100-kernel weight (g)
Line						
IPB 911-1	-6.84**(48.22)	-1.54**(23.8)	0.54**(11.8)	0.07(12.6)	0.46**(13.0)	-0.25(34.5)
IPB 911-2	5.60**(85.12)	1.18**(35.6)	-0.19**(13.7)	0.45**(16.4)	0.61**(15.7)	-1.36(33.5)
IPB 911-4	0.41(87.61)	0.69*(24.1)	0.03(12.5)	1.09**(13.8)	-0.28(13.1)	1.53(30.0)
IPB 911-12	9.03**(82.74)	0.46(33.8)	-0.15*(13.7)	-0.71**(17.3)	-0.24(15.3)	-0.58(27.3)
IPB 911-16	14.51**(121.09)	-0.98**(35.5)	-0.01(12.9)	-0.02(15.3)	-0.12(13.7)	0.25(24.5)
IPB 911-18	9.10**(73.88)	1.94**(30.6)	-0.49**(11.7)	-0.18(16.7)	-0.40*(14.7)	-1.63(29.5)
IPB 911-22	-3.21*(41.93)	0.73*(23.4)	0.51**(10.4)	-0.22(15.3)	0.01(13.0)	3.09(40.3)
IPB 911-31	-9.71**(101.05)	0.57*(23.9)	0.26**(9.90)	0.41**(13.9)	0.55**(12.7)	0.92(29.2)
IPB 911-36	-10.61**(45.46)	-0.46(33.0)	-0.69**(12.3)	-1.02**(15.3)	-0.95**(15.0)	-2.08(32.3)
IPB 911-39	-11.69**(74.69)	-1.26**(31.40	-0.35**(12.3)	0.11(15.8)	0.01(13.3)	0.14(32.0)
IPB 911-47	3.28*(79.76)	-3.55**(30.30	0.43**(14.1)	-0.31(15.1)	0.08(14.8)	-0.02(26.2)
IPB 911-50	0.14(57.61)	2.20**(26.3)	0.11(12.4)	0.33*(14.3)	0.28(13.7)	-0.02(34.5)
SE (gi)	2.14	0.26	0.08	0.18	0.23	1.15
SE (gi-gj)	4.73	0.57	0.43	0.41	0.50	2.56
Tester						
Barnali	-6.52**(98.13)	-0.28(38.6)	-0.41**(11.1)	-0.02(17.2)	-0.13*(14.5)	-0.81(30.0)
BARI Maize-7	7.74**(123.99)	0.73*(28.60	0.16(14.5)	-0.26*(16.1)	0.19*(15.1)	1.62**(33.00
E-32	-1.22*(128.92)	-0.45(26.5)	0.24(14.5)	0.28*(16.1)	-0.06(14.0)	-0.80(31.3)
SE (gj)	0.91	0.37	0.20	0.16	0.10	0.81
SE (gi-gj)	9.47	1.14	0.85	0.81	1.10	5.12
r (GCA, Mean)	0.52*	0.09	0.29	0.24	0.24	0.26

*P = 0.05; **P = 0.01

Specific combining ability (SCA)

Among the 36 crosses studied, 12 exhibited significant positive SCA effects for grain yield (Table 3). These crosses involved high × high, high × low, low × high, average × low and low × low general combining parents. Crosses with high SCA effects for grain yield, such as IPB 911-2 × E-32, IRB 911-16 × Barnali, IPB 911-16 × E -32, IPB 911 -39 × BM-7 and IPB 911-47 × Barnali were from high × low general combiner parents. This suggested an additive × dominance type of gene action. For the same trait, IPB 911-1 × BM-7 and IPB 911-4 × BM -7 were from low × high combiners, indicating a dominance × additive type of gene action. Roy *et al.* (1998) and Uddin *et al.* (2006) also found significant positive SCA effects in high × low and low × high general combiners. The hybrids IPB 911-22 × E-32, IPB 911-31 × E-32, IPB 911-36 × Barnali were from low × low general combiner parents, revealing dominance × dominance gene action. These results agreed with the results of studies by Uddin *et al.* (2006) in maize, and by Sarker *et al.* (2002) in rice. The hybrid 91-12 × BM-7 and IPB 911-18 × BM-7, suggesting high × high general combiner parents and depicting an additive × additive type of gene action. Paul and Duara (1991) reported that parents with high GCA always produce hybrids with high estimates of SCA.

A significant and positive SCA effect was observed in 11 crosses for the number of kernels per row. These crosses mostly were from high × low, low × high, high × average, average × low and low × low general combining parents. Thirteen crosses exhibited significantly positive SCA for the number of rows per ear. Eighteen and thirteen crosses showed significant and positive SCA for ear length and ear diameter. For 100-kernel weight, 12 hybrids showed a significant SCA effect. Significant positive SCA usually represents dominance and an epistatic component of variation.

From our study, five lines (IPB 911-2, IPB 911-12, IPB 911-16, IPB 911-18 and IPB 911-47) and one tester (BM-7) exhibited significant positive SCA and possessed comparatively good *per se* performance. Though these parents could be used for developing high-yielding hybrids, the crosses possessing high yield potential with significant SCA effects could be used for better hybrid selection. The information on the nature of gene action with respect to variety and characters might be used depending on the breeding objectives.

Table 3. SCA effects of F_1 hybrids for different yield and yield-contributing traits in maize.

Hybrid	Yield per plant (g)	No. of kernels per row	No. of rows per ear	Ear length (cm)	Ear diameter (cm)	100-kernel weight (g)
IRB 911-1 × Barnali	-1.32	-1.04**	-0.75**	-0.96**	-0.38**	-0.02
IPB 911-1 × BM-7	11.41**	-4.17**	0.35**	0.28**	-0.03	-0.12
IPB 911-1 × E-32	-10.10**	5.21**	0.40**	0.68**	0.42**	0.13
IPB 911-2 × Barnali	3.68	-1.19**	-0.06	0.73**	0.66**	-0.41**
IPB 911-2 × BM-7	-22.40**	-3.65**	0.41**	-0.43**	-0.26**	-1.17**
IPB 911-2 × E-32	18.72**	4.84**	-0.34**	-0.30**	-0.41**	1.58**
IPB 911-4 × Barnali	-3.43	-3.38**	-0.31**	-0.31**	0.29**	-0.13
IPB 911 -4 × BM-7	25.35**	5.34**	0.66**	0.39**	-0.30**	-1.89**
IPB 911-4 × E-32	-21.92**	-1.95**	-0.36**	-0.08	0.02	2.02**
IPB 911-12 × Barnali	-0.91	-0.22	1.14**	-0.05	-0.08	-2.19**
IPB 911-12 × BM-7	25.24**	1.98**	-1.16**	0.39**	0.00	2.05**
IPB 911 -12 × E-32	-24.33**	-1.76**	0.02	-0.35**	0.08	0.13
IRB 911-16 × Barnali	15.23**	-3.24**	0.87**	0.67**	0.53**	-0.02
IPB 911-16 × BM-7	-41.56**	4.72**	-0.35**	0.50**	0.21**	1.22**
IPB 911-1 6 × E-32	26.33**	-1.48**	-0.52**	-1.17**	-0.74**	-1.20**
IPB 911-18 × Barnali	-15.27**	-1.70**	-0.19*	0.02	0.31**	1.54**
IPB 911-18 × BM-7	21.67**	1.57**	-0.29**	-0.28**	-0.65**	-0.39**
IPB 911-18 × E-32	-6.40**	0.13	0.49**	0.25**	0.34**	-1.14**
IPB 911-22 × Barnali	-2.98	-0.98**	0.27**	0.27**	-0.14	-2.02**
IPB 911-22 × BM-7	-15.57**	-2.16**	-0.29**	-0.56**	0.08	1.88**
IPB 911-22 × E-32	18.55**	3.14**	0.02	0.30**	0.06	0.13
IPB 911-31 × Barnali	-10.61**	2.27**	-0.47**	0.23*	0.06	0.98**
IPB 911-31 × BM-7	-12.94**	0.43	-0.37**	-0.63**	-0.46**	0.55**
IPB 911-31 × E-32	23.55**	-2.70**	0.84**	0.40**	0.39**	-1.53**
IPB 911-36 × Barnali	11.74**	5.10**	0.61**	-1.20**	-1.90**	-0.02
IPB 911 -36 × BM-7	3.22	-3.57**	0.44**	0.44**	0.38**	-1.45**
IPB 911-36 × E-32	-14.96**	-1.53**	-1.04**	0.77**	1.53**	1.47**
IPB 911-39 × Barnali	0.75	-0.10	0.01	0.20*	0.60**	0.76**
IPB 911 -39 × BM-7	14.07**	0.17	0.57**	0.30**	-0.19*	0.16
IPB 911-39 × E-32	-14.82**	-0.07	-0.58**	-0.50**	-0.41**	-0.92**
IPB 911-47 × Barnali	4.64*	0.65**	-0.84**	0.75**	0.13	0.76**
IPB 911 -47 × BM-7	-6.75	-0.02	-0.07	-0.48**	0.81**	-0.34*
IPB 911-47 × E-32	2.11	-0.63**	0.91**	-0.28**	-0.94**	-0.42**
IPB 911-50 × Barnali	-1.52	3.84**	-0.26*	-0.36**	-0.07	0.76**
IPB 911-50 × BM-7	-1.74	-0.64**	0.11	0.08	0.41**	-0.50**
IPB 911-50 × E-32	3.26	-3.20**	0.16	0.28**	-0.34**	-0.25
SE (S_{ij})	3.03	0.46	0.09	0.09	0.10	0.19
SE (S_{ij}-S_{kl})	5.47	0.66	0.49	0.47	0.58	2.95

*P = 0.05; **P = 0.01.

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Use of Cluster-Based Mating in Increasing Efficiency of Finding Productive Crosses in Maize

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Abstract. Innovative methods that increase the efficiency of breeding work while reducing the cost are of great help to maize breeders. This study was aimed at evaluating the usefulness of cluster-based mating using SSR and qualitative morphological markers to find productive crosses (PCs) in maize. We used four data sets consisting of 192 crosses derived from four sets of three diallel and one design II experiments in which three sets were inbred crosses and one set variety crosses. The four sets of crosses including the control varieties were evaluated for yield in an alpha lattice design with three replications. We used 39, 40 and 30 SSR markers to establish grouping of inbred lines in sets A, B and C, respectively. For set D, we used 19 agromorphological traits to group the parent varieties. The results showed that intergroup crosses had a greater number of PCs than those in intragroup crosses. Sixty-four percent (64%) of the PCs came from the intergroup crosses in set A. In sets B, C, and D, all the PCs came from intergroup crosses. Further, cluster-based crosses appeared to be 12% more efficient in identifying PCs than noncluster-based crosses, thereby reducing the cost of extensive field crosses and tests.

Key words: Cluster-based mating, intragroup crosses, productive crosses, SSR markers

Introduction

Identification of parent lines that will give productive hybrids is a costly and time-consuming activity. The *per se* performance of maize inbred lines does not always predict the yield performance of maize hybrids (Hallauer and Miranda 1988). Conventionally, diallel analysis has been used to identify parent lines that could be used as parents for synthetic and hybrid breeding. However, this method becomes impractical when a large number of lines are involved since the number of crosses so produced increases proportionately, which would require an unmanageable extent of acreage for field testing. An alternative way to maximize utilization of genetic germplasm as well as reduce costs is to use cluster-based mating utilizing molecular and agromorphological data. Cluster-based mating is defined as crossing of lines or varieties from different groups which are established using some sets of criteria. Molecular markers are powerful tools to define heterotic groups and to assign inbred lines to existing heterotic groups (Melchinger 1999). Among molecular markers, SSR has become the marker of choice for many types of genetic analyses. SSR markers offer advantages in reliability, reproducibility, discrimination, standardization and cost-effectiveness over other markers (Smith *et al.* 1997). Cuong *et al.* (2007) and Magulama, Sales *et al.* (2007) used the SSR marker system in determining promising crosses in maize. Qualitative morphological traits have also been used in cluster-based mating of maize varieties (Magulama. Sillote *et al.* 2007). This study was aimed at evaluating the usefulness of cluster-based mating using broader sets of data derived from both SSR and morphological markers in searching for productive crosses (PC) in maize in the most effective and efficient manner that would lead to better utilization of resources.

Materials and Methods

Composition of data sets

In this study we used four data sets that involved a total of 192 crosses derived from two sets of inbred diallel crosses, one set of inbred design II crosses and one set of variety diallel crosses. Set A involved 11 yellow inbred diallel crosses with 55 single crosses. Set B included 9 white inbred diallel crosses with 36 single crosses. Set C comprised of 14 yellow QPM lines which were crossed in design II mating based on derived SSR-based grouping and resulted in 54 crosses. Set D was constituted by 10 white variety diallel crosses with 45 variety crosses.

Field trials

The crosses generated from each set of crosses were evaluated for yield in the experimental area of the University of Southern Mindanao (14°N; 30 m above sea level) during at different spans of time during 2003-2006. The four yield trials were conducted during the following periods: set A in May to Aug 2004, set B in Sep 2005 to Jan 2006, set C in May to Sep 2005, and set D in May to Aug 2003. The entries in each trial were arranged in an alpha lattice design (Patterson and Williams 1976) with three replications. The experimental units in all trials were composed of three 3 m row plots spaced 65 × 25 cm with two plants per hill. Thinning was done two weeks after sowing, keeping one plant per hill. Plants were fertilized with complete fertilizer (14-14-14) at the rate of 60-60-60 NPK ha^{-1} during planting, and urea (46-0-0) at the rate of 60-0-0 NPK ha^{-1} 25 days after planting. Other recommended cultural management practices were strictly followed.

SSR analysis

We performed SSR analyses for parent lines in sets A, B and C. Extraction of DNA was done following the AMBIONET-CIMMYT service laboratory protocol (AMBIONET 2004). Six to 8 leaves of 10-day-old corn plants were collected and bulked. DNA fingerprinting was performed with a standard marker set of 40 publicly available SSR markers which provide even coverage of the maize genome. The DNA was extracted using modified the CTAB method (Saghai-Maroof *et al.* 1984). PCR reactions were performed in 10 uL volumes containing 2 uL of template DNA, 0.25 uM each of the primers, 1 × PCR buffer, 0.25mMdNTPs, 1.5 µM $MgCl_2$ and 0.38 *U Taq* polymerase. The reactions were performed with a Peltier thermal cycler using the amplification conditions of 94°C for 2 min; followed by 30 cycles of 94°C for 30 seconds, X°C for 1 min and 72°C for 1 min; followed by extension at 72°C for 4 min. Samples containing 2.5 uL of PCR products were loaded into the well of a 96-well combs with a molecular weight marker at both ends within the gel. The bands were detected by the silver staining method. Data were scored as present (1) or absent (0) for a particular allele/maize line combination. Bands that were diffused or too difficult to score were recorded as missing data (9). In cases when a line had multiple bands of varying intensity, the most intense band was scored as 1 and the others as 9.

Data gathered

In all the field trials, only data on grain yield were presented and determined by weighing the ears harvested from three rows of each plot. Seed moisture content was recorded using an electronic moisture tester. The grain yield of each entry was calculated using the formula:

$$\text{Grain yield} = FW \times CF \times (100\text{-}MC)/100 \times (80/85) \times (10{,}000/\text{area harvested}) \times 0.001$$

where FW = field weight; CF = correction factor; and MC = moisture content.

Statistical data analysis

Data on grain yield were analyzed by the ANOVA technique using an Alpha software program (CIMMYT 1999). A matrix of binary data (0,1), with the columns equal to inbred lines, and the rows equal to the alleles of each primer was compiled in MS Excel for the SSR data. Genetic similarities (GS) among pair-wise comparisons of inbred lines were calculated using similarity coefficients developed by Jaccard (1908). Genetic similarities may range from 0 (all bands in common) to 1 (no bands in common). In developing the binary data (1 for present, and 0 for absent) for qualitative traits, a score of 1 or 0 was given to the varieties depending on the presence or absence of the trait, respectively. Scoring was done for 19 qualitative traits. Genetic similarities (GS) among pair-wise comparisons of populations were calculated using Jaccard's method. The dendrogram was constructed using the unweighted pair group method of arithmetic average (UPGMA). Cluster analysis was performed using the NTSys pc ver 2.1 software package (Rolf 2002).

Results and Discussion

Grouping of genetic materials using SSR and morphological markers

We used 39, 40 and 30 informative SSR markers for the genetic material in sets A, B and C, respectively, to establish groups in each set of inbred lines or varieties. For Set D we used 19 qualitative agromorphological markers. In Set A, cluster analysis classified the 11 maize inbred lines into 3 distinct clusters. Cluster I included 4 lines, Cluster II 5 lines, and Cluster III 2 lines. The groups of the 11 lines are shown in Fig.1a. In Set B, three clusters were noted: Cluster I had 6 lines, Cluster II 2 lines and Cluster III 1 line (Fig.1b). In Set C the 14 lines were grouped into 5 clusters where Cluster I included 2 lines, Cluster II 4 lines, Cluster III 3 lines, Cluster IV 4 lines, and Cluster V 1 line (Fig.1c). In Set D cluster analysis grouped the 10 varieties into two major clusters. Cluster I was composed of 5 varieties and Cluster

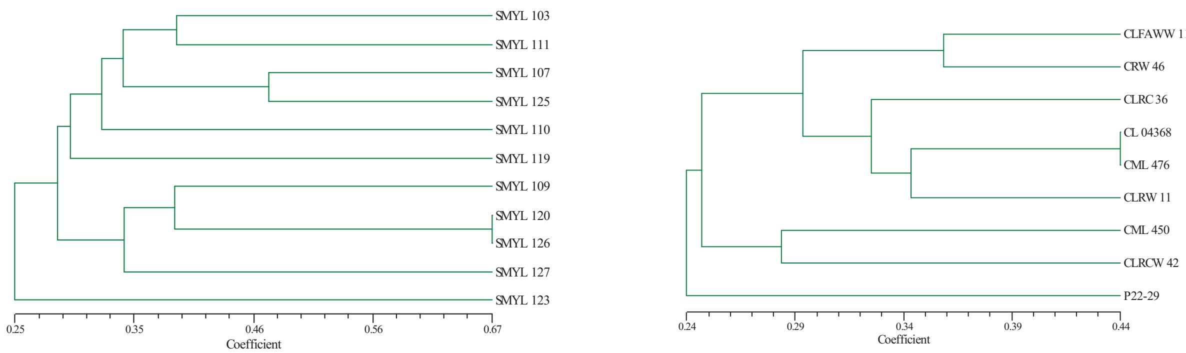

Figure 1. Dendrograms depicting the relationship of genetic materials in each data set based on markers using the UPGMA method. Sets A, B and C = grouping of inbred lines based on SSR markers; Set D = grouping of varieties based on qualitative morphological traits.

II 5 varieties (Fig.1d). The results indicated that the germplasm used in this study showed wide genetic diversity.

Comparison of intragroup and intergroup crosses in generating productive crosses

To determine which form of crosses generated more productive crosses, analyses of intra- and intergroup crosses were made. In sets A, B and C, productive crosses (PCs) were selected based on the yield superiority of crosses over the hybrid controls. For Set D, PCs were selected based on the average yield of crosses in intra- and intergroup population crosses. In Set A, 64% of the PCs came from intergroup crosses and 36% from intragroup crosses. For sets B, C and D, all the PCs were derived from intergroup crosses and none from intragroup crosses. Table 1 presents the number of productive crosses obtained from intra and intergroup crosses. Several researchers (Hallauer *et al.* 1988; Melchinger and Gumber 1988; Bernardo 2001; Magulama, Sillote *et al.* 2007) have documented the yield superiority and better agronomic performance of intergroup over intragroup crosses in maize. Lanza *et al.* (1997) and Parentoni *et al.* (2001) reported that correlation between the mean yields of single-cross hybrids and the marker-based GD estimates, which was low, became stronger when correlation analysis was performed based on the combination of lines belonging to different groups established by markers. The use of marker-based clusters as the basis for selecting inbred lines from different groups in order to make crosses may improve the chances of generating productive hybrids.

Table 1. Number and percentage of productive crosses obtained from intra- and intergroup crosses.

Set no.	Group crosses	No. of productive crosses*	Percentage of productive crosses (%)
A	Intragroup	5	36
	Intergroup	9	64
	Total crosses	14	
B	Intragroup	0	0
	Intergroup	4	100
	Total crosses	4	
C	Intragroup	0	0
	Intergroup	6	100
	Total crosses	6	
D	Intragroup	0	0
	Intergroup	22	100
	Total crosses	22	

*Productive crosses were selected based on yield superiority over control varieties.

Table 2. Comparison of cluster-based and noncluster-based mating in generating productive crosses.

Set no.	Group crosses	Total no. of crosses	No. of productive crosses (PCs)	Percent of PCs
A	Noncluster-based	55	14	25.45
	Cluster-based[1]	34	9	26.47
B	Noncluster-based	36	4	11.11
	Cluster-based	29	4	13.39
C	Noncluster-based	91	6	6.59
	Cluster-based	54	6	11.11
D	Noncluster-based	45	22	48.89
	Cluster-based	25	22	88.00

[1.] Crosses were formed by mating between lines/populations that belonged to different groups as revealed by cluster analysis.

Efficiency of cluster-based and noncluster-based group mating

Table 2 shows the comparative efficiency of noncluster-based and cluster-based mating in finding superior crosses. In Set A, using three clusters, the number of PCs was comparable between noncluster-based (25%) and cluster-based mating (26%). Considering three clusters in Set B, cluster-based mating (14%) produced more PCs than the noncluster-based mating (11%). Using five clusters in Set C, more PCs were obtained from cluster-based mating (11%) than noncluster-based (7%). Utilizing two clusters in Set D, more PCs were noted from cluster-based mating (88%) than noncluster-based mating (49%). A comparable result regarding the efficiency between noncluster-based mating and SSR-based mating in finding promising crosses was reported (Magulama, Sales *et al.* 2007), though the former has the advantage of involving fewer crosses to be made. In our study, the results support the greater efficiency of cluster-based mating over noncluster-based mating in finding productive crosses. The former type of mating could greatly save resources utilized in making crosses and field-testing of materials.

Conclusions

Cluster-based mating appeared more efficient than noncluster-based mating in finding productive crosses in maize. It can greatly help in reducing the number of crosses, thereby reducing the cost and time in making crosses and field testing. Our study demonstrates the use of marker technology in accelerating plant breeding work.

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Heterotic pattern formation for hybrid maize development in Indonesia

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Abstract. To obtain high yielding hybrid varieties, it is necessarry to develop a pair of population possessing different heterotic group. In 1993, maize breders at the Indonesian Agency for Agricultural Research and Development (IAARD) developed heterotic pattern of two population pairs, namely Malang Synthetic (MS) J1 with J2 for late maturing version and MS K1 with K2 for early maturing version. From the first cycle, eight hybrid varieties were released during 1999 – 2007 namely three-way cross hybrids (Semar-4 to Semar-10) and single cross hybrids (Bima-1, Bima-2 Bantimurung, and Bima-3 Bantimurung). This paper describes the heterotic pattern of maize inbreds in the development of hybrids released varieties conducted by the IAARD. The information of genetic relationship among inbred lines, as parents of released hybrids above, was conducted by making a matrix based on the results of molecular analysis on eight lines using 26 SSR markers in 2005. Results showed that inbred parents of hybrids Semar-4 – Semar-7 agreed with the heterotic pattern of MSK1 x MSK2 and MSJ1 x MSJ2. Inbred parents of hybrids Semar-8 – Semar-10 and Bima-1 did not agree with the heterotic pattern above. Semar-8 and Semar-9 were developed from inbreds possessed a close genetic relationship (genetic distance values 0.25 – 0.54), while Semar-10 was developed from inbreds possessed a far genetic relationship (genetic distance values 0.60 – 0.71). Bima-1 is a single cross between Mr-4 and Mr-14 having genetic distance value between two inbreds 0.71. The highest genetic distance of 0.85 was obtained between inbred Mr-14 and GM-15. Genetic distance values of the inbreds above agreed with the pedigree of those inbreds. There were eight other inbred pairs had genetic distance value relatively high (> 0.70) which give high chance in cross combinations to obtain a good hybrid, such as Mr-14 x GM-15.

Key words: maize, heterotic, pattern, inbred, hybrid, genetic, distance

Introduction

The objective of hybrid breeding program is to obtain inbred parent pirs which give excellent performance namely high specific combining ability (SCA) and able to be multiplied for seed commercial. Hybrid productivity is depend on favourable genes available in both inbred parents. Both inbreds should have complementary favourable genes in order to produce F1 hybrid possesses many loci of favourable genes and finally high yielding hybrid (Castro *et al*., 1968; Crossa *et al*., 1987).

To obtain hybrid with high productivity, it is necessary to have population pairs with high yielding potensial and from different heterotic groups. The higher yielding potensial of population pair, the higher the probability of obtaining inbreds as parents of hybrid with high productivity. Heterotic pattern information is important in hybrid formation program to reduce both time and budget required or in other words, heterotic pattern information is useful in increasing efficiency and effectiveness of hybrid development.

The most popular heterotic pattern in the USA is Mo17 and B73, which was derived from groups *Lancaster Surecrop* (LSC) and grup *Reid Yellow Dent* (RYD), respectively. Population *Iowa Stiff Stalk Sinthetic* (*BSSS*) was derived from RYD (Hallauer *et al*, 1988). In tropical countries, the heterotic pattern available are: Tuxpeno x ETO, Tuson x Tuxpeno, Cuba flint x Tuxpeno, and Suwan1 x Tuxpeno (Vassal *et al*., 1993). This paper describes the heterotic pattern of maize inbreds in the development of hybrids released varieties conducted by the IAARD.

Inbred lines formation

Improvement of two populations and formation of hybrid can be conducted together by applying a reciprocal recurrent selection (RRS) breeding method. From the improved population, there will be obtained a good combining ability lines with the partner population. In 1993, maize breeders at the Indonesian Agency for Agricultural Research and Development (IAARD) developed heterotic pattern of two population pairs, namely Malang Synthetic (MS)J1 with MSJ2 for late maturing version and MSK1 with MSK2 for early maturing version. From the cycle-1 and cycle-2 programs, eight hybrid varieties have been released during 1999 – 2002 namely three-way cross hybrid varieties (Semar-4 to Semar-10) and single cross hybrid maize varieties (Bima-1, Bima-2 Bantimurung, and Bima-3 Bantimurung). The last two hybrids were released in 2007 with grain yield productivity of 10 - 11 t/ha.

To obtain the information of the genetic relationship of inbred lines as parents of released hybrids above, cluster analysis based on the results of molecular analysis was conducted on eight lines using 26 SSR markers (Pabendon et al., 2005). Genetic distance estimation of inbred pairs is useful in determining a promising hybrid. Genetic distance estimation of several inbreds conducted by Lee *et al.* (1989) based on Modified Rogers' Distance (MRD) showed that there was a significant correlation between grain yield (t/ha) and specific combining ability (SCA).

Genetic relationship among inbred lines

Base populations of inbred parents of hybrids Semar dan Bima are presented in Table 2. Female and male parents of hybrid Semar 4 were (Mr01 x Mr02) and Mr03, respectively. The inbreds Mr01, Mr02, and Mr03 were derived from populations MSK1, MSK1, and MSK2, respectively, which agreed with the heterotic pattern of MSK1 x MSK2. Female parents of hybrids Semar 5, Semar 6, and Semar 7 consisted of inbreds Mr05, Mr06, Mr07, and Mr08, while male parents was Mr04. These inbreds were derived from populations MSJ1 and MSJ2, which agreed with the heterotic pattern of MSJ1 x MSJ2. Inbred parents of hybrids Semar 8 and Semar 9 were derived from populations Suwan2C7 and MK9, which not agree with the heterotic pattern of MSK1 x MSK2.

Inbred parents of hybrids Semar 10 and Bima 1 were derived from populations MSJ1 and Suwan3C7, which not agree with the heterotic pattern of MSJ1 x MSJ2, because Mr14 was not derived directly from MSJ2, although Suwan3 was lines source of MSJ2 (Table 1). This suggested that inbred Mr4 (derived from MSJ1) had better combining ability with lines from Suwan3C7 than lines derived directly from MSJ2 (heterotic pair of MSJ1). Bima-2 Bantimurung and Bima-3 Bantimurung are new hybrids released in 2007 which developed from crosses of B11-209 x Mr14 and Nei 9008 x Mr14, respectively (Table 2). B11-209 and Nei 9008 x

Table 1. Characteristic of MS J1, MS J2, MS K1, and MS K2 base populations.

Description	Late maturing version		Early maturing version	
	MS J1	MS J2	MS K1	MS K2
Source populations	MK 9, Kalingga, Wiyasa, Harapan, Rama, Pop 27, Muneng Sintetik 3	Genteng Kuning, Pop 24, Suwan 3, Pop 28,	MKA, MKF, Acer, Pop 31, Pool 1, Pool 2	Suwan 2
Lines	13 S6 lines	20 S3, S4 lines	24 S6 lines	20 S3 lines
Testers	GM 15	Suwan 1	Suwan 2	GM 15
Maturity (d)	100	100	90	90
Kernel color/type	Yellow / flint	Yellow / dent	Yellow / flint	Yellow / dent

Source: Dahlan *et al.* (1996).

Table 2. Grain yield of hybrid maize varieties released by AARD since 1999, inbred parents, and their source populations

Hybrids[a]	Year of released	Grain yield (t/ha)	Female parents[b]	Male parents[b]	Source Populations	
					Female parents	Male parents
Semar-4	1999	5.9	Mr01 x Mr02	Mr03	K1C1 x K1C1	K2C1
Semar-5	1999	6.8	Mr05 x Mr06	Mr04	J1C1 x J1C1	J2C1
Semar-6	1999	6.9	Mr07 x Mr08	Mr04	J1C1 x J1C1	J2C1
Semar-7	1999	6.8	Mr08 x Mr06	Mr04	J1C1 x J1C1	J2C1
Semar-8	1999	6.9	Mr09 x Mr10	GM15	Sw2C7 x Sw2C7	MK 09
Semar-9	1999	6.6	Mr11 x Mr12	GM15	Sw2C7 x Sw2C7	MK 09
Semar-10	2001	7.2	Mr13 x Mr04	Mr14	J1C1 x J2C1	Sw3C7
Bima-1	2001	7.3	Mr04	Mr14	J2C1	Sw3C7
Bima-2 Bantimurung	2007	8.51	B11-209	Mr14.	TAMNET	Sw3C7
Bima-3 Bantimurung	2007	8.27	Nei 9008	Mr14	AMBIONET,Thailand	Sw3C7

[a] Semar is Single cross (SC); Bima is Three-way cross (TWC) hybrids.
[b] GM and Mr = Inbred lines developed in Malang and Maros, respectively.

Tabel 3. Matrix of genetic distance among inbred parents of hybrids Semar-8, Semar-9, Semar-10, and Bima-1. Maros, 2005.

	Mr4	Mr9	Mr10	Mr11	Mr12	Mr13	Mr14	GM15
Mr4	0,00							
Mr9	0,65	0,00						
Mr10	0,60	0,50	0,00					
Mr11	0,55	0,38	0,56	0,00				
Mr12	0,53	0,34	0,48	0,25	0,00			
Mr13	0,62	**0,71**	0,63	**0,78**	0,68	0,00		
Mr14	**0,71**	**0,75**	**0,76**	**0,74**	**0,74**	0,60	0,00	
GM15	0,60	0,51	0,54	0,46	0,49	**0,73**	**0,83**	0,00

Source: Pabendon *et al.,* 2005

Mr14 are not derived from MSJ2, suggested that Mr14 had better combining ability with inbreds B11-209 and Nei 9008 than with Mr4.

To obtain the information of the genetic distance of 8 inbred lines as parents of released hybrids above, a matrix based on the results of molecular analysis was conducted on eight lines using 26 SSR markers (Pabendon *et al.*, 2005). Genetic distance among inbred parent pairs is presented in Table 3. Semar-8 is a three-way cross (TWC) hybrid of ((Mr-9 x Mr-10) x GM-15)). Genetic distance between inbred Mr-9 and Mr-10 was 0.50, between inbred GM-15 and Mr-9 was 0.51, and between inbred GM-15 and Mr-10 was 0.54 (value 1.0 = very far genetic distance).

Semar-9 is a TWC hybrid of ((Mr-11 x Mr-12) x GM-15)). Genetic distance between inbred Mr-11 and Mr-12 was 0.25, between inbred GM-15 and Mr-12 was 0.46, and between inbred GM-15 and Mr-12 was 0.49. This value of 0.25 was agree with the pedigree of both lines Mr11 dan Mr12 which derived from Suwan2C7 population, therefore these two lines had a close genetic relationship.

Semar-10 is a TWC hybrid of ((Mr-13 x Mr-4) x Mr-14)). Genetic distance between inbred Mr-13 and Mr-14 was 0.62, between inbred M-14 and Mr-13 was 0.60, and between inbred Mr-14 and Mr-4 was 0.71. These data showed that Semar 8 and Semar 9 were developed from inbreds possessed a close genetic relationship (genetic distance values 0.25 – 0.54), on the other hand, Semar 10 was developed from inbreds possessed a far genetic relationship (genetic distance values 0.60 – 0.71).

Bima-1 is a single cross (SC) hybrid of (Mr-4 x Mr-14) with genetic distance value between two inbreds was 0.71, suggested that the two lines had a far genetic relationship. This was agree with the fact that Mr4 was derived from J1 population, while Mr14 was derived from Suwan3C7 (one of several lines that formed J2 population).

The highest genetic distance of 0.85 was obtained between inbred M-14 and GM-15, while lowest genetic distance of 0.25 was obtained between inbred Mr-11 and Mr-12. Genetic distance values of the inbreds above were agree with the pedigree of those inbreds (Table 2). There were eight other inbred pairs had genetic distance value relatively high (> 0.70) which give high chance in cross combinations to obtain a good hybrids, such as M-14 x GM-15.

Conclusions

- Inbred parents of hybrids Semar-4 – Semar-7 agreed with the heterotic pattern of MSK1 x MSK2 and MSJ1 x MSJ2.
- Inbred parents of hybrids Semar-8 – Semar-10 and Bima-1 did not agree with the heterotic pattern of MSK1 x MSK2 and MSJ1 x MSJ2.
- Semar 8 and Semar 9 were developed from inbreds possessed a close genetic relationship, while Semar 10 and Bima-1 were developed from inbreds possessed a far genetic relationship.
- Genetic distance values of the inbreds as parents of released hybrids agreed with the pedigree of those inbreds.

Acknowledgements

The authors gratefully acknowledge the IAARD under the Indonesian Department of Agriculture which have been funding this maize MRRS program since 1993.

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Development of improved maize varieties in Indonesia

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Abstract. Maize productivity in Indonesia is relatively low (3.66 ton/ha), although in some provinces, productivity is above 4.0 t/ha. The higher productivity is due to adoption of production technology by farmers, one of which is the use of improved varieties. This paper describes the development of several improved maize varieties released by the Indonesian Agency for Agricultural Research and Development (IAARD). Two open pollinated varieties (OPVs) of normal kernel being used widely by farmers in marginal areas are Lamuru and Sukmaraga. Lamuru was developed for tolerance to drought using a Modified Reciprocal Recurrent Selection (MRRS) breeding method and released in 2000 with grain yield productivity 5.60 t/ha. Sukmaraga was selected in high aluminium saturated soil for tolerance to acid soil and released in 2003 with yield productivity of 6.00 t/ha. Both varieties were resistant to downy mildew. Two quality protein maize (QPM) varieties namely Srikandi Kuning-1 (yellow kernel) and Srikandi Putih-1 (white kernel) were released in 2004 with grain yield productivity of 5.40 and 5.89 t/ha, respectively which is similar to normal maize variety Lamuru (yellow kernel). The lysine and trypthofan concentration of Srikandi Kuning-1 were 0.477% and 0.093%, respectively, which higher thann Lamuru (0.278% and 0.064%). The lysine and trypthofan concentration of Srikandi Putih-1 were less than Srikandi Kuning-1 but higher than normal maize variety Bayu (white kernel). These QPM varieties are being programmed by the government to improve the low nutrition value in some parts of Indonesia, especially in the drought prone areas. Bima-1 was the first single cross hybrid released in 2001 with grain yield productivity of 7.30 t/ha. The inbred parents of this hybrid, Mr14 and Mr4, were developed from MRRS breeding program and become the testers of other inbreds. Bima-2 Bantimurung and Bima-3 Bantimurung, two single cross hybrid maize varieties, use inbred Mr14 as male parent and inbreds B11-209 and Nei 9008 as female parents. These two hybrids were released in 2007 with grain yield productivity 8.51 and 8.27 t/ha, respectively. Inbreds B11-209 and Nei 9008 gave better combining ability with Mr14 than Mr4 and had opportunity to be used as testers replacing Mr4 in hybrid breeding program. MRRS effectively improved grain yield of MSJ2 population.

Key-words: maize, open pollinated, hybrid, improved, varieties

Introduction

Maize in Indonesia is the second important food crops after rice. The annual maize harvested areas is 3.35 million ha and total production is 11-12 million ton. Based on survey results conducted in 1999 and 2000, about 75 - 80% of maize area in Indonesia was grown with improved varieties consisted of 47 - 56% open pollinated varieties and 24 - 28% hybrid varieties, while the rest 20 - 25% of total area was grown with local low yielding varieties (Pingali, 2001; Nugraha et al., 2002). In 2007, maize productivity was relatively low (3.66 ton/ha), although in some provinces, productivity were above 4.0 t/ha (CBS, 2008). The higher productivity is due to adoption of production technology by farmers, one of which is the use of improved varieties both open pollinated as well as hybrid varieties.

Since 1956, more than 100 improved maize varieties have been released in Indonesia, which more than 50% consisted of hybrid maize varieties. Almost all of open pollinated varieties (OPVs) were released by the Indonesian Agency for Agricultural Research and Development (IAARD), under the Indonesian Department of Agriculture. While most of hybrid varieties were released by the multinational private seed companies such as BISI, Pioneer, Monsanto, etc. Earlier, IAARD emphasized more to the development of improved OPVs, while the hybrid maize program at IAARD was started in 1987.

This paper describes the progress of breeding program conducted by the IAARD in the development of improved maize varieties in Indonesia.

Materials and Methods

In 1993, maize breeders at the Malang Research Institute for Food Crops (MARIF), under the IAARD, developed two versions of maize base population pairs as a heterotic pattern namely the late and early maturing version. The late maturing version was Malang Sintetik (MS) J1 versus MS J2, while the early maturing version was MS K1 versus MS K2. These population pairs were improved using a Modified Reciprocal Recurrent Selection (MRRS) breeding method. One cycle of selection consisted of 4 planting seasons or generations with the following steps: 1. Development of S1 lines by selfing each plant, 2. Development of top-crosses by crossing S1 lines with the opposite population as a tester (J1 vs. J2 and K1 vs. K2), 3. Evaluation of top-crosses in replicated trials, and 4. Recombination of the selected lines to produce a new cycle (n + 1) populations. Two cycles of selection were completed (Dahlan et al., 1996). Due to reorganization, this MRRS program was moved in 1995 from MARIF to the Indonesian Cereal Research Institute (ICERI) located in Maros, South Sulawesi..

The MRRS was aimed to obtain several promising lines having a good general combining abilities (GCA) and/or specific combining abilities (SCA). The combining ability of lines derived from MS J1 were evaluated using MS J2 population as a tester, and vice versa. Lines having a good GCA were recombined (intercrossed) to produce a new cycle population or synthetic varieties. Lines having a good SCA were crossed to produce a new hybrid. Therefore, in one breeding program, the OPVs and hybrids were obtained. From each improved population, several new lines were selected in each cycle having better performance than lines derived from the previous cycle. Five cycles of MRRS had been completed in 2003 for J1, J2, K1, and K2 populations. Two inbred lines, Mr4 and Mr14, were derived from MS J2 C1 and MS J1 C1 (Suwan 3 C7) populations and used as female and male parents of Bima 1 hybrid released in 2001. Since then, Mr4 and Mr14 were used as tester parents for other inbreds.

Four populations of cycle fifth from MRRS program namely K1C5, K2C5, J1C5, and J2C5 were tested in five locations along with 12 other populations and two check varieties (Lamuru and Sukmaraga) in 2004. Each genotype was tested in two rows plot of 1.5 m x 5 m, using RCB design with 3 reps.

Results and discussions

Open pollinated maize varieties

Due to limited land availability in Java, the extension of maize growing are directed to outside Java such as Sumatra, Kalimantan, Papua, Nusa Tenggara and Sulawesi.Development of maize area outside Java posses two constrains of land condition namely high soil acidity and low soil fertility as well as drought stress due to low precipitation or rain fall, therefore, OPVs are still suitable for these kinds of lands.

Nine open pollinated varieties (OPVs) were released by the IAARD since the Indonesian Maize Research Institute was moved to Maros, South Sulawesi in 1995. OPVs Palakka, Lamuru, and Gumarang were derived from MS J2C1, MS J2C2, and MS K2C2, respectively, with grain yield potential of 7.6 – 8.0 t/ha and productivity 5.0 – 6.0 t/ha. Lamuru is relatively tolerant to drought condition and being used widely by farmers, while Gumarang is early maturing variety (82 days). Lagaligo, released in 1996, is also tolerant to drought (Table 1). Sukmaraga was released in 2003 and has a good adaptation in high aluminium saturated soil, resistant to downy mildew, and yield productivity of 7.0 t/ha. Sukmaraga was selected in high aluminium saturated soil in South Kalimantan and West Sumatra.

In 2004, two quality protein maize (QPM) varieties were released namely Srikandi Kuning-1 (yellow kernel) and Srikandi Putih-1 (white kernel). Srikandi Kuning-1 and Srikandi Putih-1 showed grain yield productivity of 5.40 and 5.89 t/ha, respectively , while the normal maize varieties Lamuru (yellow kernel) yielded 5.60 (Table 1). Srikandi Kuning-1 and Srikandi Putih-1 showed grain yield potential 7.90 and 8.10 t/ha, respectively, while the normal maize varieties Lamuru and Bayu (white kernel) showed grain yield potential 7.60 and 5.60 t/ha, respectively (Table 2).

Table 1. Characteristics of open pollinated maize varieties released by IAARD since 1996.

No.	Variety	Year of released	Source Population	Yield Potential (t/ha)	Productivity (t/ha)	Maturity (days)	Specific trait
1	Lagaligo	1996	Arjuna	7,50	5,25	90	drought tolerant
2	Gumarang	2000	MS.K2(RRS)C2	8,00	5,00	82	early maturity
3	Kresna	2000	AC(FS)C7	7,00	5,20	90	early maturity
4	Lamuru	2000	MS.J2(RRS)C2	7,60	5,60	95	drought tolerant
5	Palakka	2002	MS.J2(RRS)C1	8,00	6,00	95	early maturity
6	Sukmaraga	2003	AMTL x Local	8,50	6,00	105	Acid soil tolerant
7	Srikandi Kuning-1	2004	S99TLYQ-AB	7,92	5,40	110	QPM, yellow kernel
8	Srikandi Putih-1	2004	S98TLWQ(F/D)	8,09	5,89	110	QPM, white kernel
9	Anoman (white Kernel)	2006	MS2 (Tuxpeno Sequia C6)	6,60	4,60	103	drought tolerant, short plant stature

Table 2. Grain yield potential, concentrations of protein, lysine and trypthofan of QPM varieties Srikandi Kuning-1, Srikandi Putih-1, and normal maize varieties.

Varieties	Grain yield potential (t/ha)	Protein (%)	Lysine (%)	Trypthofan (%)
Srikandi Kuning-1	7.90	10.38	0.477	0.093
Lamuru (yellow kernel)	7.60	8.49	0.278	0.064
Srikandi Putih-1	8.10	10.44	0.410	0.087
Bayu (white kernel)	5.60	-	0.252	0.062

Table 3. Characteristic of hybrid maize varieties released by IAARD since 1992

No.	Variety [a, b]	Year of released	Female parent [c]	Male parent	Yield potential (t/ha)	Produc-tivity(t/ha)	Maturity (days)
1	Semar-1	1992	GM12 x GM19	GM15	9.0	5.3	100
2	Semar-2	1992	GM25 x GM30	GM27	8.0	5.0	91
3	Semar-3	1996	GM26 x GM30	GM15	9.0	5.3	94
4	Semar-4	1999	Mr01 x Mr02	Mr03	8.5	5.9	90
5	Semar-5	1999	Mr05 x Mr06	Mr04	9.0	6.8	98
6	Semar-6	1999	Mr07 x Mr08	Mr04	8.9	6.9	98
7	Semar-7	1999	Mr08 x Mr06	Mr04	9.0	6.8	98
8	Semar-8	1999	Mr09 x Mr10	GM15DMR	9.0	6.9	94
9	Semar-9	1999	Mr11 x Mr12	GM15DMR	8.5	6.6	95
10	Semar-10	2001	Mr13 x Mr04	Mr14	9.0	7.2	97
11	Bima-1	2001	Mr04	Mr14	9.0	7.3	97
12	Bima-2 Bantimurung	2007	B11-209	Mr14.	11.0	8.51	100
13	Bima-3 Bantimurung	2007	Nei 9008	Mr14	10.0	8.27	100

[a] Bima is Single cross (SC); Semar is Three-way cross (TWC) hybrids.
[b] All hybrids are resistant to downey mildew, leaf rust, and leaf spot diseases.
[c] GM and Mr = Inbred lines developed in Malang and Maros, respectively.

The lysine and trypthofan concentrations of Srikandi Kuning-1 were 0.477% and 0.093%, respectively, while Lamuru were 0.278 and 0.064%, respectively. The lysine and trypthofan concentrations of Srikandi Putih-1 were 0.410% and 0.087%, respectively, while Bayu were 0.252 and 0.062%, respectively (Table 2). These QPM varieties are now being programmed by the government to improve the low nutrition value of children in some parts of Indonesia, especially in the drought prone areas.

Hybrid maize varieties

Thirteen hybrid maize varieties had been released since 1992, consisted of 10 “three-way cross” hybrids (Semar-1 – Semar 10) and four “single cross” hybrids (Bima-1 – Bima-3). Eight hybrid varieties (Semar 4 – Semar 10 and Bima 1) were released from the MRRS breeding program. Table 3 showed two single cross hybrid maize varieties, Bima-2 Bantimurung and Bima-3 Bantimurung, which were released in 2007 with grain yield productivity above 8.0 t/ha, while grain yield potential of 11.0 and 10.0 t/ha, respectively. Bima-2 Bantimurung was a single cross hybrid of B11-209 x Mr14, while Bima-3 Bantimurung was a single cross hybrid of Nei 9008 x Mr 14 (Table 3). Since Bima 1 was developed from Mr4 and Mr14, this fact suggested that inbred lines B11-209 and Nei 9008 were better than Mr4 as a combination with Mr14. Therefore, B11-209 and Nei 9008 inbred lines could be used as testers replacing Mr4.

Selection progress

Table 4 showed four populations (K1C5, K2C5, J1C5, and J2C5) derived from MRRS breeding program showed grain yield higher than Lamuru (6.36 t/ha). Among four populations, MSJ2C5 gave highest grain yield of 7.38 t/ha which was 16% higher than Lamuru (derived from MS J2C2) or the selection progress was 339 kg/cycle. This suggested that MRRS effectively improved grain yield of MSJ2 population (Table 4). The four populations of cycle fifth from MRRS program namely are being improved for next cycle of selection. Selection progress is depend on genetic variation of base population in RSS. Moll dan Hanson (1984) obtained 24% yield increased on Jarvis population

Table 4. Grain yield of open pollinated maize genotypes average of five locations during 2004

No.	Populations	Grain yield (t/ha)	No.	Populations	Grainyield (t/ha)
1	MS.K1(RRS)C5	6.83	10	MS.BK(S1)C1	6.58
2	MS.K2(RRS)C5	7.06	11	MS.BK(HS)C1	6.45
3	MS.J1(RRS)C5	7.14	12	Bisma(S1)C1	6.75
4	**MS.J2(RRS)C5**	7.38	13	Bisma(S2)C1	6.56
5	SATP-1(S2)C6	6.69	14	SA4-1(S1)C1	5.22
6	SATP-2(S2)C6	7.66	15	MS.QP-1	7.06
7	AMATL(S1)C3	7.42	16	MS.QK-1	6.72
8	MS.HK(S1)C3	7.22	17	**Lamuru =J2C2**	6.36
9	MS.KH(S1)C3	6.57	18	**Sukmaraga**	6.59

selected for 10 cycles of RSS on Jarvis and Indian Chief populations. Keeratinijakal and Lamkey (1993) conducted a RSS on Iowa Stiff Stalk Synthetic (BSSS, R) and Iowa Corn Borer Synthetic M1 (BSCB1, R) populations for 11 cycles and obtained yield increased on BSCB of 1.94% (0.06 t/ha = 60 kg/ha) per cycle, while there was no change observed on BSSS population.

Conclusions

- B11-209 and Nei 9008, female parents of hybrids Bima-2 and Bima-3, gave better combining ability with Mr14 than Mr4 and had opportunity to be used as testers replacing Mr4 in hybrid breeding program.
- MRRS effectively improved grain yield of MSJ2 population.
- OPVs are still needed especially for area outside Java having high soil acidity, low soil fertility, and drought stress due to low precipitation or rain fall.
- Development of QPM varieties is needed to improve the low nutrition value of children in some parts of Indonesia, especially in the drought prone areas.

Acknowledgements

Special thanks to IAARD under the Indonesian Department of Agriculture which have been funding this maize MRRS program since 1993.

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Combining ability analysis for yield and yield components among Yunnan local maize cultivars and germplasm from CIMMYT

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Abstract. Lack of new genetic resources is a major obstacle to improve maize hybrid yield all over the world. Introduction of new maize germplasm is primary method to improve local cultivars and to enhance hybrid yield to a new higher level. Maize yield is composed of yield components. Different combinations of the yield components may result in different yield in a maize inbred line or a hybrid. This study was designed to study combining ability of yield and yield components between Yunnan local cultivars and the germplasm from CIMMYT. Five local maize cultivars and five CIMMYT germplasm were chosen to make 45 crosses according to Griffing IV mating design. The crosses had been tested in three different locations where quite different weather conditions were recorded. The results had showed that Yunnan local cultivar Tengchong Yellow Waxy Corn had the highest positive average general combining ability (GCA) effect for grain yield, followed by Suwan1, Menghai Waxy Corn, Baoshan Waxy Corn, Pob32, and Pob28. The significantly positive Specific combing ability (SCA) effects were observed in the 13 crosses between CIMMYT germplasm and Yunnan local maize cultivars, indicating different genetic background of CIMMYT germplasm from our local maize varieties. The GCA effects of a same germplasm or cultivar might be quite different in different environments. Thus, the GCA and SCA information for any maize germplasm from other studies could only be used as reference. To fully explore yield potential, both GCA and SCA should be considered in a maize breeding program.

Key words: Maize germplasm introduction, yield component, CIMMYT, General combining ability (GCA), Specific combining ability (SCA) analysis; exotic maize germplasm

Introduction

Though there are about 2000 local maize germplasm collected in Yunnan Province which accounts for 17.5% of total maize germplasm in China[1-2]. These germplasm are generally not employed in commercial maize breeding programms due to their low yield potential and poor agronomic performance. Studied showed that introduction of exotic germplasm was an effective way to improve local maize varieties[3-4]. There are more than 23,000 germplasm maintained at the Maize Diversity Center in CIMMYT[5]. These germplasm have diverse genetic bases and posses valuable agronomic traits such as strong root system, tolerate to high temperature, staying green in late growing season, good plant type, better yield components and high yield potential, etc. which are lack in Yunnan local germplasm.

Perez-velasquez et al. evaluated combining ability between Colombia local cultivars and populations Suwan1, ETO and Tuxpeno from CIMMYT by diallel design[6]. They had found that two crosses, Yucatan × Tuxpeno and Yucatan × Suwan1 showed the highest yield and combing ability among all cross combinations they made. The local cultivar, Yucatan, had shown to be a promising new germplasm for tropical maize breeding program. Crossa et al. assessed yield combining ability among 25 Mexican local cultivars[7]. Their results showed that Cacahuacintle, Harinoso de Ocho and Maize Dulce were the three best local cultivars in yield and their combining ability effects. Radovic and Jelovac had made crosses between 126 Yugoslavian local cultivars and three exotic maize germplasm Mo17, B73 and V395/31[8]. Their results showed that 30, 70 and 84 out of 126 local cultivars have high combining ability with the three exotic maize germplasm, respectively. Li et al. assessed combining abilities of 10 exotic germplasm from tropical & subtropical regions and Chinese domestic elite inbred lines[9]. They found that populations Pob Stay-Green, Pob43 and Pob21 had high yield GCA and had great potential to be used for commercial maize hybrid breeding program. Fan et al. studied combining abilities between 25 inbred lines selected from five major tropical, subtropical populations and landraces and 4 temperate elite inbred lines representing four main heterotic groups (Tangsipingtou, Ludahonggu, Reid and Lancaster) in northern China[4, 10-11]. They had found significant SCA from the 100 crosses, suggesting great potential of using exotic tropical and subtropical maize germplasm in commercial maize breeding program.

Maize yield highly depends on yield components [12]. Any changes in environment conditions such as water supply, plant density, etc. may influence the yield components, which, in turn, will affect the final yield of a plant[13-14].

Thought diallel analysis is widely used for maize yield[3, 10-11, 15-17], only a few diallel analyses for both yield and yield component had been reported [15-16]. No studies have been reported on combining ability for maize yield and yield component traits between Yunnan local cultivars and germplasm from CIMMYT. The objectives of this study were (i) to estimate combining ability of yield and yield components via diallel mating design between the five Yunnan local cultivars and five exotic germplasm from CIMMYT and (ii) to assess the importance of exotic germplasm from CIMMYT for local maize germplasm improvement and enhancement.

Materials and Methods

Five Yunnan local cultivars were provided by the Yunnan Academy of Agricultural Sciences (YAAS) and five exotic tropical and subtropical germplasm were introduced from CIMMYT. The pedigree data and major agronomic characteristics of these 10 germplasm are listed in Table 1. The selected maize materials represented major germplasm types from both Yunnan and CIMMYT. The local cultivar, Menghai waxy corn (MWC), is a rare germplasm since it only has four-kernel rows per ear and still maintained primitive traits.

Forty five crosses were made among the 10 maize germplasm according to Griffing diallel Method IV[18]. During flowering time, pollen collected from 100 individual plants in one material were bulked and used to pollinate at least 50 ears in another material. The F_1 plants of the 45 crosses were evaluated in three different locations in Yunnan province in 2004 (Kunming, altitude 1960 m, longitude 120°452 , latitude 25°022 , Baoshan, altitude 1870 m, longitude 90°02 , latitude 24°552 , and Lincang, altitude 1750 m, longitude 100°022 , latitude 23°422). Based on historical data, the average total precipitations during the maize growing season (between May and September) in Kunming, Baoshan, and Lincang were 800, 700, and 900 mm, respectively. The average temperatures during the growing season in the three locations are 19 ^{o}C, 20 ^{o}C, and 21 ^{o}C, respectively. The purpose to select these three locations with such weather difference was to test if the 10 germplasm selected for the diallel experiment have different GCA and SCA effects in different environments. Randomized complete block design with three replications in each location was employed for the experiment. The experimental plot consisted of two 5 meter rows with inter-row distance at 0.75 meter and with 22 plants per row. The overall plant density was approximately 60000 plants ha^{-1}. At harvest time, equal amount of F_1 seeds from each ear in one cross were harvested and combined. Ten plants grown in the middle of each row were sampled and the following traits were recorded for each cross at each location: yield per plant (Yield), ear length (EL), ear diameter (ED), number of rows per ear (R/E), number of kernels per row (K/R), thousand-kernel weight (TKW) and shelling percentage (SP).

The following statistical model was employed for the data analysis.

$$
\begin{aligned}
Y_{ijkl} &= \mu + \alpha_l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijkl} \\
v_{ij} &= g_i + g_j + s_{ij}
\end{aligned} \qquad (1)
$$

where Y_{ijkl} = observed value from each experimental unit; μ=population mean; $á_l$ = location effect; b_{kl} = block or replication effect within each location; v_{ij} = F_1 hybrid effect

Table 1 Germplasm resources and their main agronomic characteristics used in the study

No.	Local cultivars/ Populations	Germplasm resource	Grain Texture	Grain Color	Ecology adaptation	Latitude	Altitude (m)
1	Pob32	Maize diversity center	Flint	White	Subtropical	20°322	60.0
2	Pob28	Maize diversity center	Dent	Yellow	Tropical	20°322	60.0
3	Suwan1	Maize diversity center (Thailand)	Flint	Yellow	Tropical	14°042	300.0
4	Pob401C_2	Maize diversity center	Flint	White	Subtropical	18°002	940.0
5	Pob402C_2	Maize diversity center	Flint	White	Subtropical	18°002	940.0
6	Zhaotong Waxy Corn	Zhaotong,Yunnan	Flint	Yellow	Subtropical	27°202	1949.5
7	Tengchong Yellow Waxy Corn	Tengchong,Yunnan	Dent	Yellow	Subtropical	25°072	1647.8
8	Menghai Waxy Corn	Menghai,Yunnan	Flint	White	Subtropical	21°552	1176.3
9	Qiaojia White Waxy Corn	Qiaojia,Yunnan	Flint	White	Subtropical	26°532	840.7
10	Baoshan Waxy Corn	Baoshan,Yunnan	Flint	Yellow	Subtropical	25°082	1653.5

$= g_i + g_j + s_{ij}$ (where g_i = general combining ability (GCA) for the ith parent; g_j = GCA effect of j^{th} parent; s_{ij}= specific combining ability (SCA) for the ij^{th} F_1 hybrid); $(áv)_{ijl}$ = interaction effect between ij^{th} F_1 hybrid and location; e_{ijkl} = random residual effect. Since the environments in the three locations are quite different and were selected to test how environment affect on GCA and SCA effects, the location will be considered as fixed factor. Thus all significant tests in the model will be against experimental error.

General Linear Model described in (1) was used for statistical analysis by SAS GLM procedure[19]. A trait would not be analyzed for combining ability unless the difference of a trait being statistically significant among crosses. Griffing's Method IV[18] was employed to analyze GCA and SCA. A special SAS software tool for diallel analysis developed by Zhang, et al. was used for the analysis of GCA effects, SCA effects and their interaction effects with locations[20].

Results and Analysis

The analysis of variance (Table 2) showed that the variances for all tested traits were highly significant in locations, crosses and cross x location interaction. Since the variance of crosses was significant, it was further partitioned into the variance of GCA for parental lines and the variance of SCA of the crosses. The results showed that both GCA and SCA effects on the yield and all yield components were significantly different.

The average GCA effects of the 10 parents across the three locations for yield were listed in Table 3. The germplasm with significantly positive average GCA effects for yield per plant were Tengchong Yellow Waxy Corn (TYWC), Suwan 1, Menhai Waxy Corn (MWC), Baoshan Waxy Corn (BWC), Pob32 and Pob28ÿWhereas those with significantly negative average GCA effects for yield were Zhaotong Waxy Corn (ZWC), Pob401C_2, Qiaojia White Waxy Corn (QWWC) and Pob402C_2.

Table 2 Variance analyses of 45 crosses for main agronomic traitsÿEL= ear length, ED=ear diameter, R/E= kernel row per ear, K/R=kernel no. per row, TKW=Thousand kernel weight, SP = shelling percentage

Sources	D.F	MS						
		Yield	EL	ED	R/E	K/R	TKW	SP
Rep.	6	879.63**	2.86**	0.08**	0.50*	7.41**	632.36	3.09
Env.	2	185903.98**	29.36**	3.24**	94.04**	861.51**	19368.69**	923.95**
Crosses	44	2540.77**	13.15**	0.68**	11.00**	51.26**	4607.87**	44.17**
GCA	9	4872.38**	34.54**	2.33**	35.91**	95.91**	7881.54**	117.36**
SCA	35	1941.21**	7.65**	0.26**	4.60**	39.77**	3766.07**	25.36**
Crosses×Env.	88	1631.85**	6.66**	0.18**	1.37**	31.75**	2314.13**	16.91**
GCA×Env.	18	4682.78**	15.78**	0.29**	1.42**	61.86**	4899.84**	33.60**
SCA×Env.	70	847.33**	4.31**	0.15**	1.35**	24.01**	1649.23**	12.62**
Error	264	145.00	0.46	0.01	0.18	2.38	434.42	1.59

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 3 Estimates of GCA effects for main agronomic traits

Local cultivars/Populations	Yield	EL	ED	R/E	K/R	TKW	SP
Pob32	4.21**	0.32**	0.12**	0.36**	0.58**	-2.51	-1.68**
Pob28	3.62**	-0.27**	0.12**	0.45**	0.96**	-20.62**	-1.87**
Suwan1	6.77**	0.32**	0.22**	0.79**	0.20	-1.88	-0.28
Pob401C_2	-6.61**	0.04	0.04**	0.73**	0.06	-10.54**	0.09
Pob402C_2	-14.29**	-1.10**	-0.09**	-0.69**	-1.22**	-3.20	-1.01**
Zhaotong Waxy Corn	-5.53**	-1.26**	-0.20**	0.002	-2.59**	9.73**	2.26**
Tengchong Yellow Waxy Corn	10.28**	0.42**	0.21**	0.29*	1.17**	11.53**	0.62**
Menghai Waxy Corn	5.59**	0.39**	-0.28**	-1.34**	0.04	13.93**	0.24
Qiaojia White Waxy Corn	-9.40**	0.23**	-0.19**	-0.78**	-0.23	1.22	1.23**
Baoshan Waxy Corn	5.36**	0.92**	0.05**	0.19**	1.03**	2.34	0.27
LSD0.05	2.65	0.15	0.02	0.09	0.34	4.59	0.28
LSD0.01	3.49	0.20	0.03	0.12	0.45	6.05	0.37

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

The GCA effects of the 10 parents for yield components were also listed in table 3. The results showed that the Suwan1 (0.22**), TYWC(0.21**), Pob32(0.12**), Pob28(0.12**), and BWC(0.05**) had significantly positive GCA effect on ED, while 3 out of 5 local varieties showed high significantly negative GCA effect on ED. For yield component R/E, Suwan1 (0.79**), Pob401C_2 (0.73**), Pob28 (0.45**), and Pob32 (0.36**) had higher GCA effects than all local varieties. With yield components TKW and SP, local varieties seemed to have higher GCA effect than exotic germplasm. For other two yield components, EL and K/R, some local and exotic germplasm had high and some had low GCA effects. These results clearly indicated that exotic CIMMYT germplasm had some good yield components such as ED and R/E that could be used for yield improvement of Yunnan local varieties.

The SCA effects for all traits were listed in Table 4. The crosses with significantly positive SCA effects for yield across locations were Pob28 × ZWC (30.24**),

Table 4 Estimates of SCA effects of 45 crosses for main agronomic traits

Crosses	Yield	EL	ED	R/E	K/R	TKW	SP
1×2	8.40*	-0.41*	-0.008	0.58**	-1.37**	-5.01	1.57**
1×3	11.87**	0.44*	0.089**	0.68**	1.89**	3.03	0.61**
1×4	-5.00	0.87**	0.078*	-0.25*	0.98*	-13.78*	-1.80**
1×5	-13.90**	-1.05**	0.147**	0.43**	-1.25**	-0.23	0.10
1×6	9.00*	-0.36	0.003	-0.21	-0.98*	20.69**	0.00
1×7	0.18	0.35	-0.160**	-0.77**	-0.13	6.60	0.35
1×8	-6.97	-0.08	0.144**	-0.46**	-1.71**	23.62**	0.10
1×9	-14.50**	-0.16	-0.243**	-0.35**	0.31	-18.21**	-0.11
1×10	10.92**	0.41*	-0.050	0.35**	2.27**	-16.71**	0.17
2×3	-10.51**	-0.58**	0.108**	-0.18	-0.91**	16.88**	-0.32
2×4	-7.99*	-0.58**	-0.036	-0.63**	-2.31**	-2.55	4.73 **
2×5	-22.52**	-0.63**	-0.300**	-0.59**	-1.77**	-36.67**	-4.55 **
2×6	30.24**	1.58**	0.133**	0.41**	3.62**	1.06	-0.40
2×7	5.42	0.23	-0.040	-0.17	0.64	15.93**	-0.92 **
2×8	-13.76**	-0.20	-0.047	0.52**	-1.14*	-18.47**	-1.68 **
2×9	6.15	-0.09	0.288**	0.24	1.43**	19.91**	1.88 **
2×10	4.56	0.69**	-0.097**	-0.18	1.82**	8.92	0.68 **
3×4	-16.20**	-1.51**	-0.094**	-0.26*	-2.31**	-9.19	-2.37 **
3×5	1.86	0.26	0.042	0.44**	-0.05	-12.68*	0.37
3×6	-2.07	-0.05	-0.158**	-0.93**	0.81	-0.24	0.40
3×7	-11.65**	-0.46*	-0.043	0.15	-2.12**	-11.79	-2.23 **
3×8	20.48**	1.00**	0.106**	0.38**	0.62	13.45*	1.67 **
3×9	-0.11	1.18**	-0.082**	-0.07	1.13*-	-10.51	1.32 **
3×10	6.34	-0.29	0.033	-0.21	0.94*	11.04	0.54
4×5	-14.41**	-1.38**	-0.336**	-1.09**	-4.27**	5.92	-1.32**
4×6	-6.43	-0.29	-0.014	-0.25*	0.04	-0.68	-0.80 **
4×7	16.12**	0.72**	0.090**	0.68**	2.63**	-3.48	1.66 **
4×8	9.08*	1.13**	-0.094**	-0.09	3.06**	3.79	1.07 **
4×9	11.11**	0.43*	0.218**	-0.03	0.24	32.38**	0.35
4×10	13.71**	0.61**	0.189**	1.92**	1.92**	-12.41*	-1.52 **
5×6	-15.26**	-0.33	-0.100**	-0.45**	-1.37**	2.86	1.07 **
5×7	22.89**	1.01**	0.149**	0.97**	2.68**	-18.82**	0.62 **
5×8	3.48	0.89**	0.042	-0.09	2.63**	12.56*	1.29 **
5×9	16.50**	0.68**	0.221**	0.53**	1.15*	22.48**	-0.22
5×10	21.36**	0.54**	0.136**	-0.15	2.25**	24.58**	2.63 **
6×7	2.15	0.41*	0.082**	0.15	-0.05	15.24*	0.93 **
6×8	-3.29	0.60**	-0.003	1.09**	1.77**	-29.69**	-0.61 **
6×9	5.99	-0.65**	0.032	-0.27*	-2.00**	3.88	0.19
6×10	-20.32**	-0.91**	0.025	0.47**	-1.83**	-13.13*	-0.79 **
7×8	5.28	-0.46*	-0.021	-0.60**	-0.67	42.71**	-0.76 **
7×9	-21.40**	-1.65**	-0.286**	-0.46**	-0.74	-33.03**	-0.44 *
7×10	-18.98**	-0.15	0.229**	0.05	-2.23**	-13.37*	0.78 **
8×9	-0.22	-0.86**	0.096**	0.95**	-0.48	-37.98**	-0.78 **
8×10	-14.07**	-2.02**	-0.222**	-1.70**	-4.09**	-9.99	-0.31
9×10	-3.52	1.12**	-0.243**	-0.55**	-1.04*	21.07*	-2.19 **
LSD0.05	6.97	0.39	0.06	0.24	0.89	12.06	0.41
LSD0.01	9.18	0.52	0.08	0.32	1.18	15.90	0.54

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Pob402C_2 × TYWC (22.89**), Suwan1 x MWC (20.48**), Pob402C_2 × BWC (21.36**), Pob402C_2 × QWWC(16.50**), Pob401C_2 × TYWC (16.12**), Pob401C_1 x BWC(13.71**), Pob32 × Suwan1 (11.87**), Pob401C_2 × QWWC(11.11**), and Pob32 × BWC(10.92**), Pob401C_2 x MWC(9.08*), Pob32 x ZWC(9.00*), and Pob32 x Pob28 (8.40*). The crosses with significantly negative SCA effects for yield were Pob28 × Pob402C_2 (-22.52**), TYWC × QWWC(-21.04**), ZWC × BWC(-20.32**), TYWC x BWC(-18.98**), Suwan1 × Pob401C_2 (-16.20**), Pob402C_2 × ZWC(-15.26**), Pob32 x QWWC(-14.50**), Pob401C_2 x Pob402C_2 (-14.41**), MWC × BWC (-14.07**), Pob32 × Pob402C_2 (-13.90**), Pob28 x MWC(-13.76**), Suwan1 x TYWC(-11.65**), Pob28 × Suwan1 (-10.51**), and Pob28 x Pob401C_2(-7.99*).

Table 5 GCA effects of yield in three locations

No.	Local cultivars/ Populations	Kunming	Baoshan	Lincang
1	Pob32	-10.05**	8.50**	14.17**
2	Pob28	-10.02**	1.48	19.39**
3	Suwan1	-0.45	1.38	19.39**
4	Pob401C_2	-12.19**	2.90	-10.54**
5	Pob402C_2	-19.72**	-0.95	-22.18**
6	Zhaotong Waxy Corn	10.06**	-2.29	-24.35**
7	Tengchong Yellow Waxy Corn	11.31**	6.13*	13.41**
8	Menghai Waxy Corn	17.04**	-6.60*	6.32**
9	Qiaojia White Waxy Corn	10.41**	-5.91*	-32.71**
10	Baoshan Waxy Corn Corn	3.61	-4.64	17.10**
	LSD0.05	5.12	5.82	2.06
	LSD0.01	7.64	7.71	2.73

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Since locations were treated as fixed factor, the GCA effects of the 10 maize materials and SCA effects of the 45 crosses among them for yield in the three locations were analyzed and listed in Table 5 and Table 6, respectively. The results in Table 5 showed that GCA effects of yield in the three locations were quite different. For example, Pob32 (-10.05**) and Pob28 (-10.02**) had significantly negative GCA effects in Kumming, whereas they (14.17** and 19.39**, respectively) had significantly positive GCA effects in Lincang. The GCA effects of ZWC (10.06**) and QWWC (10.41**) were highly positive in Kumming, but they were significantly negative in Lincang (-24.35** and -32.71**, respectively).

The yield SCA effects of the 45 crosses in the three locations were listed in Table 6. The results indicated that

Table 6 - SCA effects for yield of all crosses in the three locations

Crosses	Kunming	Baoshan	Lincang	crosses	Kunming	Baoshan	Lincang
1×2	-13.80*	-2.93	41.92**	3×10	-3.93	12.32	10.62**
1×3	8.86	22.51**	4.25	4×5	1.08	-14.89	-29.44**
1×4	6.81	-13.01	-8.79**	4×6	-13.1	-5.89	-0.3
1×5	-11.73	-4.82	-25.15**	4×7	13.02	7.2	28.14**
1×6	6.12	20.51**	0.36	4×8	-16.58*	12.76	31.07**
1×7	-13.66*	-3.91	18.10**	4×9	2.85	18.74*	11.76**
1×8	5.41	0.82	-27.14**	4×10	18.55**	-1.03	23.61**
1×9	2.97	-37.37**	-9.12**	5×6	-7.47	-17.37*	-20.96**
1×10	9.01	18.20*	5.57*	5×7	19.85**	20.05*	28.78**
2×3	-11.64	-7.8	-12.10**	5×8	-0.25	-1.05	11.74**
2×4	-4.59	8.34	-27.70**	5×9	4.68	14.93	29.90**
2×5	-13.23	-15.80*	-38.53**	5×10	5.58	20.32**	38.19**
2×6	46.22**	2.86	41.64**	6×7	-2.56	0.55	8.45**
2×7	6.11	4.45	5.71*	6×8	-12.56	5.95	-3.25
2×8	-12.76	4.18	-32.69**	6×9	-9.17	13.93	13.20**
2×9	8.57	2.16	7.73**	6×10	-7.7	-14.68	-38.58**
2×10	-4.89	4.55	14.02**	7×8	24.19**	-14.8	6.45*
3×4	-8.03	-12.22	-28.34**	7×9	0.49	-23.49**	-41.19**
3×5	1.47	-1.37	5.47*	7×10	-16.55*	4.74	-45.14**
3×6	0.22	-5.87	-0.56	8×9	-20.85**	30.24**	-10.06**
3×7	-30.89**	5.22	-9.29**	8×10	-7.95	-30.37**	-3.91
3×8	41.34**	-7.72	27.80**	9×10	7.88	-14.05	-4.38
3×9	2.57	-5.07	2.16				
LSD0.05	13.47	15.31	5.42	LSD0.01	17.85	20.28	7.19

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

the SCA effects, similar as GCA effects, could be significantly positive in one location, but be significantly negative in other locations or vise visa. The GCA effects of the 10 maize materials and the SCA effects of the 45 crosses for yield component traits were also quite different in the three locations (data not shown). These results strongly indicated that the GCA effects of a germplasm estimated from other studies, thought very useful, could only be used as reference, an exotic germplasm should be tested locally for their GCA effects and for their SCA effects with local germplasm before they could be used in local maize breeding programs.

The GCA effects of yield and the GCA effects of yield components should be generally agreed since yield is composed by yield components. The results in Table 3 showed that a germplasm with highly positive GCA effect for yield was generally had highly positive GCA effects on all (i.e. TYWC) or on most (i.e. BWC and Suwan1) of the yield components. The similar phenomenon was observed for the germplasm with highly negative GCA effects. For instance, Pob402C_2 had highly negative GCA effects and its 6 yield components had highly negative GCA effects. However, there were the situations that highly negative or highly positive yield GCA effect of a germplasm might be caused by only one or two yield components' GCA effects. For example, MWC had highly significantly positive GCA effect for yield, which was mainly credited to highly positive GCA effects of TKW and EL of the variety. And Pob401C_2 had highly significantly negative GCA effect for yield, which was mainly caused by highly negative GCA effect of TKW of the germplasm. This grain yield and yield component GCA information may be very useful guiding a maize breeding program. For example, based on the yield and yield component information on Pob401C_2, if its yield component TKW can be improved, we may obtain a super inbred line with much better yield GCA effect. If we want to make a high yield hybrid by using Pob401C_2 , we may have to find anther inbred line with positive GCA effect on TKW. Thus the studies of GCA effects of yield components might be very useful way for finding information to guide our breeding program for either inbred line improvement or make super high yield hybrid.

The SCA effects of the 45 crosses were listed in table 4. Similar phenomenon for SCA effect as in GCA effects had been found. If a cross had highly positive SCA effect for yield, they usually had all or most of yield component SCA effects being positive, such as the crosses of Pob28 x ZWC, Suwan1 x MWC, Pob402C_2 x BWC, Pob402C_2 x QWWC, and so on. Similarly, if a cross had highly significantly negative SCA effect for yield, all or most of their yield components would have negative SCA effects, which can be seen in the crosses of Pob28 x Pob402C_2, Suwan1 x Pob401C_2, Pob28 x MWC, Suwan1 x TYWC, Pob401C_2 x Pob402C_2, TYWC x QWWC, and MWC x BWC. However, a significantly positive or negative SCA effect in a cross might be caused by only the SCA effects of one or two yield components. For example, the SCA effects of ED (0.108**), and TKW (16.88**) were highly positive for the cross of Pob28 x Suwan1, but due to the highly negative SCA effects of EL (-0.58**) and K/R (-0.91**), the yield SCA effect for the cross turned to be significantly negative (-10.51**). In the cross of Pob32 x Pob28, the positive SCA effects of R/E (0.58**) and SP (1.57**) brought the yield SCA effect of the cross to be significantly positive (8.40**) thought the SCA effects of EL (-0.41*) and K/R (-1.37**) were significantly negative. These results suggested that specific combinations, which determine gene and gene interaction mode for yield, of yield components in a cross were the key to affect SCA effects of yield for that cross.

The SCA effects of the 45 crosses in the three individual locations were calculated and listed in table 6. As we found in GCA effect analysis, the SCA effects for a cross were usually consistent either in positive or in negative. However, there were many crosses showing inconsistent, which means the SCA effects in the three locations might be quite different either in direction (positive or negative) or size or both. For example, the yield SCA effect of Pob32 x Pob28 was significantly negative in Kumming (-13.80**), whereas it was significantly positive in Lincang (41.92**). The similar phenomenon had been observed in the crosses of Pob32 x TYWC, Pob401C_2 x MWC, and MWC x QWWC. These results further suggested that the yield potential of a cross could only be tested in a local environment condition since the yield SCA effect in one location might be quite different from those in other locations.

Discussion and Conclusions

Introduction of exotic maize germplasm had been approved to be an effective way to improve local maize in both domestic and international maize production. Several researchers had showed that genetic background of Chinese domestic maize germplasm was pretty narrow, therefore hybrid yield did not get apparent improvement in recent years in China due to lack of new genes for yield and for resistant to certain wide spread of diseases, insects, and abiotic stresses[21-23]. By using American maize germplasm, subtropical and tropical germplasm as well as domestic southwestern mountainous germplasm, Huazhong Agriculture University of China had developed four novel maize populations with a broad genetic diversity. This population had shown a promising potential for improving maize yield and other key agronomic traits in their maize breeding programs[24-25].

Our studies showed that the GCA effects of the introduced CIMMYT germplasm were much different in maize yield and yield components from our local maize varieties. Although the GCA effects of CIMMYT germplasm may not be much better than some of the local maize varieties (table 3), the most of the crosses (11 out of 13) with highly positive SCA effects were the crosses between introduced CIMMYT germplasm and local maize varieties (table 4). No one cross had significantly positive, but high negative, SCA effects among local maize varieties. If we order the yield of all 45 crosses from highest to lowest, in the top 10 crosses, only one of them was the cross among local maize varieties (data not shown). These results strongly suggested genetic narrowness among local maize germplasm and that the genetic bases of exotic CIMMYT were much different from the local maize varieties. The chance to obtain high yield hybrids by crossing local maize varieties with introduced exotic maize germplasm would be much higher than by crossing among the local varieties.

Grain yield is considered to be highly polygenic and strongly influenced by the environment. Veldboom and Lee compared the QTL for grain yield and yield components detected in two diverse environments with an elite and adapted maize population[26]. They found that about 50% of the QTLs were detected only in one or another environment. Betran et al. studied grain yield under stress and nonstress environment with a diallel design[27]. They had found that there was significant interaction for combining ability under low and high nitrogen application and the type of gene action appeared to be different under drought than under low nitrogen application with additive effects more important under drought and dominance effects more important under low nitrogen application. We had tested the 45 crosses in three different locations which had different environmental conditions in terms of longitude, latitude, temperature, rainfall, etc. We had found that both GCA effects and SCA effects were different for most of parent lines and most of the crosses. This difference could be due to soil fertility or other soil properties or due to different weather conditions such as rain fall, temperature, etc. during the maize growing season. Our research were generally consistent with previous other studies and suggested that to utilize exotic maize germplasm to obtain adapted high yield maize hybrid for a specific location (a nation or a province), a local test for their GCA effects and for the SCA effects with local varieties should be done before they can be effectively employed in a local breeding program.

Theoretically, estimates of additive (σ_A^2) and dominance (σ_D^2) variances can not be done for any diallel experiment with fixed model[17]. However, Baker (1978) had shown that 2GCA MS/ (2GCA MS + SCA MS) ratio (GSR) could be used to indicate weather additive or non-additive gene effects were more important for controlling a trait[28]. We calculated the GSR for the yield and all yield components. The GSRs for the EL, ED, R/E, K/R, TKW, and SP were 0.83, 0.90, 0.95, 0.94, 0.83, 0.81, and 0.83, respectively, indicating that both additive and non-additive genes play role for these traits studied and with additive genes being more important than non-additive genes. This result suggested that the both GCA and SCA effects for yield components should be considered in a breeding program aiming at high grain yield and individual yield component can be improved by accumulating more additive genes in an inbred line. How can we combine different better yield components into one hybrid? We all knew the famous cross between B73 (Reid Yellow Dent) and Mo17 (Lancaster Sure Crop). The Mo17 has longer EL but small ED, whereas B73 has large ED and short EL. The hybrid between B73 and Mo17 has ear with B73's ED and with Mo17's EL, which produced a super hybrid. If we look at the table 3, we may see that GCA effects between CIMMYT germplasm Pob28 and local variety ZWC are perfectly complemented each other. Their cross got second highest yield (data not shown) in the experiment. However, though local variety TYWC had better GCA effects for most of yield components than ZWC, its cross with Pob28 had lower yield than the cross between Pob28 and ZWC due to much lower SCA effects. This result suggested the importance of non-additive gene effect in determining a cross's final yield. It also indicated that we might not be able to predict a cross's yield only based on the GCA values of the yield components in any two inbred lines due to non-additive gene action and location of additive genes in each inbred line.

Based on the GCA effect size, we may find some of the maize germplasm that can be used for improving different maize yield components, which in turn will improve maize yield. For example, for improving K/R, TYWC, BWC, Pob32, and Pob28 should be selected; to improve R/E, Suwan1, and Pob401C$_2$ should be the best choices.

In conclusion, this study had shown that introduced germplasm from CIMMYT had better GCA effects on some of the yield components. Their genetic base was different from our local maize varieties. Thus these exotic germplasm should be employed in our future maize breeding program. This study also shown that environment can cause difference of GCA and SCA effects. The results from other studies generally should be used as reference when an exotic maize germplasm to be used in local breeding program. Furthermore, a cross's yield can not be predicted based only on GCA effect of any two inbred lines due to non-additive genes and the interaction between additive and non-additive genes controlling a yield component. Thus, any exotic maize germplasm to be used for improving local varieties should be tested in target location first.

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Effect of sun drying on maize seed quality

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Abstract. Two experiments were conducted in Bontobili Experimental Farm, laboratory and greenhouse of Indonesian Cereal Research Institute (ICERI) from 2005 to 2007. In the first year (2005-2006) the research was conducted to evaluate the effect of sun drying to maize seed quality on concrete floor with and without plastic sheet layer. Four maize seed varieties; MS-2, Lamuru, Srikandi Kuning and Srikandi Putih were used. In the second year (2006-2007) the research was conducted to evaluate the effect of postponed cob drying after harvest to maize seed quality. Two maize varieties; Lamuru and Srikandi Kuning were used. Data observed were: germination percentage, seedling dry weight, seed moisture content, germination rate, electric conductivity of leakage and potassium leakage. Initial observation were done directly after drying treatments at harvest time and at the period of 2, 4, 6, 8, 10, 12 months after storage in room temperature (28-32^{o}C) in Maros. The first year research showed that drying on concrete floor with plastic sheet layer showed higher seed quality in term of storability than drying on concrete floor without plastic sheet layer. The second year research showed that postponement of cob drying, especially until 9 days significantly decreased maize seed quality.

Key-words : maize, quality, seed, sun drying, concrete floor , postponed

Introduction

Proper drying is considered the biggest single factor in determining whether grain will be effectively stored without damage. Usually grain is dried while it stands in the fields, or it is spread out on concrete surfaces, roads, baskets, plastic sheets, or the ground itself.The basic methods used for drying maize seed include: a) natural drying of seed on the cob prior to harvest while plants are still standing in the field, b) sun drying of seed on the cob following harvest, and c) artificial drying (Agrawal et al., 1998). Natural drying is risky as the seed may be exposed to numerous undesirable elements including temperature extremes, rainfall, pests, etc. Sun drying involves spreading cobs on a drying floor and exposing them to the sun for certain periods (Agrawal et al., 1998). Although more labor-intensive than natural field drying, this method represents an improvement over natural drying in that it allows the seed to be covered in the event of unexpected rainfall. However, sun drying is still subject to the vagaries of the weather and extended periods of cool, cloudy, or humid weather can significantly impede the drying process. In a study conducted in Guatemala with the maize variety ICTA B-5, researchers compared drying rates over five days between a sun drying and seed dried either on concrete patios or on black plastic. Results showed that the sun drying reduced seed moisture content by 7.7%, whereas on a concrete patio and black plastic it was reduced by 11%. Percent germination remained at 90-96% with all three treatments (Cordova et al., 1999).

In these studies, two researchs were conducted to evaluate (i) the effect drying maize on concrete floor with and without plastic sheet to maize seed quality (ii) the effect of postponed drying to maize seed quality.

Methodology

Effect of sun drying on concrete floor with plastic and without plastic on maize seed quality (2005-2006)

The research was conducted in Bontobili experimental farm, laboratory and green house of Indonesian Cereal Resesarch Institute, Maros, South Sulawesi from February 2005 until April 2006. Four maize varieties were used; MS-2, Lamuru, Srikandi Putih and Srikandi Kuning-1.

Seed production was conducted in Bontobili Experimental Farm, Gowa, South Sulawesi. Maize plant were harvested 5-10 days after physiological maturity were reached. After harvest, the cob were drying and on concrete floor with the treatments : (i) drying with plastic sheet layer on concrete floor, (ii) drying without plastic sheet layer on concrete floor. After 16% moisture content were reached, the cob were shelled and grain were dried again with its initial treatment until moisture content of seed were 10-11%, Two kg of seeds were packing in the polybag for each treatment with three replications. The seeds were store in the plastic silo in the room temperature at ICERI laboratory. Observation were done before store and every two month for 12 months. Data observed were : (i) seed moisture content at harvest and at each step of drying as above by using Kitt PM 480 moisture tester, (ii) germination percentage ; 50 seeds of each treatment were sowed in plastic container with sand. Observation were done on the third, fourth, and fifth day after sowing. Observation were based on normal, abnormal and died seedling. Normal seedling were divided into strong normal seedling and weak normal seedling, (iii) seedling dry weight; seedling from germination test were dried in incubator at 60oC, for 3 x 24 hours, put into desicator, and weighed, (iv) electric conductivity of leakage; 5 g seeds from each treatments were soaked in 50 ml distilled water for 24 hours, and measure in conductometer, (v) Potassium leakage ; 50 seeds from each treatment weighed and soaked in 75 ml distilled water for 30 minutes and potassium content were measured by flame photometer.

The effect of postponed drying on maize seed quality (2006-2007)

The research was conducted in Bontobili experimental farm, laboratory and green house of ICERI from February 2006 until April 2007. Two maize varieties were used; Lamuru and Srikandi Kuning-1. As the previous research, observation were done on seed moisture content, germination percentage, seedling dry weight, electric conductivity of leakage and potassium leakage. Observation were done every two months for 12 months.

The treatments were :

A. varieties :
   (1) Lamuru,
   (2) Srikandi Kuning-1

(B) drying floor : (1) concrete floor with plastic sheet
   (2) concrete floor without plastic sheet

C. Postponed time of drying cob :
   (1) immediate cob drying after harvest, without postponed
   (2) postponed cob drying 3 days after harvest
   (3) postponed cob drying 6 days after harvest
   (4) postponed cob drying 9 days after harvest

Besides all observation above, drying temperature on drying floor with and without plastic sheet and drying time were also observed.

Discussion

Maize seed drying temperature will vary depending on (i) genotype, (ii) harvest moisture and environmental conditions. In this research, harvest moisture were about 27 – 30%. In the first research, germination percentage was remain above 90% after 12 months storage (Table 1a). These four maize varieties had different chemical characteristics, Srikandi Putih and Srikandi Kuning-1 had higher protein content compare to Lamuru and MS-2. However, from drying treatment (using plastic sheet on concrete floor and without plastic sheet), there were no significant difference in germination percentage at initial and 12 months after storage between these varieties. Average daily temperature in storage were fluctuated between 28 – 34°C.

Initial moisture content of seeds before stored were 10.8 – 11.2%. With this low moisture content, seeds can prolong its life longer, showed from low decrease of its germination percentage. At initial observation, no significant difference of its germination, electric conductivity of leakage and potassium leakage among these varieties, but after store for 12 months, its showed that the highest decrease rate of quality in term of germination percentage, electric conductivity of leakage

Table 1a. Germination percentage, Electric conductivity of leakage, Potassium leakage of maize seed of MS-2, Lamuru, Srikandi Kuning, Srikandi Putih after sun drying with and without plastic sheet on concrete floor at initial and twelve months after storage in room temperature (28-32°C) at Maros (First year, 2005-2006)

Maize Seed Varieties (Drying Treatment)	Germination Percentage (%)		Electric Conductivity of Leakage (µmhos/cm2/g)		Potassium Leakage (ppm)	
	Initial (before storage)	12 months storage	Initial (before storage)	12 months storage	Initial (beforeStorage)	12 months storage
MS-2 (PS)	99.33a	94.67a	11.92a	24.73b	56.10cd	108.89bc
MS-2 (WPS)	100.00a	94.00a	11.05a	28.79a	69.72ab	111.72bc
Lamuru (PS)	100.00a	93.33ab	10.79a	22.69bc	58.80bc	95.15d
Lamuru (WPS)	99.33a	92.67bc	11.79a	24.53b	66.42ab	111.72bc
Srikandi Kuning (PS)	100.00a	92.67bc	11.16a	22.08bc	53.59e	113.49ab
Srikandi Kuning (WPS)	99.33a	92.00bcd	11.74a	22.39bc	64.56ab	118.80a
Srikandi Putih (PS)	100.00a	91.33cd	11.28a	20.98d	58.24bcd	98.33d
Srikandi Putih (WPS)	99.33a	90.67e	11.33a	21.48cd	73.33a	113.39ab

and potassium leakage were on Srikandi Putih, Srikandi Kuning, MS-2, Lamuru respectively (Table 1a).

Use of conductivity measurement of seed leachates to determine seed viability is based on Osterhout in Mc. Donald and Nelson (1988) who established the relationship between cell death and release of electrolytes. Physiological potency of maize seed could be detect from potassium leakage of imbibed seeds (Miguel and Marcos Filho, 2002).

The results showed that no significant difference in germination percentage between these two drying treatment, but there were decreasing germination percentage with storage period. At initial observation (before storage), germination percentage of seeds that dried on concrete floor with plastic sheets were higher compare to the treatments without plastic layer sheets. The same results showed on 12 month after storage observation (Table 1b).

Seedling dryweight showed high correlation with its germination, and had negative correlation with its seed leachates (Table 3).

Seed drying involves first the transfer of moisture from the seed surface to the air around the seed and then second the movement of moisture from inside the seed to the seed surface. This is accomplished by raising the temperature

Tabel 1b. Germination percentage, Electric conductivity of leakage, Potassium leakage of maize seed of Lamuru and Srikandi Kuning with and without plastic sheet on concrete flor sun drying, Immediate drying at harvest and postponed 3, 6, and 9days after harvest at initial observation and 12 months after storage in room temperature (28-32^{o}C) at Maros (Second year, 2006-2007)

Maize Seed Varieties (Drying Treatment)	Germination Percentage (%)		Electric Conductivity of Leakage (μmhos/cm2/g)		Potassium Leakage (ppm)	
	Initial (before storage)	12 months storage	Initial (before storage)	12 months storage	Initial (beforeStorage)	12 months storage
Lamuru						
PS, PP-0	99.33a	96.67ab	4.89g	20.80f	45.92d	75.60f
PS, PP-3	99.33a	96.00ab	6.27ef	21.57ef	58.52c	83.63de
PS, PP-6	98.67a	95.33ab	7.25cde	25.10bcde	59.99bc	88.73cd
PS, PP-9	97.33a	93.33ab	10.03a	27.80a	67.05b	118.87b
WPS, PP-0	100.00a	98.00a	5.46fg	19.07f	49.11d	63.93g
WPS, PP-3	99.33a	96.67ab	6.83de	20.93ef	58.14c	83.53de
WPS, PP-6	98.67a	96.67ab	7.62bcg	22.47def	63.57bc	100.88bc
WPS, PP-9	98.00a	92.67b	10.59a	25.00bcde	76.55a	135.30a
Srikandi Kuning						
PS, PP-0	100.00a	96.67ab	5.49fg	20.80f	46.83d	71.89b
PS, PP-3	100.00a	95.33ab	6.42ef	23.90def	56.73c	89.85b
PS, PP-6	98.00a	92.67b	8.16b	24.55bcd	60.69bc	106.41ab
PS, PP-9	98.00a	90.00bc	9.95a	28.28bcd	64.91bc	135.05a
WPS, PP-0	100.00a	98.00a	5.52	21.35ef	47.37d	76.53b
WPS, PP-3	100.00a	97.33ab	6.72	24.16bcde	57.13c	96.10ab
WPS, PP-6	98.67a	94.67ab	8.52	24.90bcd	63.73bc	104.03ab
WPS, PP-9	97.33a	92.67b	10.11	29.83a	78.49a	131.05a

PS = Drying with plastic sheet on concrete floor ; WPS = Drying without plastic sheet on concrete floor
PP-0 = Direct cob drying after harvest; PP-3 = Postponed cob drying 3 days
PP-6 = Postponed cob drying 6 days; PP-9 = Postponed cob drying 9 days

Table 2. Drying temperature on concrete floor with and without plastic sheet layer and moisture content decrease in each drying treatment. Maros, 2005-2006

Maize seed varieties/ drying treatment	Drying temperature(^{o}C)					Moisture content decrease after 5 days drying (11.00 a.m – 15.00 p.m)
	11.00	12.00	13.00	14.00	15.00	
Lamuru, plastic sheet	45	50	54	56	54	14.5
Lamuru, without plastic sheet	50	55	57	60	58	11.3
Srikandi Kuning-1, plastic sheet	44	49	54	55	54	17.1
Srikandi Kuning-1, without plastic sheet	48	54	58	60	59	13.5

Table 3. Correlation value between germination percentage, seedling dry weight, electric conductivity of leakage, potassium leakage of seeds in storage.

Variable	Germination percentage	Seedling dryweight	Electric conductivity of seed leakage	Potassium leakage
Germination percentage	1	0,586**	-0,731**	-0,496**
Seedling dryweight	0,586**	1	-0,554**	-0,662
Electric conductivity of seed leakage	-0,731**	-0,554**	1	0,590**
Potassium leakage	-0,496**	-0,622**	0,590**	1

**Significant correlation at 0,01 level

and lowering the relative humidity of air and passing it through the seed mass. The heat from the air is transferred to the seed increasing the vapor pressure in the seed. Due to vapor pressure differences between the seed and air, moisture is forced out of the seed and carried away by the air. If the temperature of the air is very high, the drying process may be too violent resulting in damage to the seed. In this study, it's showed that temperature on concrete floor at 14.00 a.m. reached 60^{o}C which could damaged cell membrane of seeds and its showed from higher seed leachates (Table 2). Too low temperatures will delay the drying process often resulting in damage caused by diseases, insects, and excessive respiration. Sufficient airflow through the seed mass is essential to complete the drying operation.

Conclusion

- Drying on concrete floor with plastic sheet layer showed higher seed quality in term of storability than drying on concrete floor without plastic sheet layer.
- Postponement cob drying, especially until 9 days significantly decreased maize seed quality.
- Electric conductivity of seed leakage and potassium leakage showed negative correlation with seed germination and seedling dryweight.

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Heterosis, combining ability and phenotypic stability for yield and other characters in high quality protein maize (*Zea mays* L.)

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Abstract. Present investigation was conducted during *kharif*, 2006 at Crop Research Centre, Pantnagar (E_1); Maize Research Centre, Belipar, Gorakhpur (E_2) and KVK, Kashipur (E_3) with the objectives (i) to identify superior quality protein maize (QPM) single cross hybrids, (ii) to determine nature and magnitude of heterosis and, (iii) to estimate variability parameters, combining ability and phenotypic stability of parental maize inbred lines and hybrids over three environments for important economic characters. Experimental material consisted of 10 QPM inbred lines crossed in a half-diallel fashion. All 10 parents and 45 F_1 crosses along with two checks were evaluated in randomized complete block design. Observations on 13 characters viz., days to 50 per cent tasselling and silking, plant height, ear height, ear length, ear diameter, kernel rows/ ear, kernels /row, 100-kernel weight, grain yield, protein, lysine and tryptophan contents were recorded. The genotypes differed significantly for all characters. High per cent heterobeltiosis, standard heterosis and relative heterosis across the environments were observed in crosses, P_1 x P_2, P_3 x P_{10} and P_5 x P_8 for grain yield. High heritability and expected genetic advance were observed for grain yield, ear diameter, kernels/row, kernel rows/ear, 100-kernel weight and quality characters in all the environments. Significant variances due to GCA and SCA for most of the characters were observed in all three environments indicating the importance of both additive and non-additive genetic variances. Magnitude of SCA variance was greater than GCA variance for all characters showing preponderance of non-additive variance and suitability of material for hybrid breeding. Maximum SCA effects were observed in crosses, P_3 x P_{10}, P_1x P_2 and P_5 x P_8 in pooled environment for grain yield. Mean squares due to genotypes and environments were significant for most of the characters, which indicated substantial variability among genotypes and environments. G x E interaction was also highly significant for all characters except quality parameters. Among the lines, P_6 showed desirable stability for grain yield. Out of 45 crosses, most desirable and stable ones were P_1 x P_2, P_2 x P_5, P_2 x P_{10}, P_3 x P_7 and P_3 x P_{10} for grain yield and its components.

Key-words: Maize, QPM, heterosis, GCA, SCA, grain yield

Introduction

In India, maize occupies an area of about 8.0 m ha with total production of about 16.9 m tones. Maize has 9-12 % grain protein which is nutritionally poor due to lower contents of two essential amino acids, lysine and tryptophan and undesirable ratio of leucine to isoleucine. The opaque-2 mutant gene, which is responsible for the enhancement of lysine and tryptophan of maize protein, if incorporated into any population, inbred line or any other maize germplasm, improves the nutritional status of maize. The modified opaque-2 maize with hard endosperm and vitreous kernels is termed as Quality Protein Maize (QPM). In a highly cross pollinated crop like maize, the exploitation of heterosis can be accomplished through the development and identification of high *per se* performing vigorous parental lines and their subsequent evaluation for combining ability in cross combinations to identify hybrids with high heterotic effects. Information about the heterotic patterns and combining ability of parents and crosses both facilitate the breeders in the selection and development of single cross hybrids. Combining ability analysis provides information on the relative importance of additive and non-additive gene effects involved in the expression of the quantitative traits. In fact, the future is of hybrid cultivars. Hybrids are considered to possess a high degree of biological fitness for a given situation. In QPM also, hybrids are more successful among the farmers as these are always planted with fresh F_1 seed every year and thus, remain unaffected from contamination through normal maize cultivars. Isolation, if given, definitely safeguards the quality of grain. If the presently cultivated maize hybrids and varieties are replaced with QPM hybrids, the latter will provide a definite promise in upgrading the nutritional security of maize-based population. This study was, therefore, undertaken to assess nature and magnitude of heterosis, combining ability effects and phenotypic stability for yield and other characters in 45 single crosses developed through half-diallel system among 10 QPM inbred lines.

Material and Methods

Ten elite high QPM inbred lines abbreviated as $P_{1,}$ P_2, $P_{3.........}P_{10}$ (Table 1), were crossed in a half-diallel fashion at Pantnagar, India. These parental inbred lines and their all 45 F_1s were evaluated with two QPM checks, Shaktiman-1 and FQMH-7 in a randomized complete block design with 3 replications at three locations viz., Pantnagar (E_1), Belipar (E_2) and Kashipur (E_3) during *kharif*, 2006. The net plot area was 3.75 m^2. Data were recorded on days to 50% tasselling, days to 50% silking, plant height (cm), ear height (cm), ear length (cm), ear diameter (cm), number of kernel rows/ear, number of kernels/row, 100-kernel weight (g), grain yield (kg/ha) and quality parameters like protein (%), lysine and tryptophan (g/16g N) contents. Observations for 50% tasselling and silking and fresh ear weight (later converted at 15 % grain moisture for grain yield) were recorded on whole plot basis, whereas data on remaining characters were taken on five randomly selected competitive plants/ears from a plot. The average value of these plants/ears was calculated and used for analysis of variance, heterosis, combining ability, heritability, genetic advance and phenotypic stability using standard statistical procedures.

Results and Discussion

The analysis of variance for different characters in the three environments (E_1, E_2, E_3) indicated highly significant differences among the treatments for all characters under all the environments except days to 50% tasselling in the E_3. The partitioning of variance into various components revealed that mean squares due to environments were highly significant for all the characters except flowering and quality traits. The mean squares due to genotypes showed highly significant differences for plant height, ear height, grain yield and quality traits. It observed significant effect of environments and the genotypes and varying performance of genotypes over environments except quality traits. Here, the significant differences among parental lines and their crosses indicated inherent genetic differences for all the characters studied.

Heterosis

In the present study, heterosis has been studied with respect to superiority of hybrids over the mid parent (relative or average heterosis), the better parent (heterobeltiosis) and over the checks (standard heterosis) abbreviated as MP, BP and CP, respectively. Overall results of heterobeltiosis revealed that P_5 x P_8 (125.13 %), P_1 x P_5 (100.21%) and P_3 x P_{10} (95.09%) in E_1; P_3 x P_7 (169.23%), P_2 x P_{10} (91.20%) and P_4 x P_{10} (91.12%) in E_2; P_1 x P_{10} (237.85%), P_1 x P_8 (216.21%) and P_1 x P_2 (196.22%) in E_3; and P_3 x P_{10} (112.30%), P_5 x P_8 (140.87%) and P_1 x P_2 (140.35%) across the environments exhibited high better parent heterosis with respect to grain yield. For tryptophan and lysine contents, the crosses P_5 x P_7 (44.06%, 44.06%) and P_2 x P_3 (29.16%, 29.17%) showed heterobeltiosis across the environments. The overall results of standard heterosis for grain yield revealed that none of the hybrids showed high standard heterosis in all the environments. Only one hybrid, P_1 x P_2 exhibited high heterosis in E_2 (267.82%), E_3 (324.32%) and across the environments (178.42%). Similarly, P_5 x P_8 showed high heterosis in E_1 (139.07%) and across the environments (131.57%). For quality characters such as tryptophan and lysine contents, high standard heterosis was exhibited by the cross P_1 x P_{10} in E_1 (23.16%, 23.23%), E_2 (23.09%, 23.18%), E_3 (23.13%, 23.33%) and across the environments (23.07%, 23.19%). Overall results of relative heterosis revealed that P_5 x P_8 exhibited high relative heterosis for grain yield in E_1 (179.54%), E_2 (240.42%), E_3 (71.64) and across the environments (154.07%), however, crosses, P_3 x P_{10} in E_1 (151.30%) and across the environments (147.23%) and P_1 x P_2 in E_3 (248.88%) and across the environments (141.98%) showed high relative heterosis for grain yield. For quality traits like lysine and tryptophan contents, the crosses, P_2 x P_5 (32.24%, 32.27%) and P_5 x P_7 (52.46%, 52.33%) exhibited high mid parent heterosis. The cross, P_3 x P_{10} exhibited positive and significant heterosis (BP, CP and MP) over all the locations and in pooled analysis for the plant height and grain yield. Crosses, P_5 x P_9 for ear height, P_4 x P_{10} for ear length, P_3 x P_8 for grain yield, P_1 x P_7 and P_5 x P_8 for protein percentage showed significant heterosis (BP, CP and MP) in desired direction over all the locations.

Combining ability

Combining ability analysis is an effective tool for identifying superior parental lines for the development of hybrids (Sprague and Tatum, 1942). The analysis of variance for combining ability revealed that GCA variances were significant at all the locations for all the characters except ear length in E_1 and days to 50 per cent tasselling and silking in E_3. The SCA variance was also significant for all the characters except days to tasselling in E3. These findings are in agreement with the earlier reports of Vasal *et al.* (1992), Katna *et al.* (2005) and Singhal *et al.* (2006). Pooled analysis revealed that both GCA and SCA variances were significant for all the characters. The GCA x E and SCA x E were also significant for all the characters except tryptophan and lysine contents. These results clearly showed that both GCA and SCA variances were substantial for the economic characters included in this study. Therefore, experimental material may be exploited for the development of maize hybrids/ synthetics for a particular location as well as across the locations. Estimates of variances due to specific combining ability (s^2 SCA) were greater than general combining ability (s^2 GCA) for all the characters over all the three environments and also in pooled analysis.

The ratio s^2 GCA/s^2 SCA less than unity for all the characters also indicated preponderance of non-additive genetic variance. This suggested greater importance of non-additive gene action in their expression and further revealed very good prospects for the exploitation of non-additive genetic variation for grain yield and its component characters in hybrid breeding. Jha and Khehra (1992), Zheng and Du (1995) and Dahlan *et al.* (1997) also reported the similar results. None of the parents was excellent for all the characters across the environments. However, the lines, P_1 and P_3 in E_1 were found to be best general combiners for various characters. In E_2, P_5 and P_1 were found to be best general combiners. The GCA effects of the parents in E_3 indicated P_1 and P_8 to be the best general combiners for various characters including ear length, ear diameter and grain yield. Based on pooled analysis, P_1 and P_9 were found to be the best general combiners for several characters including tryptophan and lysine contents. Similar findings were reported by Singh and Asnani (1979), Singh *et al.* (1998), Srivastava (2001), Katna *et al.* (2005) and Singhal *et al.* (2006).

In this study, the parents showing the best *per se* performance were not the best general combiners for almost all the characters. Therefore, *per se* performance may not necessarily be the criteria in selecting the parents for hybridization. SCA effects of crosses for grain yield showed lack of any relationship between GCA effects of the parents and the SCA effects of the crosses. In majority of the cases, high SCA effects for grain yield were attributed to dominance and epistasis gene interactions. P_3 X P_{10}, P_1 X P_2 and P_5 X P_8 were some of the crosses with maximum SCA effects in the pooled environment. Overall results revealed that different crosses exhibited differential response for SCA effects in different environments for all the quantitative traits, i.e., there were very little or no reproducibility for SCA effects of the crosses in all the environments. It showed effect of the environments in the performance of the crosses. Similar results were reported by Debnath and Sarkar (1987), Zheng and Du (1995), Srivastava (2001) and Singhal *et al.* (2006).

Phenotypic stability

The pooled analysis of variance revealed that mean squares due to genotypes were significant for grain yield. Mean squares due to environments were also significant for all the characters except days to 50 % tasselling indicating sufficient differences among the environments. Highly significant mean squares due to genotype x environment interaction for all the traits indicated differential response of genotypes in different environments. The mean performance for days to 50 % tasselling ranged from 45.56 to 49.11 with an overall mean of 47.40. The results revealed that all the ten lines were stable for days to 50 % tasselling over environments. The most desirable and stable lines having low mean indicating early tasselling than overall mean (<47.4), $b_i = 1$ and $s^2 d_i = 0$ for early flowering were P_1 and P_9. Likewise the most desirable crosses which showed early flowering were P_1 x P_2, P_1 x P_4, P_1 x P_8, P_2 x P_3, P_2 x P_6, P_2 x P_7, P_2 x P_8, P_2 x P_{10}, P_3 x P_4, P_4 x P_6, P_4 x P_9, P_5 x P_8, P_7 x P_8, P_7 x P_9, P_8 x P_{10} and check, FQMH-7. The mean performance of genotypes for 100-kernel weight ranged from 14.34 to 22.08 with an overall mean of 17.89 and b_i values from -1.20 to 2.80. The most desirable and stable lines across the environments were P_1 and P_7. Similarly, the crosses showing desirable stability i.e., high mean (>17.89), bi=1 and $s^2 d_i = 0$ were P_1 x P_2, P_2 x P_5, P_3 x P_8, P_4 x P_5, P_4 x P_6, P_5 x P_8, P_5 x P_9, P_8 x P_{10} and P_9 x P_{10} across the environments. The mean performance of genotypes for grain yield ranged from 802.05 to 2752.93 with an overall mean of 1470.42. The line, P_6 was considered as most desirable and stable. Among all the crosses, the most desirable and stable i.e., having high mean (>1470.42), b_i=1 and $s^2 d_i = 0$ were P_1 x P_2, P_2 x P_5, P_2 x P_{10}, P_3 x P_7 and P_3 x P_{10}. In the present investigation, the inbred lines, P_1 and P_9 were found to be most desirable and stable for days to 50 % tasselling, however, lines, P_1 and P_7 were stable for 100-kernel weight. The inbred line, P_6 was found to be most desirable for grain yield. The stability performance of single crosses for grain yield and other characters are presented in Table 2.

Table 1. Quality protein maize inbred lines selected for the study

No.	Pedigree	Pedigree code
1.	DMR-QPM-75-⊗-⊗-#-⊗-⊗-⊗	P_1
2.	DMR-QPM-17-⊗-⊗-#-⊗-⊗-⊗	P_2
3.	DMR-QPM-18-⊗-⊗-#-⊗-⊗-⊗	P_3
4.	DMR-QPM-28-5-⊗-⊗-#-⊗-⊗-⊗	P_4
5.	DMR-QPM-03-101-#-⊗-⊗-⊗-⊗-⊗	P_5
6.	DMR-QPM-03-102-#-⊗-⊗-⊗-⊗-⊗	P_6
7.	DMR-QPM-03-121-#-⊗-⊗-⊗-⊗-⊗	P_7
8.	DMR-QPM-03-103-#-⊗-⊗-⊗-⊗-⊗	P_8
9.	DMR-QPM-28-3-#-⊗-#-⊗-⊗-⊗	P_9
10.	DMR-QPM-03-125-#-⊗-⊗-⊗-⊗-⊗	P_{10}

Table 2. Parental lines and single crosses with desirable and stable performance for grain yield and other characters

No.	Character	Parental line	Single cross
1.	Days to 50 % tasselling	P_1, P_9	P_1 x P_2, P_1 x P_4, P_1 x P_8, P_2 x P_3, P_2 x P_6, P_2 x P_7, P_2 x P_8, P_2 x P_{10}, P_3 x P_4, P_4 x P_6, P_4 x P_9, P_5 x P_8, P_7 x P_8, P_7 x P_9, P_8 x P_{10}
2.	100-kernel weight	P_1, P_7	P_1 x P_2, P_2 x P_5, P_3 x P_8, P_4 x P_5, P_4 x P_6, P_5 x P_8, P_5 x P_9, P_8 x P_{10}, P_9 x P_{10}
3.	Grain yield	P_6	P_1 x P_2, P_2 x P_5, P_2 x P_{10}, P_3 x P_7, P_3 x P_{10}

The overall results of stability revealed that none of the single cross hybrids showed stable performance for all the characters over all the environments except P_1 x P_2. In fact, the crosses, P_1 x P_2, P_2 x P_5, P_2 x P_{10}, P_3 x P_7, and P_3 x P_{10} showed desirable and stable performance for grain yield. Similar findings were advocated by Sharma and Bhalla (1982), Jha *et al.* (1986), Mahajan and Khehra (1992), Choukan (1999), Agrawal *et al.* (2000) and Reddy and Ahuja (2004).

Acknowledgements

The authors are highly indebted to the Dean Agriculture and Director, Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar for providing necessary facilities in conducting the experiment.

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Studies on multiple ears trait expression in maize (*Zea mays* L.)

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Abstract. The expression of multiple ears was studied in 18 composites/populations, 80 inbred lines and 193 experimental hybrids. Multiple ears expression varied from minimum of 0.5% to maximum of 25.33% plants with no seed set in case of bouquet when composites/populations were studied. In case of inbred lines, 30 lines did not show any expression of multiple ears whereas remaining 50 lines were found to have 1.1 to 20.0% plants with multiple ears per node. The experimental hybrids expressed multiple ears in 2.5% to 37.5% plants. All the 7 sweet corn tested were found to express multiple ears ranging from 5.0% to 33.33% plants. Variations were observed over the environments and replications which revealed differential expression of multiple ears. Among the three groups of materials used for study, composite/population was found to be severely affected. Lines which expressed multiple ears are said to be susceptible whereas those which are reluctant are considered to be resistant sources. The deviations recorded in the environmental parameters from previous two years are thought to helped genotype environment interaction suitable for expression of multiple ears. Emerging environmental stresses, changing management issues coupled with pressure on continuous yield enhancement, necessitates concerted focus to determine the cause of multiple ears per node and to identify as well to incorporate resistance sources in maize breeding programme so that undue and unpredicted losses to farmers in particular and national and global loss in general could be avoided.

Introduction

The multiple ears are not unexpected but they typically occur at different nodes, not on the same node. However, there was a report that corn hybrids from several companies have developed more than one ear at a single node across Iowa, Illinois, and Indiana during 2006. In the most extreme cases, up to eight ears have occurred at a single node. A susceptible hybrid may show bouquet ears in one location and double ears at another site. Twin ear expression on single node in maize was however observed by Hallauer in 1973 in S_2 and an S_5 progenies (Hallauer, 1984). Frank and Hallauer (1997) successfully determined its recessive nature of inheritance, yet the exact cause of multiple ears expression is not fully understood. Considering the significance of multiple ears in stabilizing yield, the present investigation was undertaken to investigate the expression of multiple ears in diverse maize genotypes.

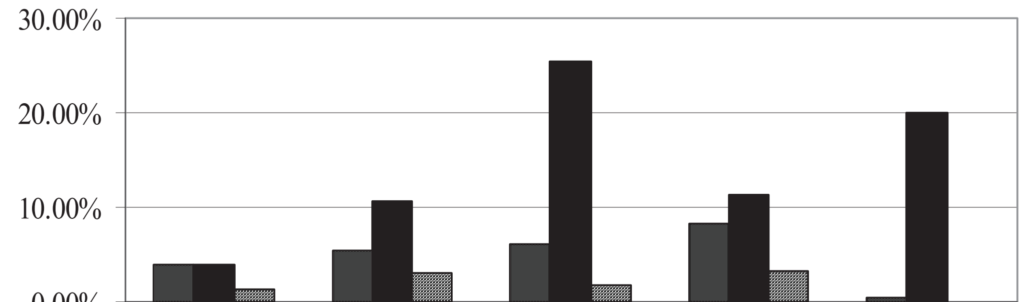
Materials and methods

The material consisted of 18 composites/populations, 80 inbred lines with different level of homozygosity and 193 experimental hybrids. Four composites were also grown at three sowing dates and under normal and rainfed high plant density conditions. Hybrids were further classified into early, medium and full maturity group. All the hybrids were planted in replicated design. Inbred lines consisted of 17 S_6 progenies and 63 S_1 progenies derived from various sources were planted in 4 row plot of 4 m length. 59 inbred lines (S_1) were also planted under rainfed and low nitrogen conditions. The investigation was carried out during the kharif 2007 at Crop Research Centre of the University. The observations on multiple ears were recorded in each line across the environments and repetitions. Data were complied and percentage of lines affected with multiple ears were calculated. Weather parameters for the last three years were collected and aligned to correlate the multiple ear expression with some specific environmental parameters.

Results and Discussion

All the composites either planted on 20th of June or 25th of June under normal conditions or on 27th June 2007 under high plant density rainfed conditions exhibited twin and multiple ears on single node with differential expression (Fig 1). Among the 11 composites planted on 20th June, plants expressing twin ears varied from maximum of 8.17%

Fig 1. Multiple ear expression in maize

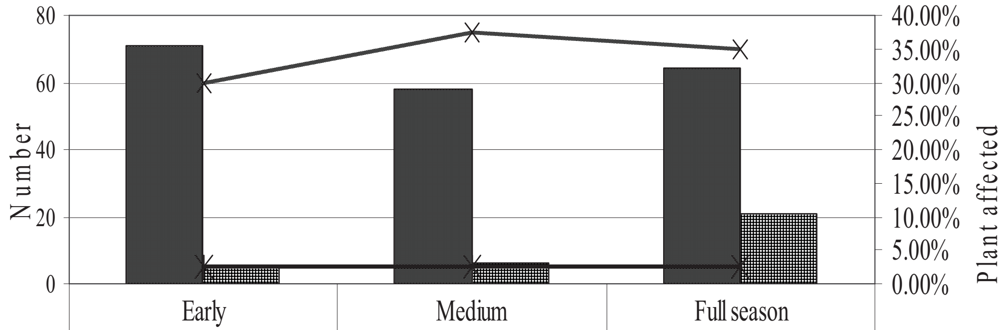


	Surya	Gaurav	D-994	D-131	DQPMC4
Normal condition 20th June	3.94%	5.51%	6.00%	8.20%	0.50%
Normal condition 25th June	4.00%	10.67%	25.33%	11.33%	20.00%
High plant density 27th June	1.30%	3.00%	1.83%	3.25%	0.00%

Fig 2. Comparison of twin ear expression in composites under different environments

plants in variety D131 to minimum of 0.6% plants in variety Shweta. Frequency of multiple ears was found to be less in comparison to twin ears and only four composites namely Surya, D994, Gaurav and D131 expressed multiple ears trait in the range varied from 0.5% to 1.0%. Four composites namely Surya, Gaurav, D994 and D131 planted on 25th June exhibited relatively higher percentage of twin as well as multiple ears (Fig 2). Twin ear expression was found maximum of 25.33% in D994 whereas it was minimum (4.0%) in Surya. The variety Surya did not show any symptom of multiple ears whereas D994 was again found to have maximum (8%) plant with multiple ears. Under rainfed and low nitrogen conditions, expression frequency of twin ear was relatively less than with those of 25th June and 20th June plantings. The variety Surya was found to have minimum of 1.3% twin ear plants where D131 exhibited maximum of 3.25% plant with twin ears. None of these populations exhibited multiple ear traits. QPM composite, $DQPMC_4$ exhibited twin ear character in 20.0% of the plant when planted on 25th of June while only 0.5% plant exhibited twin ear when planted on 20th of June. Multiple ears in 4.0% plants of $DQPMC_4$ were also observed only in 25th June planting. All the 7 sweet corn lines exhibited twin ear which varied from minimum in 5.0% plants to maximum in 33.33% plants. It was noted that expression of twin or multiple ears were not consistent which showed the differential expressivity and penetrance and seems to be a threshold character (Hallauer, 1988).

Seventy one early maturity hybrids comprised of 4 trials with 18, 7, 11 and 35 hybrids in each. 16.67% of 18 experimental hybrids in Trial No. 68 exhibited twin ears in



	Early	Medium	Full season
Total hybrids investigated	71	58	64
No. of hybrids affected	5	6	21
Maximum plant affected (%)	30.00%	37.50%	35.00%
Minimum plant affected (%)	2.50%	2.50%	2.50%

Fig 3. Twin ear expression in experimental hybrids of different maturity

2.5 % plants (Fig 3). All the 7 hybrids of Trial 67 did not express any symptom of twin ears. Trial 203 consisted of 11 hybrids where 2 hybrids exhibited twin ears and 1 hybrid multiple ears. The affected hybrids had 2.5 to 7.5% plants with twin ears. One hybrid in one replication out of the 35 in a CIMMYT trial had twin ear expression where 30% plants were found to be affected. Medium maturity maize consisted of 58 entries, were evaluated in three trials. None of the entries from Trial No. 202 and Trial No. 62 had either twin ear or multiple ears. The 100% entries of Trial 62A were found to be affected where twin ear expression varied from minimum of 2.5% to maximum of 37.5% plants. The third group consisted of 64 entries of full season maturity. All the 6 entries of Trial No. 69 exhibited twin ears in minimum of 2.5% plants to maximum of 13.3% plants. Six entries of Trial No. 201 exhibited twin ears in the range of 2.5% to 15.0%. Nine out of 44 entries of Trial No. 61 exhibited twin ears whereas the remaining entries did not show any plants with twin ears. The range of plants expressed twin ears varied from minimum of 5.0% to maximum of 35.0%. The expression of twin as well as multiple ears was not consistent as it varied across the replications. The second ear expression was too delayed to affect seed setting and ultimately yield in most of the cases is little affected.

The established 17 lines belong to CML 421, Yellow Heterotic Pool –A (YHP-A), Yellow Heterotic Pool –B (YHP-B), Pop 445 and Tarun whereas the 63 S_1 lines belong to diverse sources (Table 1). Both the lines of CML421 had twin ears expression in 3.3% of plants. 75% lines of YHP-B had twin ears expression in the range of 1.1 to 15.5% plants. Two lines of YHP-A had twin ears in the range of 1.1 to 3.3%. Lines from Pop445 and Tarun did not exhibit twin ear

Table 1. Expression of multiple ears in inbreds

Inbred lines	No. of lines	No. of lines affected	Percentage of affected plants
CML421 (S_6)	2	2, 1*	3.3, 1.1*
YHP A (S_6)	4	2	1.1 to 3.3
YHP B (S_6)	8	6, 1*	1.1 to 15.5, 3.3*
Pop 445 (S_6)	2	-	-
Tarun (S_6)	1	-	-
S_1 lines	63	40, 17*	1.67 to 20.00, 1.67 to 13.33*

character. Of the 63 S_1 lines, 23 did not express twin ears whereas 40 lines exhibited twin ears where affected plants varied from 1.67 to 20.0%. Multiple ears were also observed in 17 out of the 63 S_1 lines where 1.67 to 13.33 plants were affected. The 59 S_1 lines were also investigated under rainfed and low nitrogen conditions where only 13.56% lines were found to be affected with twin ears. The range of plants affected with twin ears was found to be from 1.17% to 3.52% which was low in frequencies in comparison to lines as well as plants affected under normal condition. No twin/multiple ears expression was found in 23 S_1 lines across both the environments and such lines are expected to be resistant to oddity of twin or multiple ears per node. Thirty S_1 lines exhibited differential expression i.e. twin or multiple ear expression in either of the environment. Six S_1 lines showed consistency in twin ear expression across the environments but with variable expression are said to be susceptible (Hallauer 1984, 1988).

The alignment of the last three years' environmental data on sunshine hour, rainfall, soil temperature, humidity and air temperature for July and August (Fig 4 & 5) revealed deviation from the previous cropping seasons. In general high humidity and continuous rainfall, lower maximum and minimum temperature, soil temperature, and less sunshine duration together-with might have coincide with the critical developmental phases of plant leading to induction of multiple ears. The cumulative environmental factors seem to be more congenial for expression of twin and multiple ears when planting was made on 25th June. Though, the contribution of other factors and cultural practices can

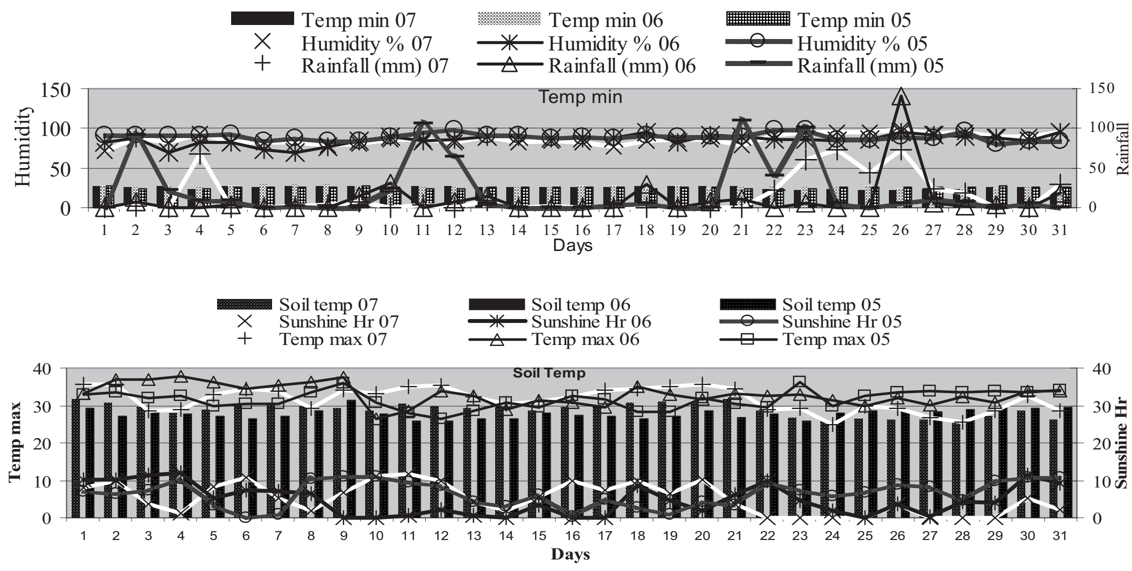


Fig 4 (a&b). Alignment of environmental parameters, July 2005-07

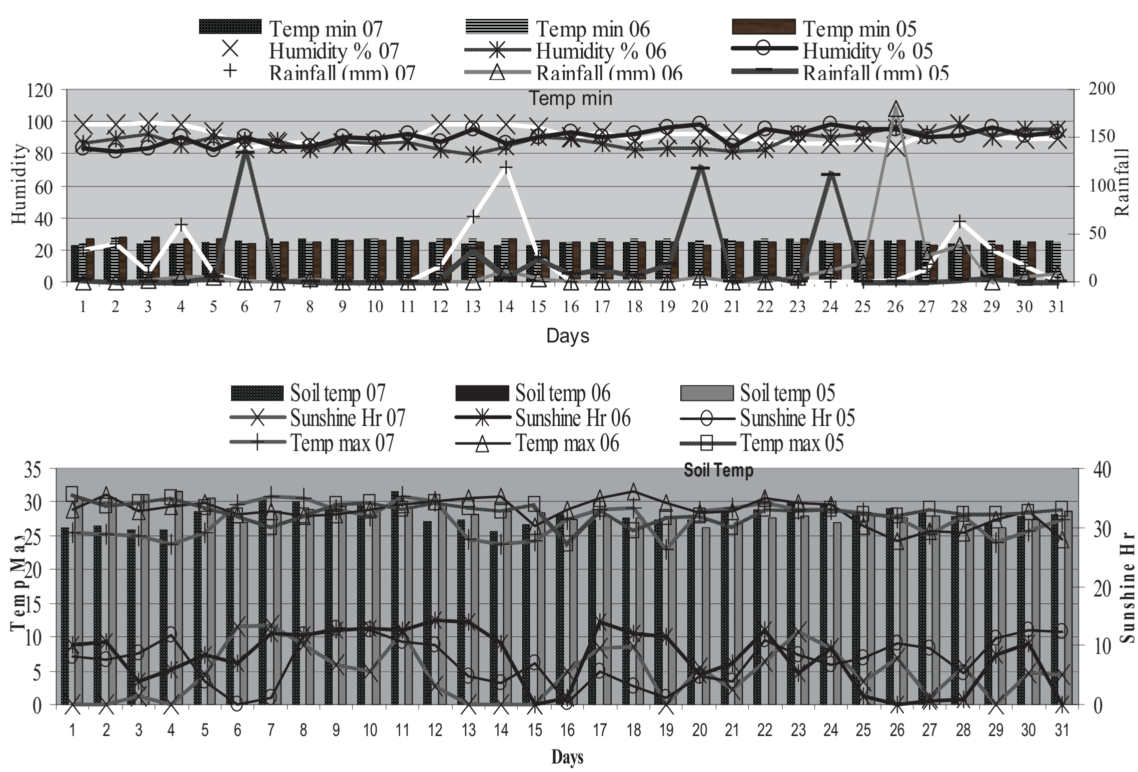


Fig 5 (a& b). Alignment of environmental parameters, August 2005-07 not be ignored in induction of twin and multiple ears (Lejeune and Bernier, 1996).

Unusual expression of twin and multiple ears were observed across the diverse materials investigated during the monsoon season of 2007. Some genotypes were found to be more prone to this oddity however its variable expression indicates the involvement of genotype-environment interaction. It is believed that this is a threshold genetic trait which may be triggered by particular stress especially during the sensitive growth duration of the plant. In terms of impact on yield, plants that had delayed development of second ear on the same shank may not affect yield substantially but in case of 'bouquet', the yield may be affected severely. The intensities of this oddity probably have now been increased and there are frequent reports from both farmers as well as from experimental plots. Global warming associated shifting in climatic conditions and human generated factors may have role in intensification of the problem. It would be better to conduct long term experiments taking unique environmental factors, cultural practices with wide range of genotypes across the locations and years to determine the exact cause of multiple ears.

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Heterosis and combining ability of Nepalese yellow maize populations

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Abstract. Knowledge about combining ability and heterotic patterns among maize germplasm is essential for hybrid breeding programs. This study was conducted to determine heterosis and combining ability of some Nepalese yellow maize populations. Forty crosses were made using a line x tester mating design with 10 parents as lines and four parents as testers. The parents and their F_1 hybrids were evaluated in an experiment conducted at CLSU in the Philippines during the2005 rainy season using a randomized complete block design with three replications. Significant differences were observed for grain yield and other traits. Population $46C_1$ x Rampur Composite showed the highest heterosis for grain yield among the crosses. Other crosses with higher heterosis were Manakamana-2/NML-2 and Rampur So3Fo2 /Rampur Composite, respectively. Crosses involving NML-2 as the male parent exhibited higher heterosis. Rampur So3Fo2 showed the highest general combining ability (GCA) value (1005.10) for grain yield, followed by Population $46C_1$ and Hill pool yellow. Pool 17E and NML-2 had higher GCA values than other testers used in the experiment. These lines and testers were thus identified as the best combiners. Upahar/Arun-4 showed the highest specific combining ability (SCA) effects (1852.24) for grain yield. Other crosses that showed higher SCA effects were Population $46C_1$ /Rampur Composite, Population $46C_1$/NML-2, and Rampur So3Eo2 /NML-2. These crosses were considered superior and parents of such crosses are ideal for developing hybrids through conventional and nonconventional methods

Key words: heterosis, combining ability, general and specific combining ability, heterotic patterns, lines and testers

Introduction

Information on genetic diversity is fundamental to hybrid breeding and population improvement programs as it enables assessment of the level of genetic diversity, characterization of germplasm, and assignment into different heterotic groups (Reif et al., 2003). Knowledge on germplasm diversity and their relationships among elite breeding materials has a significant impact on crop improvement. In hybrid maize breeding, this information is useful for developing inbred lines and planning crosses for hybrid cultivar development. Knowledge about the combining ability and heterotic patterns among Nepalese germplasm is essential in hybrid development work, but information on these aspects is limited. Thus, the immediate task of the National Maize Research Program (NMRP) is to generate information on the combining ability and heterotic patterns of available germplasm before a systematic hybrid-breeding program could be launched. Plant breeders choose parents based on per se performance, combining ability, and genetic relationships. This would help them identify superior parents and crosses to get the desired recombinants. The present study aimed to determine the heterosis and to estimate combining ability effects on grain yield and other quantitative traits of Nepalese yellow maize populations.

Materials and methods

Fourteen Nepalese maize germplasm were included in this study. Four were used as testers while 10 were used as lines. The tester parents used were Arun-4, Rampur composite, Pool 17E, and a CIMMYT-developed inbred line, NML-2 (CML-430). The lines were Population $46C_1$, Manakamana-2, Rampur So3Eo2, Population $35C_5$, Narayani, Hill pool yellow, Khumal yellow, Upahar, and Rampur So_3Fo_2. Seeds were obtained from the NMRP, Rampur, Nepal. Field experiments were conducted from February to September 2005 at the Fruits and Vegetables Seed Center (FVSC) of the Central Luzon State University ($15^0 43$ N latitude, $12^0 54$ E 30 m above mean sea level) in Munoz, Nueva Ecija, Philippines.

Production of crosses

Forty cross combinations were made using the line x tester mating design. Staggered plantings were done for the testers to synchronize flowering between lines and testers. Plantings were carried out using the recommended spacing. Fertilizer was applied at 120-60-40 kg NPK ha^{-1}. All the standard agronomic operations were followed. Crosses were made as planned and pollinations were carried out everyday from 8 o'clock in the morning to 2 o'clock in the afternoon. Seven plants were pollinated for each cross combination in order to get sufficient seed for evaluation. Seeds of parental lines were increased through bulked pollination. Mature ears from each cross, tester and line were harvested separately. Seeds from each line were shelled, dried-cleaned, and stored safely for the next-season evaluation.

Evaluation of hybrids

Forty crosses and 14 parents were evaluated. Seeds were planted in two rows of 4-m length. Two seeds per hill were planted with 25 cm spacing between hills and 75 cm spacing between rows. Plants were later thinned to one to maintain final plant density at 53,500 plants per hectare.The fertilizer rate used was 120-60-40 kg NPK ha^{-1}. All the standard agronomic operations were followed. Plants were harvested, depending on maturity, on a whole-plot basis. The ears from each plot were harvested and grain yield was taken on a plot-yield basis. Observations were taken for days to 50% silking, plant height, ear height, ear length, number of kernels per ear, 100-seed weight, and grain yield.

The step-by-step procedure described by Singh and Chaudhary (1977) was followed for the line x tester analysis. Analysis of variance, general combining ability (GCA) and specific combining ability (SCA) effects for grain yield and other quantitative traits were analyzed. The test of significance for GCA and SCA effects was done against the student t-test with error degree of freedom. Heterosis over mid-parents was calculated and estimated. Mean squares of the mid-parent heterosis were tested against the error variance by the usual F test.

Results and discussion

Results of the analysis of variance (ANOVA) for 40 crosses and 14 parents for yield and yield-contributing traits showed highly significant differences among the parents, crosses, parents versus hybrids and lines for all traits, except for plant height and ear length in parents versus hybrids. Similarly, highly significant differences were observed in line x tester for grain yield, days to 50% silking, ear height, and ear length, except for plant height, number of kernels, and 100-seed weight. The results revealed significant differences for all traits under investigation, indicating the presence of dominant effects. Rampur So3Eo2/NMIL-2 gave the highest grain yield among the crosses. Population 46C_1 x Rampur composite, Population 46C_1 /NML-2, Upahar /Arun-4, and Rampur So3Fo2 /Pool 17E, were the other superior performing crosses. The higher yield of these crosses could be due to increase in ear length, number of kernels per ear, and 100-seed weight.

Thirteen out of 40 crosses showed positive heterosis and 12 crosses exhibited significant negative heterosis for silking days (Fig. 1). Most of the crosses matured earlier than their parents. Crosses Rampur So3Eo2 /NML-2 (-10.91%), Narayani /Arun-4 (-10.06%), Population 46C_1/ NML-2 (-9.05%), and Across 9331/NML-2 (-8.75%) showed significant heterosis. All the testers demonstrated significant average heterosis for days to 50% silking. Early-maturing hybrids could be developed using these testers as male parents in hybrid breeding. Of the total number of crosses, eight displayed a significant positive heterosis for grain yield. Crosses showing higher heterosis were Population 46C_1/ Rampur composite, (63.18%), Manakamana-2 /NML-2 (52.27%), Rampur So3Fo2/Rampur composite (51.20%), Rampur So3Eo2/NML-2 (42.60%), and Rampur So3Eo2/Rampur composite (37.57%). Crosses of Rampur composite and NML-2 used as male parents showed a higher heterosis than Arun-4 and Pool 17E and thus have good potential as parents for hybrid breeding work. Parents of crosses showing higher heterosis could

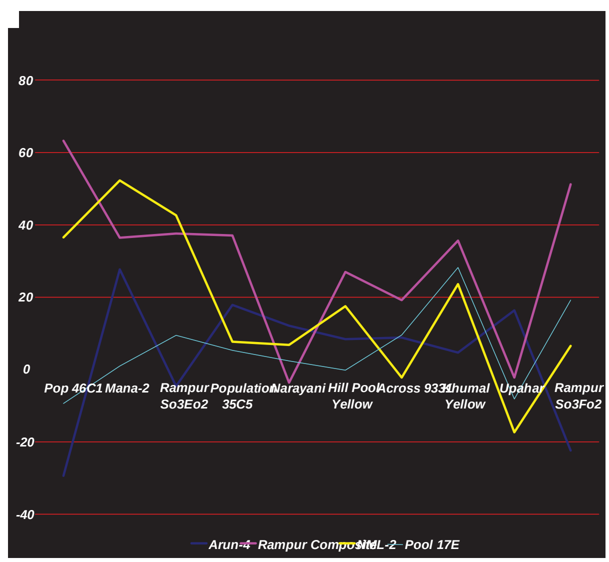

Fig. 1. Heterosis of 40 crosses for grain yield.

be used to exploit heterosis for grain yield. These germplasm could be genetically more divergent than those which exhibited little or negative heterosis.

We estimated the general combining ability (GCA) of the testers and lines for silking days, plant and ear height, number of kernels per ear, 100-seed weight, and grain yield (Table 1). Six parents demonstrated positive GCA values for silking days. NML-2, an inbred tester, had the only positive GCA value among the testers. Rampur So3Fo2 displayed the highest GCA value (3.17) among the lines, whereas Population 46C1 revealed the highest negative GCA (-2.575).Rampur, So3Fo2 was found to be the best combiner among the lines for silking days. Good combiners for silking days were Khumal yellow, Upahar, Manakamana-2, and Population $35C_5$.

Rampur So3Eo2 showed the highest GCA value (1005.10) for grain yield among the lines, followed by Population $46C_1$ (181.27), and Hill pool yellow (1.55). Pool 17E and NML-2 had shown positive GCA values (424.60 and 262.90) among the testers. Based on GCA effects, Rampur So3Eo2, Population $46C_1$, and Hill pool yellow were good combiners among the lines. Similarly, good combiners among the testers were Pool 17E and NML-2 for grain yield. These good combiners are potential parents that may be used in hybrid breeding and population improvement programs.

Similarly, we estimated specific combining ability (SCA) of the 40 crosses for silking days, plant and ear height, number of kernels per ear, 100-seed weight, and grain yield. The crosses exhibited both positive and negative SCA effects for silking days (Fig. 2). Rampur So3Fo2 /NML-2 demonstrated the highest SCA effects (4.59) among the crosses, followed by Population $46C_1$ x Arun-4 (3.34), Khumal yellow x Rampur Composite (2.84), and Narayani x Pool17E. The highest negative SCA effect was observed in Population $46C_1$/Pool 17E (-3.83), followed by Population 35C5 /Arun-4 (-2.66), and Khumal yellow and Pool 17E (Fig. 2).

Rampur So3Fo2/ NML-2, Population $46C_1$ x Arun-4, Khumal yellow x Rampur Composite and Narayani/Pool 17E proved to be the best specific cross combinations among the crosses. Parents of the crosses showing higher negative SCA effects could be employed for developing early-maturing hybrids. Nineteen crosses showed positive

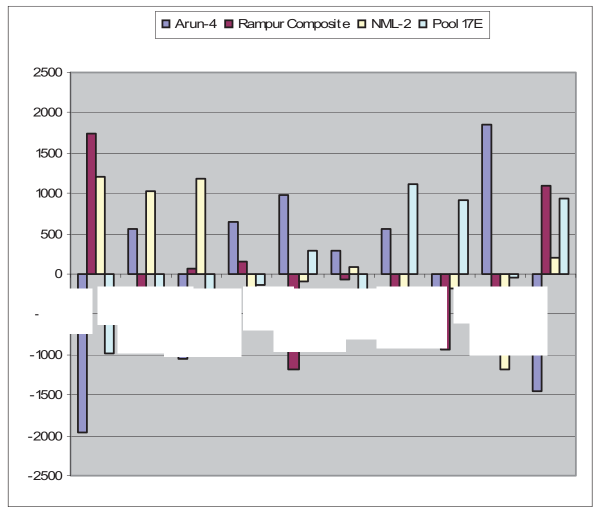

Fig. 2. Estimates of SCA effects of 40 crosses for grain yield.

Table 1. Estimates of general combining ability of yield and other important traits.

Line (Female)	Days to 50%silking	Plant height (cm)	Ear height (cm)	Ear length (cm)	Kernels/ear (no.)	100-seed weight(g)	Grain yield (kg ha^{-1})
Population $46C_1$	-2.57	-15.74	-16.55	-0.06	1.21	-0.44	181.27
Manakamana-2	0.59	-1.46	-1.83	-0.5	-23.89	-0.32	-79.09
Rampur So3Eo2	-0.82	-2.64	0.51	0.44	-22.44	2.24	1005.1
Population 35C5	0.25	-4.16	-5.25	0.03	7.08	-0.9	-80.01
Narayani	-2.24	5.01	2.24	-0.07	13.75	-1.54	-187.25
Hill pool yellow	0.01	10.2	7.81	-0.16	-5.06	0.37	1.55
Across 9331	-0.4	-1.31	1.57	-0.76	-16.89	-0.12	-609.19
Khumal yellow	1.09	7.42	10.47	0.48	-11.9	0.63	-138.66
Upahar	0.92	-2.02	-0.07	-0.28	12.12	-0.26	-9.41
Standard error (GCA for lines)	0.56	3.66	3.82	0.31	11.95	0.58	162.83
Tester (male)							
Rampur So3 Fo2	3.17	4.7	1.09	0.89	46.01	0.35	-84.28
Arun-4	-1.5	-6.45	-9.79	-0.22	-23.01	-0.56	-52.5
Rampur composite	-0.24	0.13	5.4	0.08	6.5	-0.54	-185.01
NML-2	3.75	8.59	8.34	0.21	10.86	2.14	262.9
Pool 17E	-2.01	-2.8	-3.95	-0.07	5.63	-1.03	424.6
Standard error (GCA for testers)	0.36	2.31	2.42	0.19	7.56	0.36	102.98

SCA effects and 21 crosses displayed negative SCA effects for grain yield. Population 46C_1/Arun-4 had shown the highest SCA effects (1852.24) for grain yield. Other crosses showing higher SCA effects were Population 46C_1/Rampur Composite (1740.53), Population 46C_1/NML-2 (1213.27), and Rampur So3Eo2 /NML-2.

Rampur So3Fo2/ NML-2 and Population 46C_1/ Pool 17E had higher negative SCA effect for silking days. These crosses were the best specific cross combinations for developing early-maturing hybrids. For plant height, Rampur So3Fo2/NML-2 and Rampur So3Eo2 were good specific cross combinations. Similarly, Khumal yellow /Pool 17E was a specific cross combination for ear height. Other specific combinations were Hill pool yellow /NML-2 and Hill pool yellow /Arun-4 for ear length. Population 46C_1/ Rampur composite showed the highest SCA effects for kernels per ear and was identified as the best combiner. Parents of crosses showing significant SCA effects for grain yield are ideal parents that can be used in the hybrid-breeding program.

Based on the positive GCA effects, NML-2 was the best combiner for all traits among the testers. Rampur So3Fo2 was a good combiner for all traits, except for grain yield. Khumal yellow was a good combiner, showing positive GCA effects for silking days, plant and ear height, ear length, and 100-seed weight. Rampur So3Eo2 was a good combiner for ear height, ear length, 100-seed weight, and grain yield. Similarly, Rampur composite was a good combiner for plant height, ear height, ear length, and number of kernels per ear. All these good combiners are potential parents for hybrid breeding as well as population improvement programs. Significant differences in silking, days, ear height, and grain yield for both GCA and SCA effects indicated the importance of both additive and non-additive gene action.

Conclusions

Rampur So3Eo2/ NML-2 was the highest grain yielder among the crosses. Population 46C_1, /Rampur Composite, Population 46C_1/NML-2, Upahar/Arun-4, and Rampur So3Fo2 /Pool 17E were the other high-yielding crosses. Parents of these crosses are potential parents for developing high-yielding hybrids. Crosses using NML-2 as male parent in cross combinations had shown higher heterosis and thus need to be to be exploited for heterosis.

Based on the positive GCA effects, NML-2 was the best combiner for all traits among the testers. Rampur So3Eo2, Khumal yellow, Rampur So3Eo2 and Rampur composite were identified as good combiners among the lines. These germplasm could be utilized in population improvement programs. Crosses such as Upahar/Arun-4, Population 46C_1/Rampur Composite, Population 46C_1 / NML-2, Rampur SoEo2/NML-2, Across 9331/Pool 17E, and Rampur So3Fo2 /Rampur composite were the best specific cross combinations for grain yield. They are ideal choices for developing high yielding nonconventional hybrids.

Acknowledgment

The authors are grateful to the Nepal Agricultural Research Council and the Hill Maize Research Project for providing financial assistance to this study. They are also grateful to the National Maize Research Program, Rampur, Nepal, for providing the seeds used in this experiment.

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Singh and Chaudhary

A method of increasing the efficiency of finding productive crosses in maize

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Abstract. Innovative methods in reducing the cost while increasing efficiency of breeding work are of great help to maize breeders. This study aimed to evaluate the usefulness of cluster-based mating using SSR and qualitative morphological markers in finding productive crosses (PC) in maize. In this study we used four datasets that consisted of 192 crosses derived from four sets of three diallel and a design II experiments in which three sets were inbred crosses and a set of variety crosses. The four sets of crosses including the check varieties were evaluated for yield in alpha lattice design with three replications. We used 39, 40, and 30 SSR markers to establish grouping of inbred lines in set A, B, and C, respectively. For set D, we used 19 agro-morphological traits in grouping the parent varieties. Using the four data sets, the results showed that inter-group crosses had greater number of PCs than those in intra-group crosses. Sixty-four percent (64%) of the PCs came from the inter-group crosses in set A. In set B, C, and D all the PCs were come from inter-group crosses. Further, clustered-based mating appeared more efficient in identifying productive crosses in maize by 12% than the non clustered-based mating, thereby reduced the cost of extensive field crosses and tests.

Keywords: cluster-based mating, intra group crosses, productive crosses, ssr markers

Introduction

The identification of parent lines that constitutes productive hybrids is the most costly and time-consuming activity in maize hybrid breeding. Per se performance of maize inbred lines does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988). Diallel analysis has been used as a conventional method of identifying parent lines that could be used as parents for synthetic and hybrid breeding. However, this method becomes impractical when a large number of lines is involved since the number of crosses produced increases proportionally with the number of lines used and eventually results to unmanageable size for making crosses and field testing. An alternative way to maximize the utilization of genetic germplasm at the same time reducing the cost involved is the use of cluster-based mating using molecular and agro-morphological data. Cluster-based mating is defined as crossing of lines or varieties coming from different groups which are established using some sets of criteria. Molecular markers are powerful tools to define heterotic groups and to assign inbred lines into existing heterotic groups (Melchinger, 1999). Among the molecular markers, SSRs have become the marker of choice for many genetic analyses. The SSR markers offer advantages in reliability, reproducibility, discrimination, standardization, and cost effectiveness over other marker types (Smith et al, 1997). Cuong et al (2007) and Magulama et al (2007a) used this SSR marker systems in determining promising crosses in maize. Qualitative morphology traits were also used in cluster-based mating in maize varieties (Magulama et al 2007b). This study aimed to evaluate the usefulness of cluster-based mating using broader sets of data derived from SSR and morphological markers in finding productive crosses (PC) in maize in most effective and efficient manner that would lead to better utilization of resources.

Materials and methods

Composition of data sets

In this study we used four data sets that involved a total of 192 crosses derived from two sets of inbred-diallel crosses, a set of inbred-design II crosses, and a set of variety-diallel crosses. Set A involved 11-yellow inbred diallel crosses forming 55 single crosses. Set B included 9-white inbred diallel crosses forming 36 single crosses. Set C comprised of 14 yellow QPM lines. These lines were crossed in design II mating based on derived SSR-based grouping resulting to 54 crosses. Set D constituted 10-white variety diallel crosses generating 45 variety crosses.

Field trials

The generated crosses from each set of crosses were evaluated for their yield in the experimental area of the

University of Southern Mindanao (14æ%N 30 meter above sea level) at different span of time from 2003-2006. The four yield trials were conducted with following dates, namely: set A in May to August 2004, set B in September 2005 to January 2006, set C in May to September 2005, and set D in May to August 2003. The entries in each trial were arranged in alpha lattice design (Patterson and Williams, 1976) with three replications. In all trials the experimental units were composed of three 3-m row plots spaced at 65 x 25 cm between furrows and between hills at two plants per hill. Plants in each plot were thinned two weeks after planting leaving one plant per hill. The plants were fertilized with complete fertilizer (14-14-14) at rate of 60-60-60 NPK per ha during planting, and urea (46-0-0) at rate of 60-0-0 NPK per ha 25 days after planting. Other recommended field management practices for optimum in maize production were used for each trial.

SSR analysis

We performed SSR analyses for parent lines in set A to C. DNA extraction was done following AMBIONET-CIMMYT service laboratory protocol (AMBIONET-CIMMYT, 2004). Six to 8 leaves of 10-day old corn plants were collected and bulked. DNA fingerprinting was performed with standard marker set of 40 publicly available SSR markers that provide an even coverage of the maize genome. The DNA was extracted using modified CTAB method (Saghai-Maroof et al., 1984). PCR reactions were performed in 10-*uL* volumes containing 2 ul of template DNA, 0.25 uM each of the primers, 1 x PCR buffer, 0.25mMdNTPs, 1.5 µM $MgCl_2$ and 0.38 *U Taq* polymerase. The reactions were performed with a Peltier Thermal cycler using the amplification conditions of 94^o C for 2 min; followed by 30 cycles of 94^o C for 30 s, X ^{o}C for 1 min, and 72 ^{o}C for 1 min; followed by extension at 72 ^{o}C for 4 min. Samples containing 2.5 ul of PCR products were loaded into the well of a 96 well comb with molecular weight marker at both ends within the gel. The bands were detected by silver staining method. Data were scored as present (“1”) or absent (“0”) for particular allele/maize line combination. Bands that were diffused or too difficult to score were considered as missing data (“9”). In cases when a line had multiple bands of varying intensity, the most intense band was scored as “1” and others as “9”.

Data gathered. For all the field trials, only the data on grain yield were presented and determined by weighing the ears harvested from three rows of each plot of the trial. The seed moisture content was recorded using electronic moisture tester. The grain yield of each entry was calculated using the formula as: Grain Yield = FW x CF x (100-MC)/100 x (80/85) x (10,000/area harvested) x 0.001, wherein: FW =Field Weight, CF=Correction Factor, MC=Moisture Content, CF=(PS-0.3(PS-SC))/SC, where, PS-perfect stand, SC-stand count at harvest.

Statistical data analysis

The data on grain yield were analyzed by ANOVA technique using *Alpha* software program (CIMMYT Maize Program, 1999). A matrix of binary data (0,1), with column equal to inbred lines, and rows equal to alleles of each primer, was compiled in MS Excel for the SSR data. Genetic similarities (GS) among pair-wise comparison of inbred lines were calculated using similarity coefficients developed by Jaccard (1908). Genetic similarity may range between 0 (all bands in common) to 1 (no bands in common). In developing the binary data (1 for present and 0 for absent) for qualitative traits, a score of 1 or 0 was given to the varieties depending on the presence or absence of the trait, respectively. The scoring was made on 19 qualitative traits. Genetic similarities (GS) among pair-wise comparisons of populations were calculated using Jaccard’s method. The dendrogram was constructed using the unweighted pair group method of arithmetic average (UPGMA). Cluster analysis was performed using the NTSys pc ver 2.1 software package (Rolf, 2002).

Results and discussion

Grouping of genetic materials using ssr and morphological markers

We used 39, 40, and 30 informative SSR markers for the genetic materials in set A, B and C, respectively, to establish grouping in each set of inbred lines or varieties. For Set D we used 19 qualitative agro-morphological markers. In Set A cluster analysis classified the 11 maize inbred lines into 3 distinct clusters. Cluster I included 4 lines, Cluster II- 5 lines, and Cluster III-2 lines. The grouping the 11 lines are shown in Figure 1a. In set B three clusters were noted, namely: Cluster I-6 lines, Cluster II-2 lines, and Cluster III-1 line (Figure 1b). In set C the 14 lines were grouped into 5 Clusters where Cluster I included 2 lines, Cluster II-4 lines, Cluster III-3 lines, Cluster IV-4 lines, and Cluster V-1 line (Figure 1c). In set D cluster analysis grouped the 10 varieties into two major clusters. Cluster I composed of 5 varieties and Cluster II constituted 5 varieties (Figure 1d). The results indicate that germplasm used in this study showed wide genetic diversity.

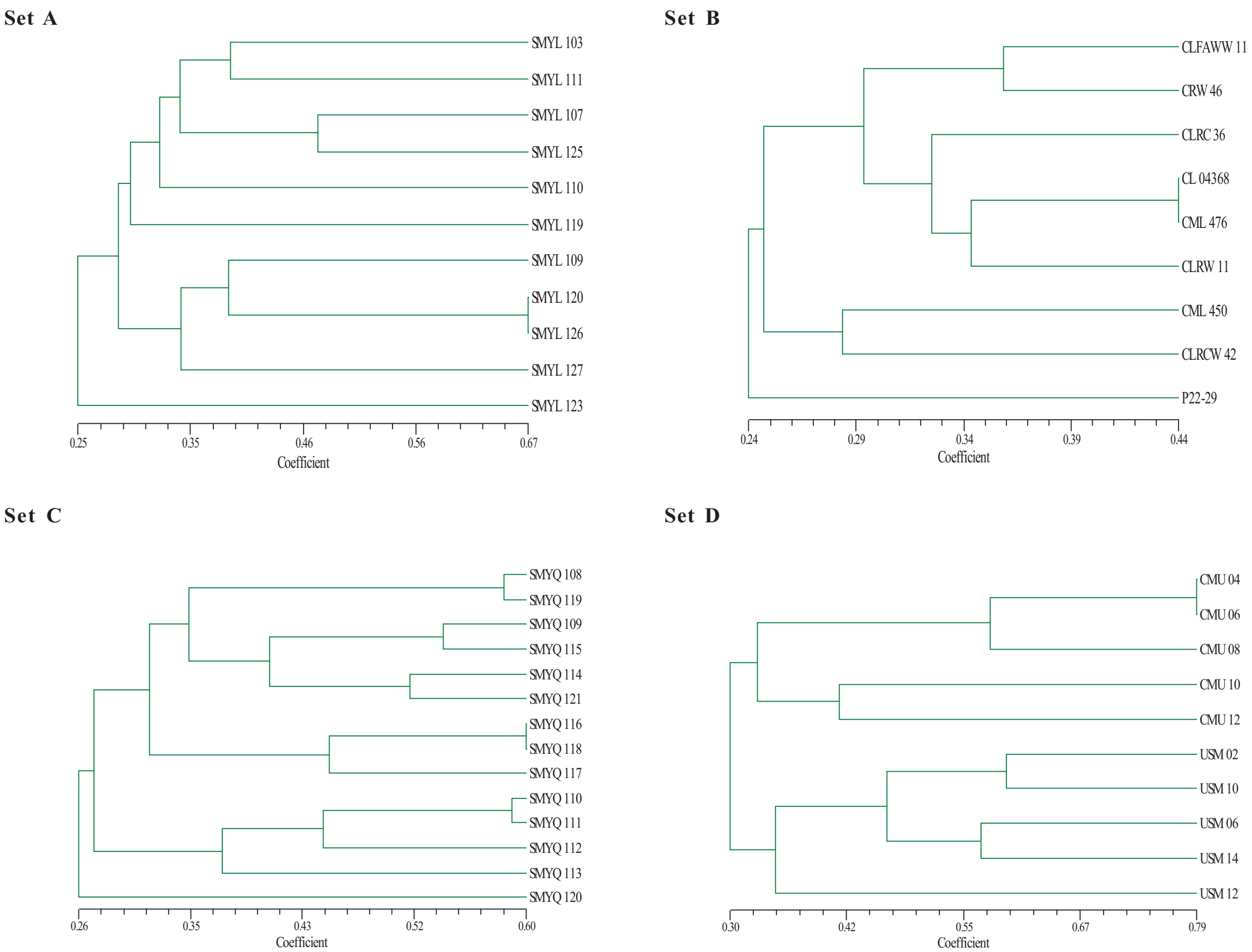

Figure 1. Dendrograms depicting the relationship of genetic materials in each dataset based on markers using UPGMA method. Set A-C=grouping of inbred lines based on SSR markers, Set D=grouping of varieties based on qualitative morphology traits.

Comparison of Intra-group and Intergroup crosses in generating productive crosses

To determine which form of crosses that generated more productive crosses, analyses on intra and inter group crosses were made. In set A to C, productive crosses (PCs) were selected based on the yield superiority of crosses over the hybrid checks. For set D PCs were selected based on the average yield of crosses in intra and inter group population crosses. In set A 64% of the PCs came from inter-group crosses and 36% from intra-group crosses. For set B, C, and D all the PCs were derived from inter-group crosses and none from the intra-group crosses. Table 1 presents the number of productive crosses obtained from intra and inter-group crosses. Several workers (Hallauer et al 1988, Melchinger and Gumber 1988, Bernardo 2001, Magulama 2007b) documented the yield superiority and better agronomic performance of inter-group over intra-group crosses in maize. Lanza et al (1997) and Parentoni et al (2001) reported that the correlation between mean yields of single-cross hybrids and the marker-based GD estimates, which was low, became stronger when correlation analysis was performed based on the combination of lines belonging to different groups established by markers. The use of marker-based clusters as basis for selecting inbred lines from different groups in order to make crosses may improve the chance to generate productive hybrids.

Efficiency between cluster-based and non-cluster-based group mating

Table 2 shows the comparison of efficiency between non cluster-based and clustering-based mating in finding superior crosses. In set A, using three clusters the number of PCs was comparable between non cluster-based (25%) and cluster-based mating (26%). Considering three clusters

Table 1. Number & percent of productive crosses obtained from intra & inter-group crosses.

Set No.	Group Crosses	No. of Productive Crosses[1]	Percent of Productive Crosses (%)
A	Intra-group	5	36
	Inter-group	9	64
	Total crosses	14	
B	Intra-group	0	0
	Inter-group	4	100
	Total crosses	4	
C	Intra-group	0	0
	Inter-group	6	100
	Total crosses	6	
D	Intra-group	0	0
	Inter-group	22	100
	Total crosses	22	

1/ The productive crosses were selected based on yield superiority over the check varieties.

Table 2. Comparison of cluster-based and non cluster-based mating in producing productive crosses.

Set No.	Group Crosses	Total No. of Crosses	No. of Productive Crosses (PC)	Percent of PC
A	Non cluster-based	55	14	25.45
	Cluster-based[a]	34	9	26.47
B	Non cluster-based	36	4	11.11
	Cluster-based	29	4	13.39
C	Non cluster-based	91	6	6.59
	Cluster-based	54	6	11.11
D	Non cluster-based	45	22	48.89
	Cluster-based	25	22	88.00

a/ The crosses were formed by mating between lines/populations that belonged to different groups as revealed by clustered analysis

in set B cluster-based mating (14%) produced more PCs than the non cluster-based mating (11%). Using five clusters in set C, more PCs were obtained from cluster-based mating (11%) than the non cluster-based (7%). Utilizing two clusters in set D, more PCs were noted from the cluster-based mating (88%) than the non cluster-based mating (49%). **A** comparable result regarding the efficiency between non cluster-based mating and SSR-based mating in finding promising crosses was reported (Magulama et al 2007a), although the former has the advantage of involving less number of crosses. In this present study, our results further support the greater efficiency of cluster-based mating over non cluster-based mating in finding productive crosses. The former mating could greatly save the utilization of resources that is making crosses and field-testing of materials.

Conclusion

Cluster-based mating appeared more efficient than non cluster-based mating in finding productive crosses in maize. It can greatly help in reducing the number of crosses thereby, reducing the cost and time in making crosses and field testing. The present study demonstrates the use of marker technology in accelerating plant breeding work.

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Development of abiotic stresses–tolerant hybrids in maize (*Zea mays L.*)

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Abstract. Maize (*Zea mays* L.) occupies a prestigious place in the world agriculture. Maize crop grown in tropics during summer-rainy season occasionally face extreme climatic conditions and various biotic/ abiotic stresses that severely limit crop growth and development and eventually yield potential. Among the abiotic stresses, low nitrogen and Excess Soil Moisture (ESM) caused by flooding, water logging or high water table or heavy soil texture are the most important constraints for maize production and productivity in the world and in Asian region. The present study was undertaken on maize in three conditions *viz.*, normal, low-N and Excess Soil Moisture (ESM) during 2007 with the main objectives of (i) Studying inter-character correlations, (ii) Estimating yield reduction under low-N and ESM conditions, (iii) Examining the combining ability of parental lines and hybrid combination (iv) Developing suitable selection indices in relation to low-N and ESM tolerance. The experimental material consisted of twelve lines and four testers and their 48 single crosses planted in Randomized Block Design in three replications in one row plot of 5 m length at Crop Research Centre of G.B.P.U.A. & T, Pantnagar, Uttarakhand, India. The observations were recorded on days to 50 per cent tasseling, days to 50 per cent silking, plant height , ear height, ear length, cob diameter, 100-kernel weight, grain yield, anthesis silking interval, leaf senescence, number of ears per plant and nodes bearing adventitious roots / stilt/brace roots. The mean values were subjected to statistical and genetic analyses, Analysis of variance, combining ability analysis, Correlation coefficient, Selection index (Smith-Hazel index).Analysis of variance revealed significance for all the traits studied in both conditions in all environments, clearly indicating there by the existence of high genetic variability in the genotypes. Reduction in yield was comparatively lower in tolerant genotypes compared to susceptible ones and the crossed generations showed varied pattern of response with regard to reduction in yield. The analysis of variance due to GCA and SCA was significant for all traits in both normal and low-N conditions and in all environments. Estimates of SCA variance were higher than GCA estimates in most of the cases under all the three conditions.

Introduction:

Among the abiotic stresses low nitrogen and excess soil moisture are the most common constraints for the maize production and productivity in the world and in Asian region. Indian soils have been characterized as being low in organic matter and N. Reasons for low N fertility in the tropics include poor soil types with low N mineralization, high run off/ leaching of applied fertilizer with heavy rainfall due to light texture soil and poor uptake of applied N due to problem of water stress. Similarly water logging or excess soil moisture (ESM) is also one of the most important constraints for crop production. In India 8.55 million hectare of arable land is in the grip of this problem. To address these problems crop improvement Programme that confer to tolerance to these environmental stresses needs to be taken up in a big way. Development of abiotic stresses tolerant hybrids is a viable option to ensure the global food security.

Material and Methods:

Twelve Inbred lines derived from source population 33 and pop.45 and four testers representing two heterotic groups (pop 445, pop 446 and pop 421 and pop 423) which were screened previously for their reaction to ESM and Low N stresses were crossed in a line × Tester fashion. Test crosses thus developed along with parental inbredlines and four testers were evaluated in three separate experiments. Experiment I was planted under optimal conditions where recommended cultural practices were applied. Experiment 2 was planted under ESM conditions where excess moisture treatment was applied by providing 5 cm of ponding water for six days. The treatment was with drawn by draining out the ponding water. Experiment 3 was conducted in a field where no nitrogen was applied.

Details of the inbred lines and Testers:

Parents	Pedigree	Coded pedigree
1.	POB. 33 C_3-12-2-1-1-2-2	L_1
2.	POB. 33 C3-12-2-1-2-2-5	L_2
3.	POB. 33 C3-142-1-6-1-1-4	L_3
4.	POB. 45 C8-86-1-3-7-6-4	L_4
5.	POB. 45 C8-45-2-6-1-2-7	L_5
6.	POB. 45 C8-269-2-4-6-3-3	L_6
7.	POB. 45 C8-86-1-1-7-5-1	L_7
8.	CLG 1708 -1-9	L_8
9.	POB. 45 C8-45-2-6-1-1-1	L_9
10.	POB. 45 C8-86-1-3-4-5-2	L_{10}
11.	POB. 45 C8-86-1-3-2-2-5	L_{11}
12.	POB. 45 C8-269-2-4-6-6-1	L_{12}
13.	POB. 455 Ä 58-6-3-B-B-B	T_1
14.	POB. 446-74-2-B-B-B	T_2
15.	CML-421	T_3
16.	CML - 423	T_4

Results and discussion

Reduction in yield

In the present study the per cent yield reduction in case of low – N condition ranged from 9.31 per cent in L_2 to 68.57 per cent in L_9. Among the testers, the range varied from 4.3 per cent in T_3 to 16.26 per cent in T_1. Among the crosses, the range was from 0.37 per cent in L_8T_1 to 83.25 per cent in L_7T_1. Similar reduction in yield was also observed by Banziger and Lafitte (1997), and Logrono and Lothrop (1997). Comparison of performance of hybrids and their parents under low –N indicated that, in general most of the hybrids showed relatively more susceptibility to low –N stress than inbred lines. One of the reasons for relatively less susceptibility of inbred lines may be because of their less requirements due to short plant stature and less yield potential in comparison to hybrid progenies, and the required amount of N demand was fulfilled through the amount of N available through N mineralization in soils (Presterl *et al*., 2002),

In ESM conditions, the per cent yield reduction varied from 20.35 per cent in L_2 to 85.88 per cent in L_9 in case of lines. For testers the range was from 3.87 per cent in T_3 to 80.46 per cent in T_4. Among the crosses the lowest reduction of 19.11 per cent was found in L_8T_2 and maximum yield reduction of 84.73 per cent was observed in $L_{12}T_2$. Similar reduction in yield was also observed, when ESM stress was given at knee – high stage by Zaidi *et al*. (2003). Carter *et al*. (1990) reported that yield form the crop stressed for 9 days of ESM during vegetative and tasseling/ silking stage were 77 and 61 per cent less, respectively, than those from drained treatment.

Combining ability analysis:

For Days to 50 per cent tasseling, inbreds L_7, L_9, L_{10} and L_{12} were found to be good general combiners and indicated earliness in crosses in normal, low –N and ESM conditions. For Days to 50 per cent silking L_7, L_{12} and T_1 were good general combiners. Tester T_1 showed negative and significant GCA effect in all three conditions. L_7 and T_3 were good general combiners for Dwarfness whereas L_3, L_9 and T_4 were good general combiners for Tallness. For Ear height, L_1, L_5, L_6, L_{11} and T_4 showed significant GCA effect in positive direction and L_7, L_{10}, L_{12} and T_3 showed significant GCA effect in negative direction in both the stress conditions. Under low – N conditions, L_4, L_9, L_{10}, L_{11}, T_1 and T_4 were found to be good general combiners for Cob length whereas in ESM condition L_6, L_9, L_{10}, L_{11}, L_{12}, T_1 and T_4 showed significant GCA effect in positive direction. For Cob diameter, L_4, L_7, L_{11}, and T_2 were found to be good general combiners in low –N as well as in ESM conditions. For 100 - kernel weight, L_1, L_2, L_7, L_8, L_9 and T_2 proved to be good general combiners in ESM conditions in low – N conditions. For yield, L_1, L_6, L_8, L_{10}, L_{11}, and T_4 exhibited the potential of being good general combiners in both low – N and ESM conditions. In low – N conditions, in addition to the above parents, L_4 and T_3 also showed good general combining ability for yield. For Leaf senescence, lines L_1, L_7, L_9, L_{11}, and L_{12} showed significant GCA effect in positive direction whereas L_3, L_6, L_8 and L_9 showed significant GCA effect in negative direction in low – N conditions. However, none of the testers were observed to be good general combiners for leaf senescence. Parent L_{10} and T_1 were good general combiners for nodes bearing adventitious roots in ESM conditions. For number of Ears per plant, L_1, L_2, L_4 and T_4 were good general combiners in low – N conditions while in case of ESM conditions L_4, L_5 and T_4 were good general combiners for this trait.

The SCA effect represents mainly dominance, additive × dominance, and dominance × dominance effect. The crosses showing SCA effects involving parents with good GCA could be exploited in the form of hybrid varieties. Crosses L_2T_1, L_3T_4, L_5T_4, L_9T_4 and $L_{10}T_2$ showed good GCA estimates for Days to 50 per sent tasseling in normal, low-N and ESM conditions. For Days to 50 per cent silking, L_1T_2, L_3T_4, L_5T_4, L_6T_3, L_7T_2 and L_8T_2 showed good GCA estimates in all the situations. The higher SCA estimates for 100 kernal weight in L_1T_3, L_3T_2, L_5T_2, L_5T_3, L_6T_3, $L_{10}T_1$, $L_{10}T_4$, $L_{11}T_1$, $L_{11}T_3$, and $L_{12}T_4$ in ESM conditions and crosses

L_2T_4, L_4T_1, L_4T_4, L_5T_1, L_5T_2, L_6T_2, $L_{10}T_1$, $L_{11}T_3$,and $L_{12}T_4$ in low –N conditions resulted the high SCA estimates for their yields correspondingly. For leaf senescence, L_1T_2, L_2T_2, L_3T_2, L_4T_1, L_5T_3, L_6T_2, L_6T_4, L_7T_4, L_8T_3, L_8T_4, L_9T_3, $L_{10}T_1$, $L_{10}T_4$, $L_{11}T_1$, $L_{12}T_1$ and $L_{12}T_2$ were good combiners indicating delayed leaf senescence. Genotype with delayed leaf senescence are relatively more low – N tolerant (Banziger et al., 1997). Higher positive SCA estimates were found for number of Ears per plant in crosses L_2T_4, L_4T_1, L_6T_2 L_7T_2, L_8T_1, L_8T_2, L_9T_3, $L_{10}T_1$ and $L_{11}T_4$ in both low – N and ESM conditions. In the present study as the results revealed that combining ability varied in both normal and low –N condition, many inbred lines themselves may have high GCA effects or may have poor GCA effects but showing high SCA effects may combine well with other inbreds.

Inter character correlation

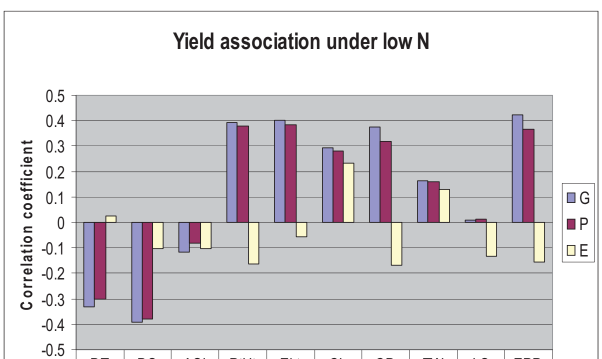
The yield showed significant and positive correlation with Plant Height, Ear height and Cob length in all three conditions i.e., normal, low –N and ESM conditions. However under normal condition, yield was also positively and significantly correlated with Cob diameter and 100 kernal weights. Positive and non – significant correlation of yield was found with nodes bearing adventitious roots in normal and ESM conditions. In low–N conditions yield showed positive and non significant correlation with leaf senescence, this was also the case with normal condition. Kebede (2007) also reported positive correlation between yield and leaf senescence.

Selection Index

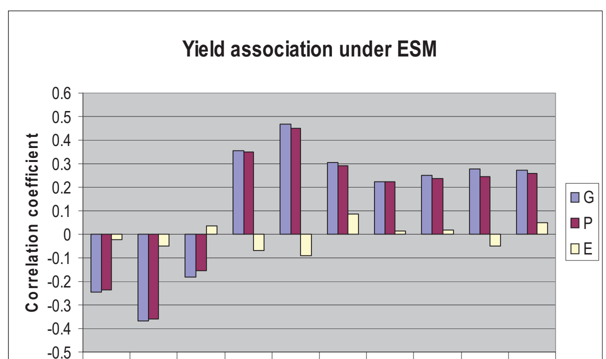
It has been recognized that most rapid improvement in the economic value is expected from selection applied simultaneously to all the characters which determine the economic value of a plant, provided appropriate weights are assigned to each character according to their relative economic importance, quality considerations, heritability and correlation between characters. Assigning of economic weights is thus a complex matter.

Among the parents, none of the parents ranked high among all genotypes according to the assigned selection criteria in normal low – N and ESM conditions. Among the crosses aggregate score were higher in most of the cases but general trend was that ESM traits had the lower score values. Crosses $L_{11}T_4$, L_8T_1, $L_{11}T_3$, L_7T_2 and L_6T_2 performed well in both normal and low –N conditions for the assigned selection criteria while crosses L_5T_4 and $L_{12}T_2$ performed poorly in low –N conditions. In ESM trials, L_6T_4, L_7T_2 and L_4T_1 performed excellent while crosses L_5T_1, $L_{12}T_2$, L_5T_4 and L_3T_3 ranked very low in ESM trials.

In case of ESM tolerance we can deduce from the results that crosses L_6T_4 and L_7T_2 are the best available crosses according to the given selection criteria. For low – N tolerance, $L_{11}T_4$ and L_8T_1 were the best crosses. Similar studies with different objectives were conducted by Brim et al. (1959), Mulamba and Mock (1978).

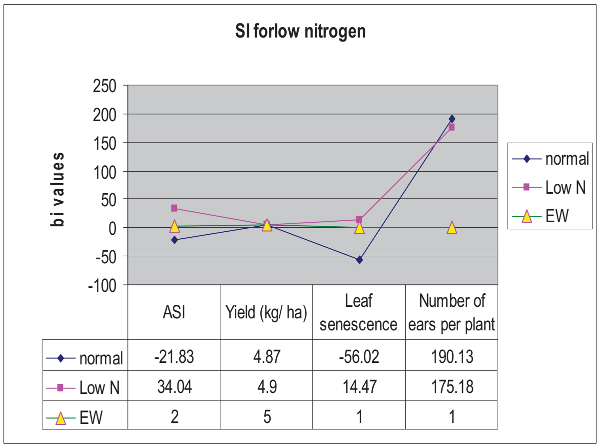


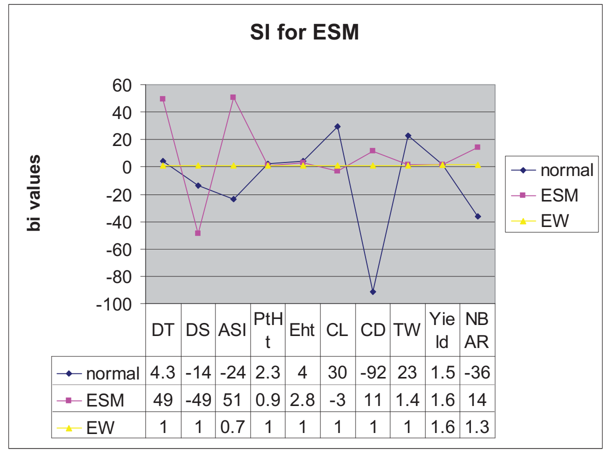
	DT	DS	ASI	PtHt	Eht	CL	CD	TW	LS	EPP
G	-0.333	-0.392	-0.118	0.394	0.4	0.292	0.376	0.162	0.007	0.422
P	-0.303	-0.378	-0.083	0.379	0.384	0.279	0.319	0.159	0.011	0.365
E	0.026	-0.104	-0.104	-0.164	-0.054	0.232	-0.17	0.129	-0.135	-0.154



	DT	DS	ASI	PtHt	Eht	CL	CD	TW	LS	EPP
G	-0.245	-0.368	-0.181	0.356	0.468	0.303	0.225	0.25	0.276	0.275
P	-0.237	-0.358	-0.153	0.348	0.449	0.293	0.221	0.236	0.245	0.257
E	-0.021	-0.048	0.036	-0.07	-0.089	0.085	0.013	0.018	-0.048	0.049

Figure 1.Character Association under low –N and ESM Conditions





DT = Days to 50 percent Tasseling, DS = Days to 50 percent Silking, ASI = Anthesis Silking Interval, Pt Ht. = Plant height in cms, E ht = Ear height in cms, CL = Cob length in cms, CD = Cob diameter, T W = 100 kernel weight in gms, LS = leaf Senescence rating, EPP = Number of Ears per plant, ESM = Excess Soil Moisture, SI = Selection Index, EW = Economic Weight

Figure 2. Indices for low – N and Excesses Soil moistures conditions

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Performance stability of quality protein maize genotypes across hill environments of Nepal

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Abstract. Maize (*Zea mays* L.), the second most important staple food crop after rice in Nepal and a major food crop in the hills. Out of total maize production area (8, 70, 401 ha) of Nepal, hill and high hill environments occupies 80.5 % (6, 84,990 ha.). The majority of hill farm families are suffering from protein malnutrition. Considering this problem present study was undertaken since 2004 summer season. QPM synthetics have nearly double the essential amino acids: lysine and tryptophan than the normal maize. This study conducted in the hills of Nepal in three years to determine performance stability of open pollinated QPM genotypes in comparison to open pollinated cultivars of normal maize (non-QPM). Replicated field experiments were conducted in 13 environments using selected 6 QPM genotypes. The normal maize included released and pre released cultivars (Manakamana-3, Rampur Composite, Sitala and Population 45 C10) and one local check (farmers' variety). Stability and genotype superiority for grain yield showed that the highest yielding QPM genotype: S99 TLWQ-HG-AB in each year had significantly higher grain yield than the local check and higher or comparable yield to the improved check. Across years, QPM genotypes i.e. Corralejo S99 SIWQ, Celaya S91 SIWQ and S 99 TLYQ-B produced significantly higher grain yield than the local check. The findings of this study provide new information on stability of the open pollinated QPM genotypes tested. These cultivars are also adapted to other developing countries, and this information could be useful for international and national QPM improvement programs.

Key-words: Genotypes, Lysine, Quality protein maize, Synthetics, Tryptophan.

Introduction

Maize (*Zea mays L*) is the second most important cereal crop after rice in Nepal, and a major food crop in the hills. The total area under maize is 8, 70, 401 hectare in Nepal. Out of this area, 70.1 % (6,09,870 ha.) belongs to mid hills and 8.8 % (90,480 ha.) is under high hills, with the productivity of 2094 kg^{-ha} and 1770 kg^{-ha} respectively. In general, out of total maize area 80.5 % occupied by hill environments of Nepal. The majority of hill farm families especially infants, pregnant women, lactating women and elderly persons are suffering from quality protein malnutrition. Considering this problem present study was undertaken for the identification, development, and promotion of QPM maize varieties for commercial cultivation in the hills. All the OPVs and hybrid varieties of maize released so far are normal type. Their nutritional quality is poor as it is deficient of two essential amino acids Lysine and Tryptophane. New QPM synthetics have special characteristics features such as low and uniform ear placement, resistance to ear rot and root lodging and most notably levels of tryptophan (0.11% of the whole grain), lysine (0.475% of the whole grain) and protein (11.0% of the whole grain) far beyond those contained in normal maize (0.05%, 0.225% and 8.5%).These features make the QPM synthetics / OPVs particularly attractive to farmers (Cordova H., 2000). Lysine and tryptophan are the most essential amino acids for protein synthesis in human and monogastric animals. For humans, lysine is the most limiting amino acid in maize protein (Kies et al., 1965) and tryptophan the second.

The nutritional benefits of QPM for people who depend on maize for their energy and protein intake, and for other nutrients, are in did quite significant Several researchers later demonstrated the superior protein quality and protein digestibility of QPM over normal maize (Paes, M. *et al* 1995, Bressani, 1995 and Graham, G. *et al.* 1980. At least four studies on children and four on adults have found that subjects eating QPM had significantly higher nitrogen retention than those who ate normal maize

(Bressani,1991), indicating that QPM protein is more "bioavailable" (NRC, 1988). The biological value of QPM protein is about 80% that of milk is about 90% and that of normal maize is about 45% (FAO 1992).

Materials and Methods

Exotic QPM germplasm (from CIMMYT) being tested in Nepal since 1998. Series of Observation Nurseries were evaluated. Superior 20 OPVs of QPM were evaluated from Observation Nurseries to IYT, CVT, and CFFT respectively. On farm, experiments i.e. Coordinated Farmers' Field Trial (CFFT) conducted across hills in Nepal using 6 most superior QPM OPVs under farmer-managed rain fed condition in RCB Design during 2004 to 2006 summer seasons (March to September). There were 13 different environments (year-site combinations) in the three years. Each experimental plot of 13.5 m^2 was seeded at the standard seed rate of 20 kg ha^{-1} and net area harvested was 9.0 m^2. The spaces between row-to-row and plant to plant were 75 and 25 cm. respectively. Two seeds per hill were planted and thinned to single plant per hill after first weeding. Fertilizer were applied at the rate of 120:60:40 kg ha^{-1} N: P_2O_5 : K_2O respectively in addition to 15 t farm yard manure (FYM) ha^{-1}. Half dose of N and full dose of P and K applied as basal dose. The remaining half of the N applied as a side dressing at knee-high stage. The plots were kept free of weeds manually. Traits in each plot were recorded: days to 50 % tasseling and silking (5 plants in each plot), plant and ear height. Data on plant aspect, (plant and ear height, uniformity of plants, disease and insect damage and lodging) in each plot was recorded at the brown husk stage on a scale of 1 to 5, where 1 and 5 represents excellent and poor respectively. At maturity, husk cover was rated on a scale of 1 to 5. After harvest, all ears from a plot were placed in a pile and ear aspect (size, disease and insect damage, grain filling and uniformity) was recorded on a scale of 1 to 5. The plots were harvested individually. Grain yield adjusted to 80% shelling recovery from the de-husked cob weight /plot. Grain moisture content for each plot was recorded and grain yield was adjusted to 15 % percent moisture basis. MSTATC and Excel programme were used for statistical analyses.

Results and Discussion

Results of 2004 summer season revealed that out of 6 genotypes evaluated, the genotype S99 TLWQ-HG-AB produced the highest grain yield (7158 kg ha^{-1}) followed by Celaya S91 SIWQ (6466 kg ha^{-1}) and Manakamana-3 (Improved check normal maize) 6412 kg ha^{-1} respectively across hill environments. These QPM genotypes were statistically at par with Improved Check and significantly superior (P=>0.01) then the Farmers' Local Check variety. However all the tested QPM genotypes were significantly higher grain yielder then the Farmers' Variety. For days to 50 % flowering Farmers' Variety was earliest followed by Corralejo -S99 SIWQ and Celaya S91 SIWQ respectively. Genotype S99 TLYQ-B was significantly earlier and S99 TLWQ-HG-AB was at par for days to 50 % flowering than the Improved Check. Genotype Corralejo -S99 SIWQ had the significantly shortest ear height (110 cm) followed by Celaya S91 SIWQ (121 cm) and Farmers' Variety (127 cm) respectively. Manakamana- 3 had the tallest ear height (139 cm) followed by S99TLYQ-B (137 cm) and S99 TLWQ-HG-AB (135 cm) respectively. All the tested genotypes were statistically at par for plant height. (Table 1).

Table 1. Combined Analysis of QPM genotypes on CFFT across hill environments (Pakhribas, Lumle, Dailekh and Kabre) 2004 summer.

S. No.	Genotypes	Days to 50% Silking	Plant ht. (cm)	Ear ht. (cm)	*E. tur.* (1-5)	Plant aspect (1-5)	Ear aspect (1-5)	Grain yield ha^{-1}
1	S99TLYQ-B	74	251	137	2.9	1.6	2.8	5732 bc
2	S99TLWQ-HG-AB	76	257	135	2.3	1.6	2.4	7158 a
3	Celaya S91 SIWQ	71	243	121	2.3	1.6	2.6	6466 ab
4	Corralejo -S99SIWQ	69	236	110	2.8	2.1	1.9	6412 ab
5	Mana-3 (Imp.Chk.) (non QPM)	78	268	139	2.7	2.5	1.8	5848 bc
6	Farmers' Var. (non- QPM)	67	250	127	2.8	2.5	2.3	5457 c
	Grand Mean	**73**	**251**	**128**				**6179**
	F-test	**	NS	**				**
	CV%	4.3	10.4	13				15.4
	LSD 0.05	2.5		14				777
	Mean of Locations	4	4	4	2	4	4	4

Note: The data summarized from 16 replications.

From the results of 2005 summer season, it is realized that out of 7 genotypes evaluated, the genotype S99 TLWQ-HG-AB produced the highest grain yield (5299 kg ha^{-1}) followed by Pop. 45 C_{10} (5135 kg ha^{-1} normal maize) and Manakamana-3 (Improved check normal maize) 4961 kg ha^{-1} respectively across hill environments. These QPM genotypes were statistically at par for grain yield except Celaya S 91 SIWQ. All the tested genotypes were statistically superior (P= > 0.01) then the Farmers' Local Check. However all the tested QPM genotypes were significantly higher grain yielder then the Farmers' variety. (Table 2).

From the results of 2006 summer season, it is realized that out of 8 genotypes evaluated, the genotype S01 SIWQ-3 produced the highest grain yield (6038 kg ha^{-1}) followed S99 TLWQ-HG-AB (5927 kg ha^{-1}) and S99 TLYQ-B (5486 kg ha^{-1}) respectively across hill environments. These QPM genotypes were statistically at par for grain yield. All the other genotypes evaluated were statistically superior (P= > 0.01) then the Farmers' Local Check and Corralejo S99 SIWQ. However all the tested QPM genotypes were significantly higher grain yielder then the Local Check. Farmers' Local Check was the tallest genotype followed by Rampur Composite and S 99 TLYQ-B for both plant and ear height (Table 3).

Table 2 Combined Analysis of QPM genotypes on CFFT across hill environments (Pakhribas, Lumle, Dailekh and Kabre) 2005 summer.

S. No.	Genotypes	Days to 50% Silking	Ear ht. (cm)	Plant aspect (1-5)	Ear aspect (1-5)	*E. tur.* (1-5)	Husk Cov. (1-5)	Grain yield kg ha^{-1}
1	S99 TLYQ-B	80	121	1.7	2.7	2.2	2.9	4844 AB
2	S99 TLWQ-HG-AB	82	118	1.9	2.3	2.1	2.8	5299 A
3	Celaya- S-91 SIWQ	79	105	1.9	2.1	2.2	2.3	4614 BC
4	Corralejo-S99 SIWQ	78	108	1.8	2.0	2.4	2.2	4855 AB
5	Pop-45 C_{10} (Imp.Chk.) (non-QPM)	78	112	2.1	2.4	2.3	2.2	5135 AB
6	Mana-3 (Imp. Chk.) (non-QPM)	80	135	2.1	2.0	2.1	2.2	4961 AB
7	F. Local (Check) (non-QPM)	73	136	3.0	2.4	2.4	1.1	4124 C
	Grand Mean	**79**	**119**	**2.1**	**2.3**	**2.2**	**2.2**	**4833**
	F- Test	**	**	**	NS	NS	**	**
	C V %	5.4	21	35.2	45.95	17	37	17.1
	LSD (0.05)	2.7	16	0.47			1.0	517
	Mean of Locations	4	4	4	4	2	2	4

Note: The data summarized from 16 replications.

Table 3. Combined Analysis of QPM genotypes on CFFT across hill environments (Pakhribas, Lumle, Dailekh, Kabre and Khumaltar) 2006 summer.

Genotypes	Days to 50%	Plant Ht Silking	Ear ht. (cm)	*E. tur.* (1-5)	Grain yield (1-5)	DMR Test (kg ha^{-1}
S01 SIWQ-2	73	227	112	1.9	5214	BC
S01 SIWQ-3	76	236	121	2.1	6038	A
S99 TLYQ-B	74	238	129	2.2	5486	ABC
S99 TLWQ-HG-AB	**76**	**230**	**122**	2.0	**5927**	**AB**
Celaya S91 SIWQ	73	232	119	2.0	5266	ABC
Corralejo S99 SIWQ	72	222	108	2.5	4904	C
Ram. Com. St. Chk. (non-QPM)	73	241	132	2.2	5459	ABC
F. Local Check. (non-QPM)	70	249	143	2.5	4813	C
G. Mean	73	234	123	2.2	3388	
F-test	**	NS	**		**	
CV %	4	13	15		21	
LSD 0.05	2	19	11		706	
Mean of locations	5	5	5	3	5	

Mean table of 21 farmers as 21 replications.

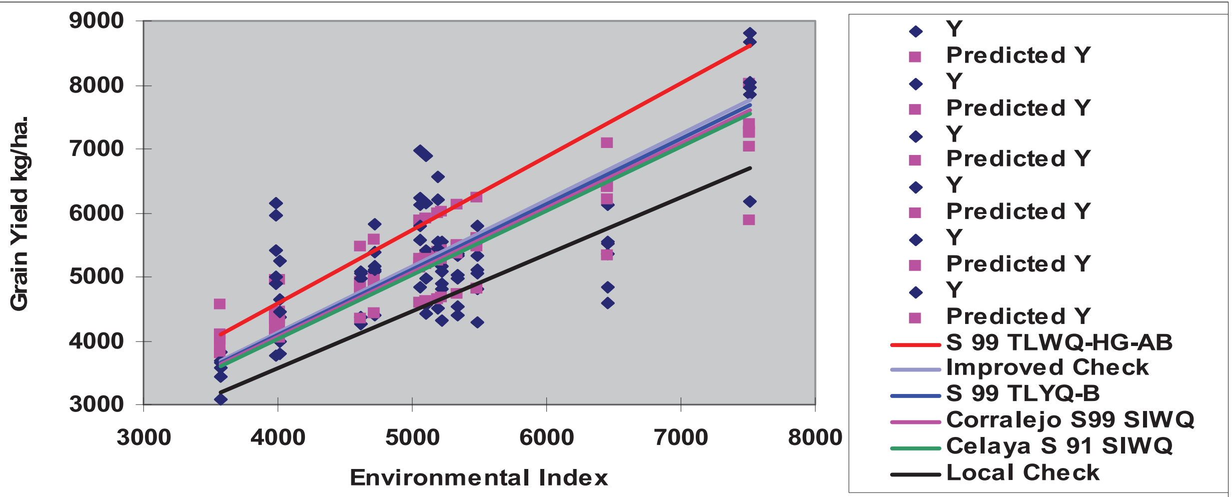

Fig.1: Pattern of stability for grain yield of QPM maize menotypes in CFFT across 13 hill environments of Nepal.

Table 4. Mean Grain Yield and Estimate of Stability Parameters in Selected Maize Genotypes.

Genotypes	Mean	b	R^2	SE
S99 TLYQ-B	5098	0.83	0.95	458
S99 TLWQ-HG-AB	5904	0.87	0.90	778
Celaya- S-91 SIWQ	5177	0.76	0.89	764
Corralejo-S99 SIWQ	5180	0.92	0.99	849
Imp. Chk. (Mana-3/RC)	5308	0.80	0.91	918
Farmers' Var. (L. Chk)	4609	0.52	0.48	601
Grand Mean	**5213**			

Stability analysis showed that genotypes S99 TLWQ-HG-AB and Improved check have higher genotypic mean for grain yield than grand mean across environments. These genotypes were near to average stability. Farmers' Local Check and Celaya- S-91 SIWQ observed below average stability (Table 4). However, in general, all the tested genotypes produced low grain yield in poor environments and higher grain yield in rich environments (Fig. 1).

Conclusion

Exotic QPM genotypes tested under 13 hill environments of Nepal showed significant variation for grain yield, days to flowering, plant and ear height, husk cover tightness and plant aspect. There were QPM genotypes out yielded the normal checks, were also highly stable. QPM genotype, S99 TLWQ-HG-AB was observed stable and higher grain yielder than the normal checks. Concluding results revealed that the genotype S99 TLWQ-HG-AB performed very well across hill environments and liked by farmers due to its' higher productivity, white grain colour, stay green and non lodging plant type. It has been proposed for release as a variety for commercial cultivation to the hill farmers of Nepal.

Acknowledgements

The authors are grateful to the Hill Maize Research Project (HMRP) due to which support this study was conducted. We would like to appreciate the work of Mr. J. B. Chhetri, Mr. N. L. Dhakal, and Mr. R. P Neupane., Mr. R. P.Ghimire of NMRP Rampur for the involvement on the QPM research. Finally, we would also like to express our sincere thanks to all collaborating station chiefs and technical staff.

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Study on the use of Quality Protein Maize (QPM) silage for dairy cattle in urban area of DKI Jakarta

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Abstract. A series of experiment has been conducted to investigate the effect of the addition of Quality Protein Maize (QPM) silage in the ration on the milk quality and production of dairy cattle which are reared in urban area of DKI Jakarta Province. The first step of the experiment was aimed at comparing silage quality after 15, 45 and 90 days of preservation period. It was revealed that the silage was best preserved at 45 days. This silage was then use in the second experiment using a total of 10 dairy cattle which were divided into 2 groups of 5 cattle and given either one of two rations provided, i.e. a) the Control or farmers' ration, and b) the Silage of the QPM maize. The investigation lasted for 6 weeks, comprising of a 2 weeks of preliminary period and a 4 weeks of measurement period. The data were collected 3 times a week and 2 times daily according to the milking time. All data were statistically analysed using the *t-test* method. It was found that the use of the QPM maize silage increased milk production from 11.1 to 11.5 kg 4% FCM/head/day, and milk protein from 2.63 to 2.73%. However, it did not have any effect to the milk density, but caused a decrease in the fat content from 3.92 to 3.35%. Therefore, the silage of QPM maize may be recommended to be used as an alternative feed resources for dairy cattle reared in DKI Jakarta area.

Keywords: Dairy Cattle, Silage, QPM Maize, Milk Production

Introduction

Dairy cattle have been raised, in urban area of DKI Jakarta, for many generations by the local dairy farmers, known as the "Betawi" Tribes. There are more than 4.000 heads of dairy cattle in this area, where most of them are located at Pondok Rangoon, east of Jakarta, situated right at the border between Jakarta and Bogor area. However, the amount of milk produced by these cattle is relatively low, only between 5 to 10 litres/head/day (LFM Office, 2007). These are due to the fact that they are usually fed with low quality field grasses collected from the surroundings area of Jakarta, as well as with various agricultural by products or wastes collected from traditional markets, such as corn peelers, cabbages, cassava leaves, etc.

The biomass of the quality protein maize (QPM), comprises of the stems, leaves and the young corns, is potential to be used as an alternative source of roughage for dairy cattle. It is assumed that the biomass would have a better protein quality as the maize is known to contain high level of essential amino acids particularly the lysine and tryptophan (Vasal, 1994; Cordova, 2001). Therefore, it could be used to improve the feed quality of the dairy cattle and it would then be expected to be able to improve the quality and the amount of milk produced by the cattle. It has been reported that the amount of biomass of the whole QPM plant, when harvested at 70 days after planting, is up to 70 ton/ha (Akil *et al*., 2004). In order to provide a continuous supply of the roughage for dairy cattle reared in the urban area, it would be better if the biomass is preserved in the form of silage.

This paper reports the result of experiment utilizing the silage made from the biomass of the yellow QPM maize, called the Srikandi Kuning-1, which is on of the newly released QPM maize variety in Indonesia (CRIFC, 2005). The aim of this experiment was to investigate the effect of the addition of this silage in the ration on the milk quality and production of dairy cattle.

Methods

The QPM maize was first planted on an approximately 3.000 m^2 of land and was harvested at 70 days after planting. The first step of the experiment was aimed at comparing the silage quality between 15, 45 and 90 days of preservation period. The silage was made using a starter mixture comprising of rice bran, molasses and probiotics. It is evaluated based on its quality, texture, odour and appearance.

The best quality silage from the first experiment was then used in the second experiment, conducted at Pondok Rangoon dairy farm, using 2 groups of 5 dairy cattle which were kept in individual pens. The cattle were fed with either one of the two rations provided, i.e. the Control and the silage of QPM maize. The Control ration is the ordinary ration used by the farmer, consisted of 30% field grass, 30% tofu by product from tofu industry, 15.4% corn mills, 9.4% rice bran, 14% by product from beer factory and 12% protefeed (Table 1). The silage ration was formulated using similar ingredients as for the Control ration, but 50% of the field grass was replaced by the QPM silage. The experiment lasted for 6 weeks, comprising of a 2 weeks of preliminary period and a 4 weeks of measurement period, in order to collect data on the quality and quantity of milk produced by the animal. The data were collected 3 times a week and 2 times daily according to the milking time. All data were statistically analysed using the *t-test* method.

Results

It was revealed from the first experiment that the best silage was produced after preserving the QPM maize biomass for 45 days. This silage had a better appearance, texture and odour as well as had a better quality as it contained 33.2% dry matter and 9.2% crude protein (Table 2). The level of protein is comparable to the protein content in the grain of similar type QPM maize (9.95%) as reported by Suarni and Firmansyah (2005), but it was slightly lower than (10.4%) those reported by Azrai (2004).

There was no effect of the QPM silage inclusion in the diet to the milk acidity (pH) and density, but it caused a decrease in the milk fat content from 3.92 to 3.35% and an increase in the milk protein from 2.64 to 2.73% (Table 3). This shows that the increase in feed quality, due to the inclusion of the QPM silage in the ration, had a high correlation with an increase in the protein contained in the milk. Furthermore, the QPM silage also caused an increase in the amount of milk produced by the dairy cattle from 11.1 to 11.5 kg 4% FCM/head/day. The amount of milk produced was similar to those reported by Suryahadi *et al.* (2003), which was 11.4% kg 4% FCM/head/day for dairy cattle reared in south Jakarta and fed with a similar diet as the Control ration.

The daily fluctuation of milk produced by the two groups of animal is shown in Figure 1. The milk production at the beginning of observation was similar for both groups, with an average of 10.57 kg 4% FCM/head/day. Then the amount of milk produced increased on the third day to 12.03 kg 4% FCM/head/day, but it decreased on the fifth day to 10.57 kg 4% FCM/head/day for the Control group, and it only slightly increased for those received the Silage ration. The fluctuation of milk produced had a similar pattern for both groups on the 12th to 19th days, but the figure for the Silage group (average of 11.91 kg 4% FCM/head/day) was always higher than for the Control group (11.08 kg 4% FCM/head/day). However, during the period of 19th to 24th days, the milk produced by the Silage group decreased to as low as 10.04 kg 4% FCM/head/day and there was a significant increased to 11.71 kg 4% FCM/head/day by the Control group. Finally, at the end of the observation period, the amount of milk produced was similar for both groups with an average of 10.76 kg 4% FCM/head/day.

Table 1. Crude protein content in the feed ingredients, percentage of the ingredients in the ration and crude protein content of the rations used in the experiment

No.	Feed ingredients	Crude protein (%)	Type of ration (%)	
			Control	QPM silage
1.	QPM silage	9,30	-	15,0
2.	Field grass	8,40	30,0	15,0
3.	Tofu by product	18,2	30,0	30,0
4.	Corn mills	9,06	15,4	15,4
5.	Rice bran	5,55	9,40	9,40
6.	Beer by product	16,3	14,0	14,0
7.	Protefeed	9,89	1,20	1,20
8.	Crude protein content (%):			
	· Control ration			12,31
	· QPM silage			12,45

Table 2. Dry matter and crude protein contents of QPM silage after preserving at four different periods

No.	Preservation time (days)	Dry matter (%)	Crude Protein (%)
1.	0	26.2	10.4
2.	15	32.8	8.8
3.	45	33.2	9.2
4.	90	26.8	9.3

Table 3. The quality and the amount of milk produced by dairy cattle fed either the Control or QPM silage ration

No.	Milk quality and quantity	Type of ration[1)]	
		Control	QPM silage
1.	Acidity (pH)	6.618^{a}	6.550^{a}
2.	Density	1.0280^{a}	1.0272^{a}
3.	Fat (%)	3.92^{a}	3.35^{b}
4.	Protein (%)	2.636^{a}	2.726^{b}
5.	Milk production (kg 4% FCM[2)]/head/day)	11.1^{a}	11.5^{b}

Note: [1)] A different letter in a same row showed a significantly different (P<0.05) between treatments
[2)] FCM = Fat Corrected Milk

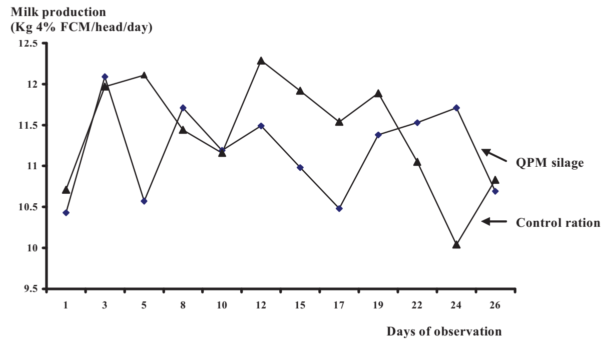

Figure 1. Fluctuation of daily milk production of dairy cattle fed with Control or QPM silage rations during experiment

Conclusions

It can be concluded from this experiment that a) a good quality QPM silage was best produced after 45 days of preservation; b) the addition of QPM silage in the ration of dairy cattle did not have any effect on the acidity level and density of the milk, but it reduced the milk fat content and increased the milk protein content as well as the quantity of the milk produced by dairy cattle; c) the QPM silage may be recommended to be used as an alternative source of roughage for dairy cattle reared in the DKI Jakarta area.

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Inbred flowering control for hybrid maize seed production

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Abstract. Synchronization in male and female flowering is very important for high yielding of hybrid maize seed production; however, it is occasionally found that some hybrids have a different maturity of parent inbreds. In 2006, an experiment was conducted at Nakhon Sawan Field Crops Research Center to investigate some practical flowering-control methods to improve synchronization of inbreds for hybrid seed production. Split-plot design with 3 replications was set up, main plot consisted of 4 inbreds namely - Tak Fa 1, Nei 452006, Nei 452015 and Nei 452026; sub plot consisted of 7 flowering-control methods: 1) Deep planting at 10 cm 2) Half-stem cutting at V5 3) Foliar herbicide spraying at V5 4) Soil fertilizer application at 7 days after planting 5) Foliar-fertilizer spraying at V4-V6 6) Early detasseling and 7) Normal practice. It was found that half-stem cutting and herbicide spraying significantly delayed both male and female flowering from 2-7 days. The delay flowering was more pronounced in half-stem cutting than that of herbicide spraying. Agronomic characteristics showed that half-stem cutting and herbicide spraying decreased numbers of tassel branches, silk dry weight, leaf area, plant height and yield but an increase of main tassel spike was interestingly observed. A response to these two flowering-control methods differed among inbreds and a yield decreased approximately 30%, thus these methods were not recommended for female parent inbreds. Other methods which were soil fertilization, foliar fertilization, deep planting and early detasseling did not show a significant increase or delay male and female flowering.

Key-words: Hybrid, seed production, maize, flowering control, inbred

Introduction

Synchronization of male and female flowering (tassel shed and silking) of parent inbred is very important for high yield of hybrid maize seed production. Good synchronization results in a high seed yield, occasionally, the difficulty has been found in seed production for some good hybrids due to a different flowering time of parent inbreds. In addition, unfavorable conditions possibly cause a low yield causing a delay or stimulation of the inbred flowering. Thus, many techniques have been used to control inbred flowering to promote good synchronization (Shoultz, 1999; Beck, 2004). Objective of this experiment is to study some practical techniques to control flowering of female and male parent inbred of promising drought-tolerant hybrid.

Material and methods

An experiment was conducted at Nakhon Sawan Field Crops Research Center (NSFCRC) in 2006; split-plot design with 3 replications was set up, main plot consisted of 4 parent inbreds namely – Tak Fa 1, Nei 452006, Nei 452015 and Nei 452026; sub plot consisted of 7 flowering-control methods, 1) Deep planting at 10 cm 2) Half-stem cutting at V5, stem was cut off and 2 leaves above the ground remained intact 3) Foliar herbicide spraying at V5, 100 ppm of paraquat 20% (w/v ai) was sprayed at V5 4) Soil fertilizer application at 7 days after planting, a rate of 94 kg ha^{-1} P_2O_5 of 0-46-0 fertilizer was applied 5) Foliar-fertilizer spraying at V4-V6, a rate of 0.5% w/v of 0-52-34 foliar fertilizer was sprayed at V4, V5 and V6 6) Early detasseling, tassels or tassels including 1-3 leaves were cut off as plant showed four fully expanded leaves above ears and 7) Normal practice. Inbreds were planted in each sub plot, 2 m wide by 6.5 m long; spacing was 0.65 cm between rows and 0.15 cm between plants. Ammonium sulphate fertilizer (21-0-0) at a rate of 188 kg ha^{-1} were applied at 7 and 21 days after planting; urea fertilizer (46-0-0) at a rate of 63 kg ha^{-1} was applied at 50 days after planting. The following observations were recorded - days of 50% tassel shedding, days of 50% silking, numbers of tassel branches, tassel main axis length, silk dry weight, plant height, leaf area and yield.

Results and Discussion

Effect of flowering control methods on days of flowering

It clearly showed that half-stem cutting and foliar herbicide spraying significantly delayed days of flowering, 50% tassel shed and silking (Table 1). The delay varied from 2 to 7 days among inbreds, from 2 to 7 days in silking and from 3 to 6 days in tassel shedding. The delay in silking and tassel shed days of Tak Fa 1 and Nei 452006 was more pronounced than those of Nei 452015 and Nei 452026. Half-stem cutting showed a delay of flowering more than that of a herbicide spraying. These two methods also significantly decreased numbers of tassel branches from 2 up to 5 branches depending on inbreds, Nei 452006 exhibited a decrease of tassel branches in both stem cutting and herbicide spraying methods more than other inbreds (Table 2). A decrease of tassel branch numbers was approximately 24% compared with that of normal practice. It was interesting that stem cutting and herbicide spraying significantly increased main tassel spike length of all inbreds, the increase was 20% and 16% respectively compared with that of normal practice. This may be a reproductive adaptation of tassels in stress conditions for survival since these two methods obviously decreased silk dry weight in all inbreds (Table 3).

Vegetative growth i.e. plant height and leaf area were drastically decreased by stem cutting and herbicide spraying methods (Table 3). Average decrease of height by stem cutting and herbicide spraying was 38% and 28% respectively; similarly, a dramatic decrease of leaf area was 72% and 53% compared with those of normal practice. According to a great decrease in vegetative growth by these two methods, average yield of all inbreds decreased approximately 34% by stem cutting and 32% by herbicide spraying. Thus, the methods that decreased seed yield should not practice with female inbred (Beck, 2004). Berzy *et al.* (1996) reported that mild damage of tassel did not seriously affect on seed yield especially removal of tassel

Table 1 **Effect of flowering control methods on days of tassel shed and silking of inbreds**

Methods	Days of 50% flowering									
	Tak Fa 1		Nei 452006		Nei 452015		Nei 452026		Average	
	tassel shed	silking	tassel shed	silking	tassel shed	silking	tassel shed	silking	tassel shed	silking
1) Deep planting	0	1	-1	0	0	0	-1	-1	0	-1
2) Half-stem cutting	6	7	5	7	5	6	5	5	6	6
3) Herbicide spraying	6	6	5	7	3	4	3	2	5	4
4) P_2O_5 soil fertilizer	1	1	-1	1	0	1	0	0	1	0
5) Foliar fertilizer	1	1	-1	1	1	1	-1	-1	0	0
6) Early detasseling	-	1	-	1	-	1	-	0	-	0
7) Normal practice	0	0	0	0	0	0	0	0	0	0

(+) numbers of days delayed from normal practice method
(-) numbers of days earlier from normal practice method
(0) numbers of day as check (normal practice) with 1)-6) control methods

Table 2 **Effect of flowering control methods on numbers of tassel branches and main tassel spike length**

Methods	Tassel characteristics									
	Tak Fa 1		Nei 452006		Nei 452015		Nei 452026		Average	
	No. of branches	main spike length (cm)	No. of branches	main spike length (cm)	No. of branches	main spike length (cm)	No. of branches	main spike length (cm)	No. of branches	main spike length (cm)
1) Deep planting	1	17.7	-1	20	0	24.7	0	21.7	0	21.0 *b*
2) Half-stem cutting	-2	22.1	-5	24.9	-5	26.8	-5	24	-4	24.5 *a*
3) Herbicide spraying	0	20.3	-5	24.7	-3	24.3	-3	23.4	-3	23.2 *a*
4) P_2O_5 soil fertilizer	1	17	-1	19.8	0	23.8	-1	20.6	0	20.3 *b*
5) Foliar fertilizer	2	17.2	0	19.8	0	23.6	0	21.7	0	20.6 *b*
7) Normal practice	0	16.1	0	19.7	0	23.7	0	20.5	0	20.0 *b*

Means within a column followed by the same letter were not different according to DMRT at $P \leq 0.05$

Table 3 Effect of flowering control methods on some agronomic characteristics of inbreds

Inbreds	Methods	Silk dry weight (g ear^{-1})	Yield (kg ha^{-1})	Leaf area at 49 DAP ($x10^3cm^2$ $plant^{-1}$)	Plant height at 49 DAP (cm)
Tak Fa 1	1. Deep planting	0.83	4,501 *f-j*	3.6 *de*	162
	2. Half-stem cutting	0.75	3,451 *i-m*	1.0 *hi*	103
	3. Herbicide spraying	0.72	3,868 *g-l*	1.9 *fh*	123
	4. P_2O_5 soil fertilizer	0.78	5,428 *c-f*	3.8 *cde*	160
	5. Foliar fertilizer	0.81	4,729 *e-j*	3.6 *de*	163
	6. Early detasseling	0.81	9,211 *klm*	3.7 *de*	167
	7. Normal practice	0.79	4,695 *e-j*	4.0 *bcd*	170
Nei 452006	1. Deep planting	0.43	7,121 *ab*	4.1 *bcd*	163
	2. Half-stem cutting	0.26	5,113 *c-h*	1.3 *ghi*	110
	3. Herbicide spraying	0.33	4,004 *f-k*	1.7 *fg*	112
	4. P_2O_5 soil fertilizer	0.33	6,317 *a-d*	4.1 *bcd*	164
	5. Foliar fertilizer	0.31	6,937 *ab*	3.8 *cde*	164
	6. Early detasseling	0.48	7,125 *abc*	4.1 *bcd*	163
	7. Normal practice	0.37	7,491 *a*	4.4 *abc*	167
Nei 452015	1. Deep planting	0.50	5,130 *c-g*	3.5 *de*	146
	2. Half-stem cutting	0.38	2,481 *lm*	1.0 *i*	85
	3. Herbicide spraying	0.37	3,659 *h-m*	1.6 *fgh*	102
	4. P_2O_5 soil fertilizer	0.49	5,308 *c-g*	3.6 *de*	142
	5. Foliar fertilizer	0.44	5,481 *c-f*	3.3 *e*	129
	6. Early detasseling	0.49	3,370 *j-m*	3.6 *de*	145
	7. Normal practice	0.46	5,983 *b-e*	3.5 *de*	145
Nei 452026	1. Deep planting	0.50	4,895 *d-i*	4.7 *a*	157
	2. Half-stem cutting	0.41	4,874 *d-i*	1.1 *hi*	94
	3. Herbicide spraying	0.39	5,029 *c-h*	2.1 *f*	121
	4. P_2O_5 soil fertilizer	0.47	6,035 *b-e*	4.0 *bcd*	157
	5. Foliar fertilizer	0.51	5,299 *c-g*	4.5 *ab*	163
	6. Early detasseling	0.56	2,390 *m*	4.0 *bcd*	161
	7. Normal practice	0.45	6,079 *b-e*	3.7 *de*	150
	C.V. (Inbreds) (%)	15.0	26.8	18.8	7.5
	C.V. (Methods) (%)	13.5	15.2	10.9	5.6
	Inbreds	**	*	ns	**
	Methods	**	**	**	**
	IxM	ns	**	*	ns

* Means within a column followed by the same letters were not significant different according to DMRT at $p \leq 0.05$
**significant different at $p \leq 0.01$; ns = not significant

branches since undamaged branches continued to shed pollen. However in this experiment with the stem cutting and herbicide spraying, plants were damaged resulting in reduction of vegetative growth causing yield loss. Thus, after stem cutting and herbicide spraying, it is necessary that fertilizer and irrigation need to be applied since the treated plants showed vulnerable symptoms especially burned leaves only 1 day after spraying.

Although stem cutting delayed flowering, a great decrease in both vegetative and reproductive growth was clearly reported here. This technique was the most use which was about 21% used from a survey accomplished by seventy companies out of 150 member companies reported by Shoultz (1999). He also pointed out from the survey that the most used technique was also the technique that being the most unsuccessful. However, in the production field even under uncontrollable conditions, we still need the technique that gave a consistent result possibly 60-70% success out of every attempt. So many techniques used in seed production should be confirmed in various conditions to check their consistency.

Other flowering control methods which were deep planting, P_2O_5 soil fertilizer, foliar fertilizer and early detasseling did not show a significant effect on a delay or stimulation flowering (silking and tassel shed) (Table 1, 2 and 3). For fertilizer application, it was possible that soil contained sufficient phosphorus since the experiment was conducted in black soil with moderate to high fertility. Shoultz (1999) also indicated that results of fertilizer application were much better in the lower fertility soils. Thus, an experiment in this field must get rid of environmental factors i.e. soil, weather, precipitation etc. that could interfere the results.

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Composite Line Method for the Development of Early Generation Hybrids of Maize (*Zea mays* L.)

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Abstract. Six commercial single crosses were used for the improvement of composite and inbred lines. Modified S_1-full sib selection was applied to improve the three sister line composite. Lines were visually selected under low-competition environment in honeycomb arrangement with equilateral triangular side 0.866m. Testcross as well as diallel cross were applied to identify high combining lines. All yield trials were conducted in randomized completed block design with 4 replications, 1 row plot of 5m long and 0.75 x 0.25m plant spacing. Standard cultural practices were regulated and irrigation was applied as needed. Statistically, there was no clear advantage of yield between composite and inbred lines in early generation testcrosses. Besides, the diallel sets of both groups of lines gave similar results. However, the top hybrids of overall trials came from composite crosses even though it was not significant. In addition, composite lines were superior to S_3 lines in yield, earliness and plant height. Modified S_1-full sib selection is a flexible breeding method but its merit for the construction of early generation hybrids must be thoroughly investigated even though the positive results were observed.

Key Words: Maize breeding, testcross, honeycomb, composite line.

Introduction

Development of single cross hybrid of maize is the ultimate goal of most of maize breeding programs. However, finding stable high yield inbred lines to ensure the high level of economic return for commercial hybrid seed production is the main obstacle of small and new emerged single cross development program. Combined line selection and testing for combining ability is time and space consuming processes. Instead of five or six generations of selfing usually practiced in the development of inbred lines, composite-sibbing lines from individual of S_1 progenies have been proposed (Kinman, 1952). The method fixed the composite-sibbed lines since the first selfing and therefore improvement in the combining ability or other characteristics of composite-sibbing lines can not be made after several generations of mass sibbing unless effective selection is practiced. In other way, line selection from cross between closely related parents has been proved to be an effective method for inbred line development (Rasmusson and Phillips, 1977; Troyer, 1999). Selection for high and low yield lines effectively separated lines into high and low combining ability groups but yield of lines within group can not be used as criterion for combining ability of lines (Lamkey and Hallauer, 1986). In addition, for effective differentiation of lines, Fasoula and Fasoula (1997) proposed line selection under nil-competition environment in honeycomb designs. In order to improve yield and combining ability of population, Landi and Frascaroli (1993) applied full-sib selection in F_2 population of single cross. The method proved to be very effective for several cycles of selection. However, the previous study of Genter (1976) which applied the same method suggested that using S_1 instead of S_0 to form full-sibs was more effective to identify high yielding full-sibs as well as improvement of population per se. This finding is well agreed with suggestion of Lonnquist (1950) that testing for combining ability after one generation of selfing is desirable when the composite sib-breeding method is used.

The above finding suggested that alternate selfing and full sibbing among few closely related lines under low-competition environment should lead to uniform, high yield and high combining composite lines as high level of homozygosity is approached and provide a chance for continuous improvement of composite lines in the successive cycles.

The present study therefore aim to formulate the effective breeding method for the development of composite lines and evaluate its merit as compare to the conventional line selection with early generation testing for combining ability. The modified S_1-full sib selection within related lines is proposed.

Materials and methods

Six commercial single cross hybrids comprised of Monsanto 949, Monsanto 919, Pioneer A33, Pioneer 3012, Pacific 984 and Syngenta NK 48 were planted in normal plant spacing (0.75 x 0.25m) and selfed to obtain S_1 ears. Nine S_1 ears within each family were randomly grouped in to 3 ear sets, 3 sets per family and therefore resulted in 18 sets of 3 S_1 and 54 individual S_1 lines. They were separately ear-row in honeycomb arrangement (HC) with equilateral triangular side of 0.866m. Three best S_1 plants within each set were intercrossed (full sibbing) to form 18 intra-set diallel crosses which will be refered to as full sib sets while 3 best S_1 plants from each family were also selfed to obtain 18 S_2 lines.

Consequently, they were ear-row in HC, the 18 S_2 plants were selfed as well as testcrossed to the inbred tester, KRi 208 to obtain 18 S_3 lines and 18 testcrosses, S_2 x KRi 208 hybrids. The best S_2 lines by visual selection, one from each family were also intercrossed to form 15 diallel crosses of 6 S_2. In the meantime, the best 3 F_1 plants from each full sib set were crossed in all possible combinations to form 18 composite lines and they will be refered as composite line cycle-1 (C#1). The method is essentially similar to S_1 and full-sib selection of which it will be refered to as modified S_1-full sib selection for composite line development. Afterward, C#1 were testcrossed to KRi 208. As a result, 18 C#1 testcrosses were obtained. In addition, the best C#1 by visual selection, one from each family were intercrossed to form 15 diallel crosses of 6 C#1.

Yield trials of 18 S_3 lines, 18 C#1, 18 testcrosses of S_2 x KRi 208, 18 testcrosses of C#1 x KRi 208, 15 diallel crosses of 6 C#1 and 15 diallel crosses of 6 S_2 lines were conducted in separated trials in adjacent areas in randomized completed block design with 4 replications, 1 row plot of 5m long and 0.75 x 0.25m plant spacing. Five original hybrids were included as common checks in all hybrid yield trials. Pacific 984 was excluded and replaced by Suwan 4452 because it was dropped out from the market and there was no seed available.

All experiments were conducted from September 2004 to March 2006 at National Corn and Sorghum Research Center, Suwan Farm, in Nakhon Ratchasima province ($14^0 30'$N, $101^0 30'$E, and 356m asl.), Thailand under standard cultural practices. Basal fertilizers were applied at planting time at the rate of 75 kg ha^{-1} of N and 100 kg ha^{-1} of P_2O_5. Top-dressing was done at the 6 to 8 leaf stages with the rate of 75 kg N ha^{-1}. Pre-emergence herbicides, Atrazine and Alachlor were used by mixing at the rate 1.5 and 1 kg a.i. per ha, respectively. Thinning was done at 14 days after sowing. Irrigation was applied when necessary.

Results and discussion

Mean grain yields and other agronomic traits of top-10 S_3 lines are presented in Table 1. All selected lines were statistically not different except line 406-3 and only line 401-6 showed significant difference over the inbred check, KRi 208. However, the KRi 208 had higher level of homozygosity and therefore, if further inbreeding were applied, all lines were expected to be similar in yield level. There was no clear evidence for the advantage or disadvantage of other agronomic traits among the top ten lines but line 403-5 demonstrated a superior shelling percentage over other lines including the checks.

Table 1. **Grain yields at 15 percent moisture and other agronomic traits of top 10 S_3 lines and KRi 208 conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

S_3 lines	Source of germplasms	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
401-6	Pac.984	4.21 a	68.7 a-d	69.0 bcd	21.9 a-d	131.5	54.2 bc	76.6 a-d
402-6	Mon.949	3.77 ab	68.3 a-e	67.3 edf	25.6 a	140.2	60.3 abc	73.6 bcd
404-4	Pio.A33	3.71 ab	67.7 b-f	67.3 edf	23.7 abc	140.3	63.5 abc	73.9 bcd
402-8	Mon.949	3.63 ab	66.7 ef	67.0 ef	24.9 ab	134.3	60.7 abc	75.6 bcd
401-9	Pac.984	3.40 ab	70.0 a	68.8 b-e	23.4 a-d	131.3	54.3 bc	79.1 ab
402-7	Mon.949	3.38 ab	66.3 f	66.3 f	23.9 abc	138	52.3 c	75.7 bcd
405-4	Syn. 48	3.36 ab	67.0 def	67.7 c-f	22.8 a-d	134.7	60.5 abc	71.8 cd
403-5	Mon.919	3.21 abc	68.0 b-f	68.0 b-f	20.2 cd	126	52.3 c	82.2 a
401-7	Pac.984	3.08 a-d	70.0 a	69.0 bcd	22.0 a-d	131.5	58.7 abc	78.0 ab
406-3	Pio.3012	2.89bcd	70.0 a	68.8 b-e	24.5 ab	151.5	68.2 ab	74.6bcd
KRi 208	Pio.3012/3013	3.01bcd	69.0 abc	69.7 ab	25.5 a	120.3	50.2 c	73.5 bcd
	Mean	3.48	68.2	68.0	23.4	132.8	56.7	76.0
	F-value[1/]	**	**	**	**	ns	**	**
	CV(%)	19.568	1.338	1.353	8.489	10.561	13.461	3.973

[1/] ns: non significant, * : significant, ** : highly significant

In comparison S_3 with C#1 lines, the C#1 lines were consistently superior in the characteristics used as measures of vigor; grain yield, earliness of anthesis and silking, plant and ear height. They were earlier, taller and higher yield regardless of germplasm sources. Moreover, better distribution of germplasm sources of top-10 C#1 was evident. All six germplasm sources were presented in the top-10 C#1 while in the top-10 S_3 lines, visual selection leaned toward Monsanto 949 and Pacific 984. The results indicated that C#1 was more stable by outcrossing. On the other hand, inbred lines from each germplasm source should have different level of inbreeding depression and thus selection for performance per se was biased toward the less inbreeding depression germplasm. In this case, Pioneer 3012 was lost from the top-10 S_3 lines.

The present results were well agreed with report presented by Kinman (1952) of which selective mass sibbing within individual S_1 progenies was used. In Kinman's words, the population is closed at the time of first sibbing, it should not be expected that improvement in the combining ability or other characteristics of composite-sibbed lines will be made even after several generations of mass sibbing unless effective selection is practiced. Unlike Kinman's method, the modified S_1-full sib selection employed in the present study provided a more flexible approach. Selection for S_1 performance per se alternate with diallel crossed of individual of 3 selected S_1 lines (full sibbing) should improve general combining ability as well as specific combing ability of S_1 lines from successive cycles. In the meantime, the new emerged individual S_1 as well as full sib of each cycle can be fixed by mass sibbing method and used in early generation hybrid combinations while the successive cycles of composite sets move slowly toward higher level of homozygosity and hence more uniform lines and hybrids in later stage.

Lamkey and Hallauer (1986) founded that inbred line performance per se can be used as criterion to differentiate combining ability between high and low yield inbreds. However, yield per se within high or low yielding groups can not be used to predict line performance in hybrid combinations. Yielding ability of line per se in Table 1 and their testcross performance in Table 3 clearly supported the above finding. Since all 18 inbred lines came from the top-3 high yield lines of each original hybrid therefore they should be considered as high yield lines. However, their yielding ability did not represent the combining ability of lines in the testcross combinations with the inbred tester (KRi 208), line 403-4 which was excluded from the top-10 lines gave the highest yield in the testcrosses while the top yield line, 401-6 ranked 9th in testcrosses. Besides, only two Pioneer lines, 406-1 and 404-4 were presented in the top-10 testcrosses. This is not unexpected because the tester line, KRi 208 derived from Pioneer 3012/ Pioneer 3013. Therefore, genetic background of tester played an important role in the combinations with tested lines. However, 406-1/KRi 208 is essentially a backcross to sister line and ranked 6th in the top-10 testcrosses indicated a strong additive effect in this hybrid combination. Since different testers gave different performance with the same group of lines (Castellanos *et al*., 1998), all high yield lines should be tested their hybrid combinations directly to their counterpart parental lines to identify the best hybrid combination.

Table 2 **Grain yields at 15 percent moisture and other agronomic traits of composite lines of cycle 1 conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

S_3 lines	Source of germplasms	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
Set 4	Mon.949	6.13 a	67.3 abc	67.3 bc	25.5 a	168.7	82	77.2
Set 5	Mon.949	5.53 ab	65.0 d	67.0 bc	25.6 a	165.6	71.2	79.2
Set 18	Mon.919	5.26 abc	63.0 e	66.0 c	21.4 cd	157.4	63.9	80.8
Set 10	Syn. 48	4.91 bc	66.0 cd	66.7 bc	21.9 bcd	173.3	80.3	79.6
Set 11	Syn. 48	4.87 bcd	66.0 cd	67.0 bc	22.9 bcd	161.2	67.7	72.9
Set 2	Pac.984	4.81 bcd	68.3 ab	67.7 bc	23.8 abc	171.8	75.2	79.4
Set 8	Pio.A33	4.66 bcd	66.7 a-d	67.0 bc	22.8 bcd	165.8	80.8	80.2
Set 14	Pio.3012	4.64 bcd	68.0 abc	67.7 bc	22.0 bcd	158.1	69.2	80.2
Set 3	Pac.984	4.63 bcd	68.7 a	68.3 ab	23.8 abc	159.3	65.5	79.7
Set 7	Pio.A33	4.58 bcd	66.3bcd	67.0 bc	22.6 bcd	150.3	67.7	79.5
	Mean	5.00	66.5	67.2	23.2	163.2	72.4	78.9
	F-value[1/]	*	**	*	**	ns	ns	ns
	CV(%)	14.076	1.73	1.575	5.815	7.111	15.454	3.299

[1/] ns: non significant, * : significant, ** : highly significant

Table 3 **Grain yields at 15 percent moisture and other agronomic traits of top 10 testcrosses between selected S_2 x KRi 208 and original hybrids conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

S_3 lines	Source of germplasms	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
403-4	Mon.919	8.96 a	61.3 h	61.7 j	22.6 def	165.8 b-f	81.7 a-f	75.9 h-k
405-5	Syn. 48	8.82 ab	62.3 e-h	64.0 d-i	23.0 c-f	153.3 fgh	70.3 fg	77.1 f-i
402-6	Mon.949	8.40 a-d	62.0 fgh	62.7 hij	24.9 abc	161.0 d-h	80.3 a-f	75.3 k
405-4	Syn. 48	8.14 a-e	61.7 gh	62.3 hij	23.2 c-f	158.0 d-h	79.0 b-g	77.5 efg
405-6	Syn. 48	8.08 a-f	63.3 b-g	64.3 b-g	24.3 bcd	157.6 d-h	82.7 a-f	75.6 ijk
406-1	Pio.3012	8.07 a-f	63.0 c-h	63.7 e-i	24.1 bcd	164.2 b-f	81.7 a-f	78.3 def
402-7	Mon.949	8.00 a-f	61.3 h	62.0 ij	24.0 bcd	150.0 gh	73.7 d-g	77.0 f-i
404-4	Pio.A33	7.89 a-f	65.0 ab	65.3 c-f	23.6 b-f	159.8 d-h	76.8 b-g	75.4 jk
401-6	Pac.984	7.70 a-g	62.7 e-h	63.3 f-j	23.4 c-f	156.3 e-h	72.7 d-g	79.5 cd
403-5	Mon.919	7.66 a-g	62.0 fgh	62.7 hij	21.9 ef	162.1 c-h	89.8 a-g	81.1 bc
Check	Pio.A33	8.47 abc	64.7 bc	66.0 bcd	21.9 ef	182.0 a	91.9 a	79.8 bc
Check	SW 4452	8.24 a-e	66.7 a	68.7 a	25.0 abc	177.3 ab	84.2 a-e	76.3 g-k
Check	Pio.3012	8.08 a-f	66.7 a	68.0 ab	22.4 def	165.2 b-f	89.1 ab	77.2 fgh
Check	Syn. 48	7.86 a-f	64.0 b-e	66.0 bcd	22.7 def	171.3 a-d	74.4 dfg	77.9 ef
check	Mon.949	7.57 b-g	62.7 e-h	64.3 d-g	26.7 a	178.3 ab	81.6 a-f	77.9 ef
Check	Mon.919	7.32 c-g	63.3 b-g	65.0 c-g	23.9 b-e	176.1 abc	88.1 abc	82.9 a
	Mean of top 10 topcrosses	8.17	62.5	63.2	23.5	158.8	78.9	77.3
	F-value[1/]	**	**	**	**	ns	*	**
	CV(%)	11.006	1.836	1.985	5.198	5.296	9.685	1.179

[1/] ns: non significant, * : significant, ** : highly significant
[2/] Pedigree of KRi 208 is Pio.3012/Pio.3013

Statistically, all top-10 testcrosses yielded as high as the top-4 checks but somewhat better than the Monsanto 949 and Monsanto 919. However, 403-4/KRi 208 gave an outstanding feature of yield and earliness even though it was taller and lower in shelling percentage than the average.

Top S_2 lines, one from each of six original hybrids were intercrossed and the top-10 interfamily hybrids are presented in Table 4. As expected, the average of top-10 S_2 interfamily diallel hybrids was lower than the average of top-10 S_2 testcrosses because both parental lines of S_2-interfamily hybrids were more heterogeneous than the tester line, KRi 208 in S_2 testcrosses. Therefore, the specific combining ability of lines were more pronounced. Seven out of 10 S_2-interfamily hybrids were involved with Pioneer 404-6 and Pioneer 406-1 and 6 out of 10 were crosses between Pioneer and Monsanto lines. Evidently, both germplasm sources were complimented each other of which they showed a good heterotic pattern. Although most of S_2-interfamily hybrids were significantly not different from the checks, 404-6/402-6 (Pioneer A33/Monsanto 949) gave outstanding features for yielding ability, earliness, plant and ear height while retained good shelling percentage. Therefore, beside the conventional testcross program, diallel cross between the top high yield lines is necessary for thoroughly used of germplasms and identification of new unique hybrid combination.

The numbers of original germplasm sources involved in top-10 S_2 and C#1 testcrosses in Table 3 and 5 were almost the same; 4:5 (Monsanto), 3:3 (Syngenta), 2:1 (Pioneer) and 1:1 (Pacific) indicated that they responded similarity to the same tester, even though each S_2 line derived from visual selection within each composite set. However, average yield of S_2 testcrosses was higher than that of C#1 testcrosses but top testcross yields of both groups as well as the best check were more or less the same.

The average yield of S_2 diallel crosses in Table 4 and average yield of C#1 diallel crosses in Table 6 were almost the same but with the higher trend toward the C#1 lines. Evidently, general combining ability of S_2 and C#1 were somewhat the same even though the C#1 were more heterogeneous. Surprisingly, the top-2 hybrids of C#1 gave higher yield over other hybrids and checks tested in the present studies even though they were statistically not different.

Evidences from previous studies: Genter (1976); Landi and Frascaroli (1993); Rasmusson and Phillips (1997) and

Table 4 **Grain yields at 15 percent moisture and other agronomic traits of interfamily diallel hybrids of selected S_2 lines and original hybrids conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

Source of germplasm	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
Pio.A33 x Mon.949	8.86 a	62.3 d	63.3 g	25.1 ab	171.9 de	80.5 d-g	76.1 gh
Pio.A33 x Mon.919	7.82 abc	62.7 cd	64.3 efg	23.1 b-f	170.3 de	81.7 c-g	77.2 efg
Pio.3012 x Syn.48	7.61 bcd	66.3 a	67.3 abc	24.0 b-e	202.3 a	106.9 a	74.8 h
Pio.3012 x Syn.48	7.53 b-e	64.0 bcd	65.3 def	24.3 bcd	197.5 ab	96.7 ab	77.4 efg
Mon.919 x Pac.984	7.51 b-e	62.3 d	63.7 fg	21.8 efg	169.2 de	77.0 fg	80.6 b
Pio.3012 x Pio.A33	7.47 b-e	65.7 ab	66.7 bcd	23.8 b-f	194.7 abc	97.2 ab	78.2 c-f
Pio.A33 x Pac.984	7.11 cde	64.0 bcd	66.3 bcd	23.3 b-f	175.4 de	84.1 c-f	80.0 bc
Pio.3012 x Mon.919	6.59 de	63.7 bcd	65.0 d-g	22.1 def	183.5 bcd	93.3 bc	77.8 efg
Syn.48 x Mon.949	6.55 de	63.3 cd	65.3 def	24.6 abc	178.3 de	79.9 efg	75.0 h
Pac.984 x Mon.949	6.32 e	63.0 cd	64.3 efg	23.4 b-f	180.9 cde	77.3 efg	78.2 c-f
Pio.A33	8.47 ab	64.7 abc	66.0 cde	21.9 ef	182.0 bcd	91.9 bcd	79.8 bcd
SW 4452	8.26 abc	66.7 a	68.7 a	25.0 ab	177.3 de	84.2 c-f	76.3 fgh
Pio.3012	8.08 abc	66.7 a	68.0 ab	22.4 def	165.2 e	89.1 b-e	77.2 efg
Syn. 48	7.86 abc	64.0 bcd	66.0 cde	22.7 c-e	171.3 de	74.4 g	77.9 d-g
Mon.949	7.57 bcd	62.7 cd	64.3 efg	26.7 a	178.3 de	81.6 c-g	77.9 d-g
Mon.919	7.32 b-e	63.3 cd	65.0 d-g	23.9 b-e	176.1 de	88.1 b-f	82.9 a
Mean of top 10 inter-family cross	7.34	63.7	65.2	23.6	182.4	87.5	77.5
F-value[1/]	**	**	**	**	**	**	**
CV(%)	10.322	1.9	1.839	5.706	5.327	8.393	1.494

[1/]ns: non significant, * : significant, ** : highly significant
[2/] Crosses between two sets of composite lines.

Troyer (1999) showed that selections in a very narrow base populations were very effective for the improvement of the populations as well as inbred line per se. The method for composite line improvement used in the present studies is very similar to that suggested by Genter (1976) for population improvement but only 3 S_1 lines were used to form new population of each cycle, aiming to get uniform, high yield and high combining ability composite lines for better hybrid combinations. The method is simply a modification of S_1 and full-sib selection and therefore designated as modified S_1-full sib selection method. Data presented in this study did not show any clear advantage of line selection over the composite line method. More advanced cycles of S_1-full sib selection are underway to prove the merit of the method as compare to the conventional line selection.

The composite-sibbed lines as proposed by Kinman (1952) is clearly had an advantage over line selection method when time and space are involved. Composite-sibbed lines are ready for final testing without five or six generations of selfing usually practice in the development of inbred lines. In the modified S_1-full sib selection, composite-sibbed lines can be derived from composite sets as used in this study or using the individual S_1 and full-sib of each successive cycle. In addition, S_1 lines may be selfed for one or two additional generations in order to eliminate the undesirable alleles and several desirable sister lines

Table 5 **Grain yields at 15 percent moisture and other agronomic traits of top 10 testcrosses between composite lines of cycle 1 x KRi 208 and original hybrids conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

S_3 lines	Source of germplasms	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
set 4	Mon.949	8.74 a	62.7 fg	63.3 ghi	24.9 abc	172.5 a-d	84.4	76.1 def
set 3	Pac.984	8.31 abc	65.7 a-d	67.0 a-d	23.8 b-g	165.5 c-g	84.9	78.9 bc
set 11	Syn. 48	7.80 a-d	64.0 c-f	65. d-h	22.9 b-h	163.9 d-g	78.5	74.6 fg
set 5	Mon.949	7.65 a-d	61.7 g	62.7 i	23.8 b-g	168.8 b-g	80.5	76.5 c-f
set 12	Syn. 48	7.44 b-d	63.3 efg	64.7 e-h	22.9 b-g	160.7 efg	77.2	76.3 def
set 17	Mon.919	7.37 b-f	63.0 efg	65. d-h	21.1 h	161.9 d-g	81.9	76.2 def
set 10	Syn. 48	7.34 b-f	62.7 fg	63.0 hi	22.2 fgh	160.5 fg	81.2	77.1 cde
set 15	Pio.3012	7.24 c-g	66.0 abc	67.0 a-d	24.0 b-g	189.9 g	82.3	77.2 cde
set 16	Mon.919	7.20 c-g	63.3 efg	64.7 e-h	21.7 gh	161.9 d-g	83.7	75.3 efg
set 18	Mon.919	7.20 c-g	63.7 efg	65.3 c-g	23.9 b-g	169.3 b-g	85.4	77.3 cde
Check	Pio.A33	8.47 ab	64.7 b-f	66.0 b-f	21.9 gh	182.0 a	91.9	79.8 b
check	SW 4452	8.26 abc	66.7 ab	68.7 a	25.0 ab	177.3 ab	84.2	76.3 def
Check	Pio.3013	8.08 a-d	66.7 ab	68.0 ab	22.4 e-g	165.2 d-g	89.1	77.2 cde
Check	Syn. 48	7.86 a-d	64.0 c-f	66.0 b-f	22.7 c-g	171.3 a-e	74.4	77.9 bcd
Check	Mon.949	7.57 a-d	62.7 fg	64.3 ghi	26.7 a	178.3 ab	81.6	77.9 bcd
Check	Mon.919	7.32 b-f	63.3 efg	65. d-h	23.9 b-g	176.1 abc	88.1	82.9 a
	Mean of top 10 topcrosses	7.63	63.6	64.8	23.1	167.5	82.0	76.6
	F-value[1/]	**	**	**	**	**	ns	**
	CV(%)	10.175	1.923	1.921	5.964	3.873	7.455	1.93

[1/] ns: non significant, * : significant, ** : highly significant

Table 6 **Grain yields at 15 percent moisture and other agronomic traits of interfamily diallel hybrids of composite lines (cycle 1) and original hybrids conducted at Suwan Farm, Thailand in November, 2005 (dry season).**

Source of germplasms	Grain Yield (ton/ha)	Days to Anthesis (days)	Days to Silking (days)	Moisture Content (%)	Plant Height (cm)	Ear Height (cm)	Shelling (%)
Pac.984 x Mon.949	9.33 a	65.0 c-h	66.3 b-e	22.9 b-e	190.1	95.3 abc	78.8 b-e
Mon.949 x Pio.A33	9.18 ab	63.7 g-j	64.6 def	24.4 bc	181.4	92.9 a-d	78.5 b-e
Pac.984 x Pio.3012	7.98 c-f	65.7 a-f	66.3 b-e	19.3 f	182.9	91.9 b-e	78.9 b-e
Mon.949 x Syn. 48	7.98 c-f	63.0 ij	64.0 f	23.0 b-e	184.9	89.3 b-f	77.5 de
Pio.A33 x Mon.919	7.62 c-h	63.7 g-j	65.0 def	21.4 ef	177.6	95.7 ab	77.9 cde
Syn. 48 x Pio.3012	7.20 d-i	65.3 b-g	66.3 b-e	22.8 c-e	187.5	100.5 a	77.6 de
Pac.984 x Mon.919	7.06 e-i	66.0 a-e	66.7 a-d	20.5 ef	189.5	86.3 e-g	77.3 de
Pio.A33 x Pio.3012	6.97 e-i	66.7 abc	68.3 ab	21.4 ef	169.7	87.1 c-g	77.8 de
Mon.949 x Pio.3012	6.86 f-i	64.3 e-j	65.0 def	23.1 b-e	186.5	92.4 a-e	77.8 de
Pio.3012 x Mon.919	6.75 ghi	63.3 i-j	65.0 def	21.0 ef	175.6	89.1 b-f	81.2 abc
Pioneer A33	8.47 abc	64.7 d-i	66.0 d-f	21.9 def	182	91.9 b-e	79.8 a-d
Suwan 4452	8.26 a-d	66.7 abc	68.7 a	25.0 ab	177.3	84.2 efg	76.3 e
Pioneer 3012	8.08 b-e	66.7 abc	68.0 abc	22.4 c-f	165.2	89.1 b-g	77.2 de
Syngenta NK 48	7.86 c-g	64.0 f-j	66.0 d-f	22.7 c-f	171.3	74.4 h	77.9 cde
Monsanto 949	7.57 c-h	62.7 j	64.3 ef	26.7 a	178.3	81.6 fgh	77.9 cde
Monsanto 919	7.32 c-i	63.3 hij	65.0 def	23.9 bcd	176.1	88.1 b-g	82.9 a
Mean of top 10 interfamily cross	7.69	64.7	65.8	22.0	182.6	92.1	78.3
F-value[1/]	**	**	**	**	ns	**	*
CV(%)	9.449	1.825	1.941	5.827	7.565	5.779	2.557

[1/] ns: non significant, * : significant, ** : highly significant

[2/] Crosses between two sets of composite lines.

may then be composited to establish the composite-sibbed lines.

Conclusion

Line selection combined with early generation testing for combining ability is an effective method. It gave higher average yield of top-10 S_2 testcrosses over the composite testcrosses. However, statistically, there was no clear advantage of yield between both groups of lines in early generation testcrosses. Besides, the selected S_2 and composite lines showed similar results in diallel cross sets. Visual selection under low-competition environment proved to be a very effective method to identify good combining and relatively high yield lines. However, testcross and dialel cross should be applied for thorough test of combining ability of lines.

Composite lines had clear advantages over S_3 lines in yield, earliness and plant height. The modified S_1-full sib selection for the improvement of composite lines is a flexible method of which can be applied to improve the composite as well as inbred lines. However, further investigation is required to prove its merit for the construction of early generation hybrids as well as for the improvement of inbred lines.

Acknowledgment

We are grateful to Higher Education Project of Nong Lam University, Vietnam for financial support and to the staff of National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand for their kind helps during the time we did experiments.

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Genetic diversity, heterosis, and combining ability within Nepalese maize varieties

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Abstract. Information on the genetic diversity of germplasm is important in breeding programs, enabling the assessment of the breadth of their diversity and assigning them into different heterotic groups. Four maize populations, each represented by15 individual plants, were fingerprinted with 30 SSR markers to analyze their genetic relationships. The average number of alleles equal to 3.45 per locus was observed among the populations. Gene diversity was >0.5, ranging from 0.51 (Manakamana-2) to 0.56 (Khumal Yellow and Arun-4).Forty crosses among the populations were made using Line x Tester mating design with 10 parents as lines and four parents as testers. The parents and their F_1 hybrids were evaluated to estimate heterotic effects, determine general and specific combining ability, and establish heterotic groups. Population $46C_1$/ Rampur Composite showed the highest heterosis for grain yield among the crosses. The crosses; Manakamana-2 /NML-2 and Rampur So3Fo2/Rampur Composite also showed high heterosis. Based on the GCA effects, Rampur So3Fo2 was identified as the best combiner among the lines whereas good combiners were NML-2 and Rampur Composite among the testers. Upahar/Arun-4, Population $46C_1$/Rampur Composite, Population $46C_1$ /NML-2 had shown higher SCA effects for grain yield.Based on SCA effects for grain yield, the germplasm were assigned into A, B, and AB heterotic groups. These heterotic groupings could serve as a useful guide to the maize breeders in inbred line development for hybrid cultivar development. The groupings of the four populations as determined by molecular markers were consistent with their placement in the heterotic groups based on SCA effects.

Keywords: genetic diversity, heterosis, combining ability, heterotic groupings, SSR markers, alleles, genetic distance

Introduction

Information on genetic diversity is fundamental for hybrid breeding program in assessing the level of genetic diversity, characterizing the germplasm and assigning them into different heterotic groups (Reif *et al.,* 2003). Knowledge of germplasm diversity and their relationships among the elite breeding materials has a significant impact on the improvement of crop plants. This kind of information is very useful and helpful in the development and evaluation of inbred lines for planning crosses for hybrid cultivar development.

In addition, the choice of heterotic groups is fundamental in hybrid maize breeding. Heterotic group has been considered as the most important tool for exploiting heterosis in germplasm. Genetic diversity in crop plants can be analyzed at different levels. The development of molecular markers has made possible to achieve the degree of precision in the analysis of plant genetics at DNA level (Melchinger and Gumber, 1998). A number of molecular markers systems are currently available for the analysis of genetic diversity of the germplasm at the DNA level. Of the molecular markers, SSRs or microsatellites have become the marker of choice for many genetic analyses because of their high level of polymorphism, repeatability, low cost, and amenability to automation. SSR markers are codominant in nature, which allows the allelic contribution of each parent to be detected, is particularly important in hybrid maize testing. Characterization of Nepalese maize germplasm based on the molecular analysis is essential. There is limited information on the genetic diversity; heterosis and combining ability of the Nepalese maize germplasm. Information generated so far was solely based on the morphological data. Studies in this regard are needed to make a hybrid program more systematic so that heterosis could be exploited and utilized in hybrid breeding program.

Realizing the important aspects of genetic diversity and heterosis, and combining ability of maize, the study was undertaken in order to determine heterotic effects on yield and other quantitative traits, to estimate general and specific combining ability of parental lines and their crosses, to establish different heterotic groupings and to analyze the relationships of the Nepalese maize germplasm.

Materials and methods

Two studies were carried out in this study. The first study was to estimate heterosis of the crosses and to determine combining ability of parents included in the study. The second study was to fingerprint some of the selected maize populations to analyze their genetic relationship.

Fourteen maize germplasm were used in this quality. Arun-4, Rampur composite, Pool 17E, and NML-2 (CML-430), a CIMMYT developed inbred line were used as testers. Ten germplasm; Population 46$C_{1,}$ Manakamana-2, Rampur So3Eo2, Population 35$C_{5,}$ Narayani, Hill pool yellow, Khumal yellow, Upahar, and Rampur So_3Fo_2 were used as lines. Seeds of these germplasm were obtained from the National maize research program, Rampur, Nepal. Field experiments were conducted from February to September 2005 at the Fruits and Vegetables Seed Center of the Central Luzon State University (15 ^{0}43 N latitude, 12^0 54 E ^{30} amsl) in the Philippines.Four open-pollinated varieties?Rampur Composite, Khumal Yellow, Manakamana-2, and Arun-4?were included in molecular analysis. Rampur Composite and Arun-4 are the varieties recommended for both terai and hill environments, whereas Khumal Yellow and Manakamana-2 are suitable to the hill environments. Khumal Yellow and Rampur Composite are still very popular varieties in the mid hills, even though these varieties were released in 1966 and 1975, respectively.

Thirty SSR marker loci, possessing a repeat unit greater than two nucleotides and representing six bins per chromosome, were selected based on bin location and PIC value for uniform genome coverage (Maize DB, www.agron.missouri.edu). Markers were chosen from the 43 priority markers used by the Asian Maize Biotechnology Network (AMBIONET). Primers were synthesized through Research Genetics, Inc. (Huntsville, AL, USA). Seeds were grown in plastic trays for DNA isolation. The DNAs were isolated/extracted following the CTAB procedure (CIMMYT, 2001). The protocol for DNA extraction; amplification, and detection, as described in George et al. (2004), was followed. PCR products were separated using 4.5% PAGE and visualized using silver staining (Promega). A laboratory protocol used by the AMBIONET Service Laboratory was followed for running the PAGE. A laboratory protocol developed and used by the AMBIONET was followed for the molecular analysis of the four populations. Cluster analysis was done with the unweighted pair group method using arithmetic averages (UPGMA) and relationships between populations were visualized in a dendrogram.

Production of crosses

We made forty-crosses using the line x tester mating design. Staggered plantings were done for the testers to synchronize flowering between the lines and the testers. Plantings were carried out using the recommended spacing. Fertilizer was applied at 120-60-40 kg NPK ha^{-1}. All the standard agronomic operations were followed. Crosses were made as planned. Seven plants were pollinated for each cross combination in order to get sufficient seed for evaluation. Mature ears from each cross and their parents were harvested separately. Seeds from each line were shelled, dried, cleaned, and stored safely for the next-season evaluation.

Evaluation of hybrids

Forty crosses along with their 14 parents were evaluated for yield and other important traits. Seeds were planted in two rows of 4-m length. Two seeds per hill were planted with 25 cm spacing between hills and 75 cm spacing between rows. Plants were later thinned to one to maintain final plant density at 53,500 plants ha-^{1}. The fertilizer rate used was 120-60-40 kg NPK ha^{-1}. All the standard agronomic operations were followed. Plants were harvested depending upon the maturity, on a whole-plot basis. The ears from each plot were harvested and grain yield was taken on a plot-yield basis. Observations were taken for days to 50% silking, plant height, ear height, ear length, number of kernels per ear, 100-seed weight, and grain yield.

The systematic procedure as described by Singh and Chaudhary (1977) was followed for the line x tester analysis. Analysis of variance, general combining ability (GCA) and specific combining ability (SCA) effects for grain yield and other quantitative traits were analyzed. The test of significance for GCA and SCA effects was done against the student t-test with error degree of freedom. Heterosis over mid-parents was calculated and estimated. Mean squares of the mid-parent heterosis were tested against the error variance by the usual F test.

Results and discussion

Results of the analysis of variance (ANOVA) for 40 crosses and 14 parents for yield and yield-contributing traits showed highly significant differences among the parents, crosses, parents versus hybrids and lines for all traits, except for plant height and ear length in parents versus hybrids. Similarly, highly significant differences were observed in line x tester for grain yield, days to 50% silking, ear height, and ear length, except for plant height,

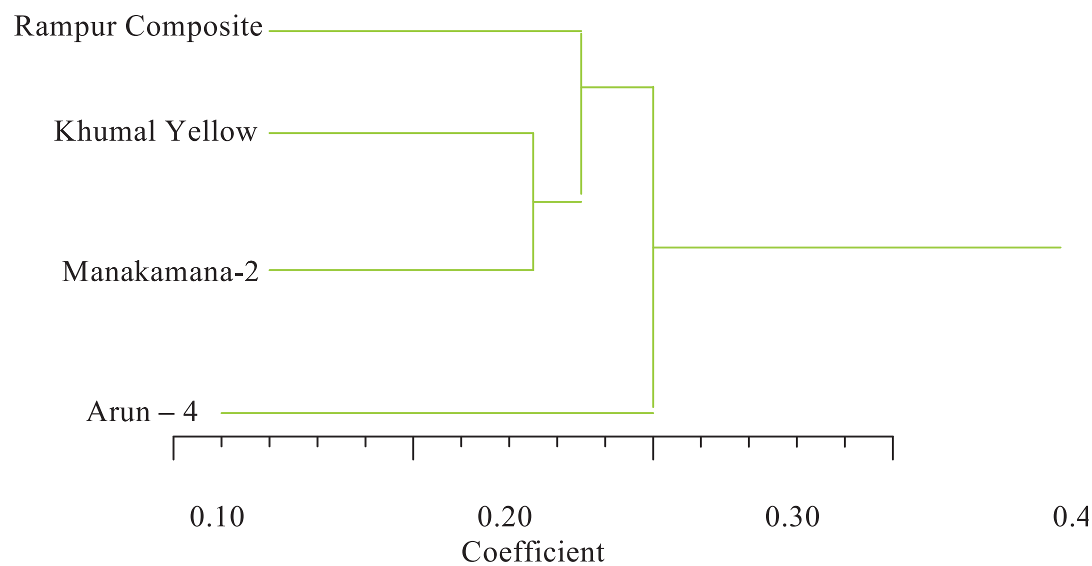

Figure 1. Genetic distance of four Nepalese maize populations based on modified Rogers' distance.

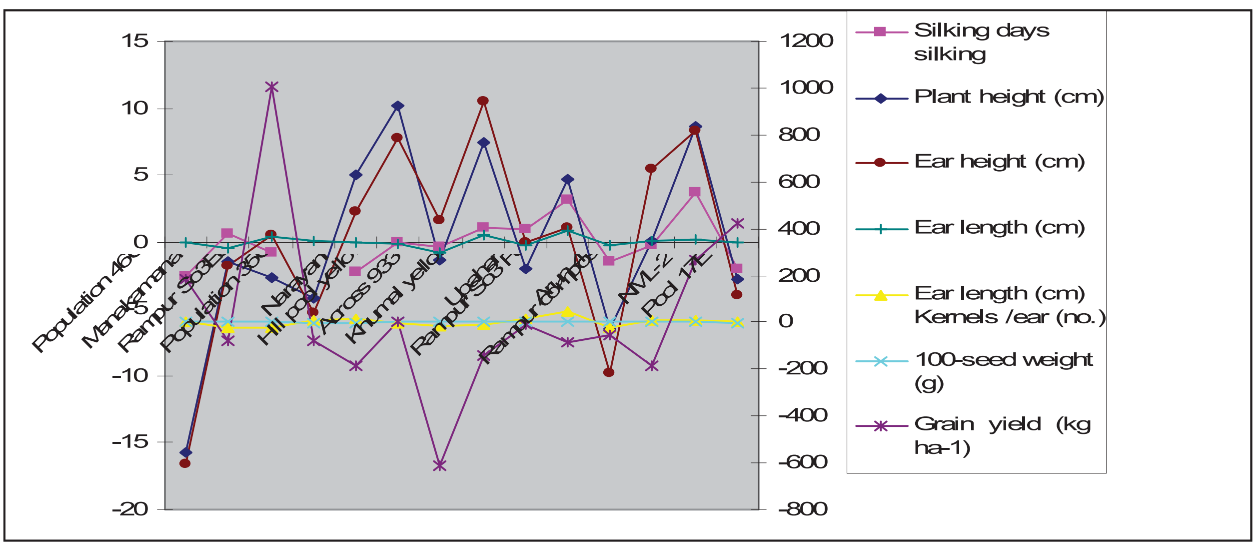

Figure 2. Estimates of general combining ability of germplasm for yield & other traits

number of kernels, and 100-seed weight indicating the presence of dominant effects.

Thirteen out of 40 crosses showed positive heterosis and 12 crosses exhibited negative heterosis for silking days (fig.3). Most of the crosses matured earlier than their parents. Of the total crosses, eight showed a significant positive heterosis for grain yield. Crosses using Rampur composite and NML-2 as male parents showed a higher heterosis than Arun-4 and Pool 17E.These parents are potential parents for hybrid breeding work. We estimated the general combining ability (GCA) of testers and lines for silking days, plant and ear height, number of kernels per ear, 100-seed weight, and grain yield. Rampur So3Eo2 showed the highest GCA value (1005.10) for grain yield among the lines, followed by Population 46C_1 (181.27), and Hill pool yellow (1.55). Pool 17E and NML-2 had shown positive GCA values (424.60 and 262.90) among the testers (fig.2).

Based on the positive GCA effects, NML-2 was the best combiner for all traits among the testers. Rampur So3Fo2 was a good combiner among the lines for all traits, except for grain yield. Rampur So3Eo2, Population 46C_1,

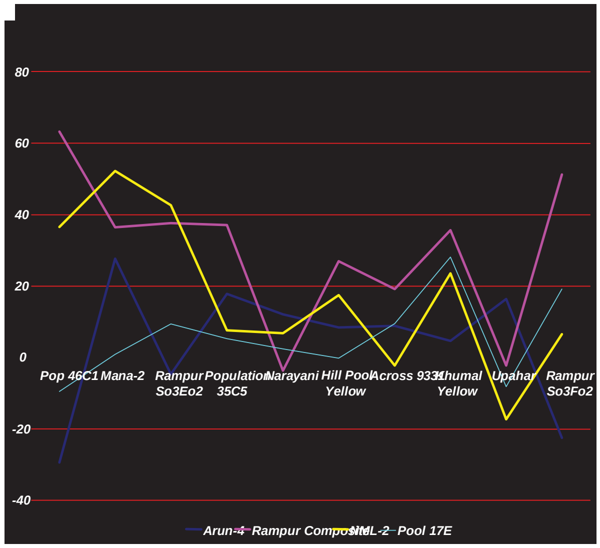

Figure 3. Heterosis of 40 crosses for grain yield

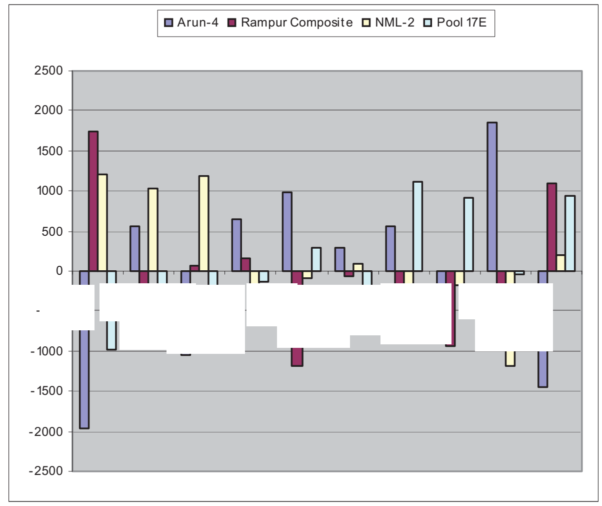

Figure 4. Estimates of SCA effects of 40 crosses for grain yield

and Hill pool yellow were good combiners among the lines. Similarly, good combiners among the testers were Pool 17E and NML-2 for grain yield. These good combiners are potential parents for hybrid breeding work. Significant differences in silking, days, ear height, and grain yield for both GCA and SCA effects indicated the importance of both additive and non-additive gene action.

Similarly, specific combining ability (SCA) of the 40 crosses was estimated for silking days, plant and ear height, number of kernels per ear, 100-seed weight, and grain yield. Crosses exhibited both positive and negative SCA effects for silking days (fig.4). The highest negative SCA effects was observed in Population 46C$_1$/Pool 17E (-3.83), followed by Population 35C5 /Arun-4 (-2.66), and Khumal yellow and Pool 17E respectively. Parents of the crosses showing higher negative SCA effects could be employed for developing early-maturing hybrids.

Nineteen crosses showed positive SCA effects and 21 crosses displayed negative SCA effects for grain yield (fig.4). Population 46C$_1$/Arun-4 had shown the highest SCA effects (1852.24) for grain yield. Other crosses showing higher SCA effects were Population 46C$_1$/Rampur Composite (1740.53), Population 46C$_1$/NML-2 (1213.27), and Rampur So3Eo2 /NML-2. Parents of crosses showing higher SCA effects for grain yield are ideal parents that can be used in the hybrid-breeding program.

Germplasms were classified into different heterotic group based on the SCA effects for grain yield. Germplasm showing positive SCA effects with NML-2, an inbred tester with full season maturity and negative SCA effects with Arun-4, an OPV tester with early maturing were assigned into heterotic group A. Germplasm exhibiting negative SCA effects with NML-2 and positive SCA effects with Arun-4 were classified into heterotic group B. Germplasm showing positive SCA effects with both the testers were assigned into heterotic group AB and germplasm displaying negative SCA effects with both the testers kept undetermined (Table1). Germplasms under heterotic group A were Population 46C$_1$, Rampur So3Eo2, and Rampur So3Fo2 while Upahar, Across 9331, Population 35C$_5$ and Narayani were under heterotic group B. Manakamana-2, and Hill Pool Yellow were kept under AB heterotic group. Khumal Yellow was kept under undetermined. Potential inbred lines could be developed from these heterotic groups. These heterotic groups could serve as the potential sources for inbred line development for hybrid breeding.

We estimated the PIC values of each of the polymorphic SSR locus detected in the study. Average PIC value at the population level was 0.50 and the range was from 0.47 in Manakamana-2 to 0.52 in Khumal Yellow and Arun-4 (Table 2). SSR loci; phi064, phi109188, and phi053 showed the best average PIC values (0.76 and 0.67) respectively. The total number of alleles detected was 415 for 30 SSR markers in 60 genotypes from the four maize populations. The alleles ranged from one in phi089 to seven in bnlg118, phi109188, and umc 1152.The average number of alleles among the populations was 3.45 (range: from 3.21 alleles in Manakamana-2 to 3.76 in Khumal Yellow). The unique alleles detected were 27, whereas the total common alleles were 71 in 60 genotypes from the four populations.

Table 1. Classification of germplasm into different heterotic groups based on SCA effects for grain yield.

Lines	Grain yield		SCA effects		Proposed heterotic group
	NML-2	Arun-4	NML-2 (A)	Arun-4 (B)	
Population 46C_1	8164.18	4217.52	1213.27	-1967.98	A
Rampur So3Eo2	8957.65	5961.44	1182.91	-1047.88	A
Rampur So3Fo2	6012.81	4458.39	195.96	-1461.54	A
Upahar	5572.98	7847.06	-1187.23	1852.24	B
Across 9331	5352.96	5958.17	-807.47	563.14	B
Population 35C_5	6012.81	6571.37	-676.804	647.16	B
Narayani	6489.93	6802.61	-92.44	985.64	B
Manakamana-2	7713.14	6474.07	1022.60	548.94	AB
Hill Pool Yellow	6867.28	6292.98	96.08	287.20	AB
Khumal Yellow	6449.50	5458.63	-181.47	-406.93	Undetermined

Table 2. Mean PIC values, number of alleles per locus, heterozygosity percent, gene diversity and variance of gene diversity in four maize populations as revealed by SSR markers

Population	PIC	Number of alleles/locus	Heterozygosity (%)	Gene diversity	Variance of gene diversity
Rampur Composite	0.48	3.41	35.23	0.52	0.06
Khumal Yellow	0.52	3.76	48.21	0.56	0.03
Manakamana-2	0.47	3.21	42.19	0.51	0.04
Arun-4	0.52	3.41	54.64	0.56	0.04
Across the populations	0.50	3.45	45.07	0.54	0.04
Range	0.00-0.84	1.00-7.00	21.00-68.97	00.00-0.93	0.00-0.31
SE±	0.35±0.64	3.05±3.84	44.23±45.90	0.39±0.68	0.02±0.10

The average heterozygosity at the population level was 45.07%; the range was from 35.23% in Rampur Composite to 54.64% in Arun-4. phi109188 showed the maximum heterozygosity (81.03%), while umc1109 SSR exhibited the minimum (1.69%). At the population level, Arun-4 showed the highest heterozygosity (54.64%), followed by Khumal Yellow (48.21%), Manakamana-2 (42.19%), and Rampur composite (35.23%).Manakamana-2 and Khumal yellow had higher heterozygosity percentage, reflecting greater gene diversity than the other two populations.

Khumal yellow showed the highest gene diversity (0.62) among the populations, followed by Rampur Composite (0.59), Arun-4 (0.57), and Manakamana-2 (0.56). The observed average gene diversity was 0.59 among the populations.. The gene diversity in Rampur Composite ranged from 0.13 in umc1304 to 0.93 in phi064.The minimum gene diversity was observed in phi423796 (0.07) and the maximum was noted in phi072 (0.83).

Based on the Modified Rogers Distance, a dendrogram was constructed according to Wright (1978) and Goodman and Stuber (1983). The highest genetic distance observed was 0.290, between Manakamana-2 and Arun-4 showed the highest genetic distance, followed by Rampur composite and Arun-4 (0.271), and Khumal yellow and Arun-4 (0.265) (Fig. 1). Khumal yellow and Manakamana-2 displayed the lowest genetic distance (0.220) among the four populations(fig.1).

Based on the genetic distance, Rampur composite, Khumal yellow, and Manakamana-2 are considered closely related, sharing similar genetic backgrounds, whereas Arun-4 is genetically distant from the three populations.

Conclusions

Rampur So3Eo2/ NML-2 was the highest grain yielder among the crosses. Population 46C_1, /Rampur composite, Population 46C_1/NML-2, and Upahar/Arun-4 were the other high-yielding crosses. Parents of these crosses could be utilized for developing high-yielding hybrids. Population 46C_1/ Rampur Composite showed the highest heterosis for grain yield among the crosses. The crosses Manakamana-2 /NML-2 and Rampur So3Fo2/Rampur Composite also had high heterosis. Based on the GCA effects, Rampur So3Fo2 was identified as the best combiner among the lines whereas NML-2 was best combiner among the testers. Rampur So3Eo2, Khumal yellow, Rampur So3Eo2 and Rampur composite were identified as good combiners among the lines. These germplasm could be utilized in population improvement programs. Crosses such as Upahar/Arun-4, Population 46C_1/Rampur Composite, Population 46C_1 /NML-2, Rampur SoEo2/NML-2, Across 9331/Pool 17E, and Rampur So3Fo2 /Rampur composite were the best specific cross combinations for grain yield. Parents of these crosses are ideal choices for developing high yielding non-conventional hybrids.

Three heterotic groups; A, B, and AB have been established based on the SCA effects for grain yield. Genotypes within the same group are genetically similar and between the two groups are dissimilar. Potential inbred lines could be developed from these heterotic groups for developing hybrids.

The highest number of alleles per locus was observed in Khumal yellow and Arun-4. Khumal yellow had the highest percentage of the heterozygosity. The results suggest that a considerable amount of allelic, gene diversity exists among the four populations. Manakamana-2 and Arun-4 had the maximum MRD among the four populations. Arun-4 was more distantly related compared with the other three populations. Rampur composite, Khumal yellow, and Manakamana-2 are closely related to each other and share a similar genetic background.

Acknowledgement

The authors are very grateful to the Nepal Agricultural Research Council and the Hill Maize Research Project for providing the financial assistance to this study. The authors are also thankful to the AMBIONET, CIMMYT, Philippines for providing all the materials and technical support required in the conduct of this study at the AMBIONET Service Laboratory, IRRI, Los Banos, Philippines. The authors would also like to thank to Ms. Ellen S. Regalado and Ms. Meggy Lou Katimbang of AMBIONET, CIMMYT, Philippines, for their help and cooperation received during the conduct of the molecular analysis of this work. The authors also express their sincere thank to the National Maize Research Program, Rampur, Nepal for providing the seeds of the germplasm used in this study.

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Performance of Grain Protein, Starch and Oil Contents in Maize (*Zea mays* L.)

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Abstract. The objectives of the present study were to investigate the heterosis and the parent-offspring correlations for protein, starch and oil content in maize (*Zea mays* L.). Ten parental inbred lines and resulting 90 reciprocal F_1 single crosses were grown in a randomized complete block design at two locations with two replications in 2002. The seed samples were determined for protein, starch and oil contents by near infrared reflectance spectroscopy (NIRS). Protein content invariably showed a negative heterosis, whereas starch content expressed a positive heterosis. Oil content consistently expressed an average positive heterosis over the lower parent. In all comparisons, there existed a highly significant and the largest correlations between protein, starch and oil contents of the hybrid and the means of two parents, the *r*-values ranged from 0.60^{**} to 0.53^{**}, 0.52^{**} to 0.62^{**}, and 0.57^{**} to 0.37^{**} at two locations, respectively. It is indicated that gene action was primarily additive for these traits. Therefore, the proximate values for protein, starch and oil contents of the hybrid could be predicted from the means of the two parents.

Key words: maize (*Zea mays* L.); heterosis; parent- offspring correlation; protein; starch; oil

Introduction

Maize (*Zea mays* L.) is widely used for animal feed, human food, and industrial products, thus increasing kernel protein, oil and starch concentrations may provide added values (Letchworth and Lambert, 1998). Feeding trails with poultry, swine and dairy cattle all show advantages of high oil hybrids over the normal maize (Han et al., 1987; Berke and Rocheford, 1995; Lambert, 2001), high protein hybrids would offer potential as feed for ruminant animals, while high starch hybrids may be valuable for the production of starch and ethanol (Dudley et al., 2007). The great importance of maize encouraged breeders to develop hybrids with value added traits for various end-uses. Therefore, information on the performance of these quality traits in hybrid progeny would be helpful.

Several researchers have studied the heterosis and parent-offspring relationship of maize quality traits. Ryšavá (1994), Wang et al (1998) and Duan et al (2003) reported a negative heterosis for protein content. Moreover, Ryšavá (1994) found that heterotic effect depended on combinations of inbred lines, the direction of crossing and the year. Oil content showed a negative heterosis (Miševiæ et al., 1989) or no obvious heterosis (Li and Song, 1991). But Duan et al (2003) found a positive heterosis for oil content. Wang et al (1998) and Duan et al (2003) observed an average positive heterosis for starch content. The correlations of protein or N, starch and oil contents between the hybrids and their parents were investigated, but no consensus were obtained (Genter et al., 1957; Russell and Pierre, 1980; Wang et al., 1998; Duan et al (2003). The correlations coefficients (*r*) were highly significant for oil and protein content (Genter et al., 1957; Russell and Pierre; Wang et al., 1998). Furthermore, Genter et al (1957) and Russell and Pierre (1980) concluded that little dominance is expressed in the inheritance of protein or N and oil content in kernels. Wang et al (1998) observed no significant parent-offspring correlations for starch content. However, a low relationship between the hybrids and their parents for the three traits were found by Duan et al (2003). The objectives of the present study were to determine the heterosis and the correlations of inbred lines with their single-crosses for protein, oil and starch contents.

Material and methods

Ten maize inbred lines (P_1=Ye 478, P_2=Mo17, P_3=Chang 7-2, P_4=E28, P_5=Ye 107, P_6=BT_1, P_7=Ji 446, P_8=Chuan 48-2, P_9=Huang C, and P_{10}=P138) were crossed using a complete diallel mating design to produce 90 F_1 reciprocal crosses. Parents and 90 F_1 crosses were grown in a randomized complete block design with two replications at each of two locations (Beijing, and Changzhi city in Shanxi province) in 2002. Plots consisted of single row for F_1 crosses, and four rows for parental lines. Each row was 5 m long and 0.667 m apart with 16 plants. All plots were thinned to approximately 57,000 plants per hectare. At anthesis, eight to ten plants from the middle part of each plot were self-pollinated, and equal numbers of seeds from each selfed ear within a plot were bulked for chemical analysis. Protein, starch and oil concentrations (%, on dry weight basis) in the kernel were measured on an intact-kernel sample of the bulked seed by near infrared reflectance spectroscopy (NIRS) (Wei et al., 2005). Protein, starch and oil contents (%) of the parental lines and their single crosses are summarized in Table 1. The heterosis values for protein, starch and oil were estimated using the following equations: Mid-parent heterosis (%) = 100 × (F_2 - MP) / MP, Lower-parent heterosis (%) = 100 × (F_2 - LP) / LP; Higher-parent heterosis (%) = 100 × (F_2 - HP) / HP, where MP, LP, and HP is the Mid-parent, the lower parent and the higher parent, respectively. The simple correlations of hybrids with their parents were also calculated.

Results

The heterosis of protein, starch and oil contents

It was indicated from Table 2 that the average heterosis of protein content over the mid-parent, the lower parent and the higher parent are all negative values; suggesting protein content of the hybrid generally lower than that of either parent. In contrast, the starch content consistently showed an average positive heterosis over the mid-parent, the lower parent and the higher parent. Therefore, the starch content of the hybrid would higher than that of either parent. Oil content invariably showed a positive heterosis over the lower parent at two locations, therefore, the oil content of the hybrid is intermediate that of the parents.

Table 2 further revealed that growing site affected heterotic effect in the magnitude or direction. For example, in Beijing experiment, the average heterosis of oil content over the mid-parent and the higher parent were positive values (23.99, 17.09), whereas in Changzhi they were negative (-0.17, -2.83). The similar phenomenon was observed for protein and starch content.

The parent- offspring correlations

It is evident from Table 3 that most of the correlations of hybrids with their parents were highly significant. In all cases, the correlations of hybrids with their mid-parents were the largest, the *r*-values ranged from 0.602** to 0.531**, from 0.520** to 0.620** and from 0.566** to 0.370** for protein, starch and oil contents, respectively. In addition, the correlations were also affected by the locality of growing. For example, in Beijing experiment, the correlation of oil content between the hybrid and its lower parent was highly significant (*r* = 0.540**) while in Changzhi experiment it was not significant (*r* =0.067).

Table 1. Phenotypic means and ranges of protein, starch and oil contents (%) in ten parents and 90 single crosses grown at two locations in 2002.

Generation		Protein		Starch		Oil	
		Beijing	Changzhi	Beijing	Changzhi	Beijing	Changzhi
Parents	Mean	12.81	11.09	66.41	67.96	3.67	4.78
	Range	11.37~15.02	9.20~12.50	63.41~68.91	63.89~70.98	3.07~4.16	4.58~5.17
F_2	Mean	10.48	9.92	69.34	69.46	4.55	4.77
	Range	8.99~12.18	8.19~11.61	65.46~71.34	66.82~72.44	3.94~5.56	3.99~5.39

Table 2. Average heterosis values (%) for protein, starch and oil contents.

Item	Protein		Starch		Oil	
	Beijing	Changzhi	Beijing	Changzhi	Beijing	Changzhi
Mid-parent heterosis	-18.05	-10.43	4.42	2.22	23.99	-0.17
Range	-27.46~-7.41	-23.45~6.86	0.76~8.33	-1.47~7.00	6.70~46.78	-16.11~28.21
Lower-parent heterosis	-13.89	-4.27	6.04	4.13	32.51	2.74
Range	-23.98~-2.44	-19.37~13.76	1.97~10.99	-1.07~11.70	12.42~65.37	5.51~32.74
Higher-parent heterosis	- 21.69	-15.58	2.87	0.42	17.09	-2.83
Range	-30.81~-10.61	-32.39~3.28	-1.56~8.57	-3.69~5.10	-0.46~42.95	-16.70~23.98

Table 3. The parent-offspring correlation coefficients for three quality traits.

Item	Hybrid-higher parent		Hybrid-lower parent		Hybrid-midparent	
	Beijing	Changzhi	Beijing	Changzhi	Beijing	Changzhi
Protein	0.596**	0.368**	0.383**	0.527**	0.602**	0.531**
Starch	0.425**	0.596**	0.452**	0.465**	0.520**	0.620**
Oil	0.410**	0.230*	0.540**	0.067	0.566**	0.370**

*,** Significant at the 0.05, 0.01 probability level, respectively.

Discussion

It was indicated by this and previous study (Ryšavá, 1994; Wang et al., 1998; Duan et al, 2003) that protein content invariably expressed a negative heterosis. Thus, it would be difficult to exploit heterotic effect to improve protein content of the hybrid. On the contrary, starch content consistently showed a positive heterosis over their parents. This was in close agreement with the results of Wang et al (1998) and Duan et al (2003). Therefore, starch content of the hybrid can be increased by using heterosis effect while the parental lines should be properly chosen. Oil content showed a positive heterosis over the lower parent. This was consistent with the result of Duan et al (2003); however Miševiæ et al (1989) and Li and Song (1991) observed a negative or no obvious heterosis. Thus, development of parental inbred lines with increased oil content is important. The negative heterosis for protein, while a positive heterosis for starch content possibly was due to the hybrids usually have larger kernels than their parent lines, primarily an increase in endosperm, which is mostly starch; consequently, hybrids would be expected to have lower protein, and high starch content (Russell and Pierre, 1980; Ryšavá, 1994).

In this study, the correlations of hybrids with their parental means consistently were the largest for three quality traits, suggesting primarily additive gene action for these quality traits (Genter et al., 1957; Russell and Pierre, 1980). However, heterosis analysis in present and previous study (Miševiæ et al.,1989; Wang et al., 1998; Duan et al., 2003) gave the appearance of the complete dominance of low protein, high starch and low oil. Genter et al (1957) concluded that these extreme dominances may be partially caused by hybrid vigor, environmental factors or confounding effects, which may mask the true nature of gene action and give the appearance of dominance in direct parent-progeny comparisons, whereas correlations of parent-offspring can eliminate the confounding effects. Correlation data obtained in this study along with those of Genter et al (1957) and Russell and Pierre (1980) consistently confirmed the absence of complete dominance in protein, starch and oil contents inheritance. Therefore, the relative performance of the hybrids can be predicted from the mean of parental lines (Genter et al., 1957; Russell and Pierre, 1980).

It was revealed from this study that locality affected heterosis and correlations of parent-offspring of three quality traits. Protein, starch and oil contents are quantitatively inherited and may be influenced by environmental factors and cultural practices (Ryšavá, 1994; Lambert, 2001). Several researchers have observed significant genotype × environment (GE) interaction on these traits (Genter et al., 1956; Pollmer et al., 1979; Ryšavá, 1994; Berke and Recheford, 1995). This suggested that in maize production, the hybrids should be planted in suitable regions according to their adaptation.

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Traits

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Evaluation of improved maize populations for yield

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Abstract. Broadening genetic base and increasing the yield potential of the maize breeding populations are the prime importance for maize breeders. One potential source is the use of exotic germplasm by incorporating exotic sources into adapted sources. The objective of this study was to evaluate the breeding potential of ten maize populations for the hybrid breeding program. Ten breeding populations, diallel crosses among all ten populations, and nine check hybrids were evaluated in a 8 x 8 triple lattice conducted at six locations in 2007. Sources of variation for entries, populations, general combining ability (GCA) and specific combining ability (SCA) were highly significant. KS23(S)C5 had the highest positive significant GCA and variety effects of all the populations tested. Suwan5(S)C6 x KS23(S)C5 had the highest yield of 7.11 Mg ha^{-1}. KS23(S)C5 was also included in the parents of the other top four high-yielding crosses: Suwan3(S)C7 x KS23(S)C5 (6.94 Mg ha^{-1}), KS24(S)C3 x KS23(S)C5 (6.92 Mg ha^{-1}), Suwan1(S)C14 x KS23(S)C5 (6.86 Mg ha^{-1}), and KS23(S)C5 x KS27(S)C3 (6.79 Mg ha^{-1}). Suwan1(S)C14 x KS6(S)C4 had the highest high-parent heterosis (22.67%) with the yield of 6.44 Mg ha^{-1}. KS23 was superior to the other exotic maize populations and it seemed to be a new potential source in crosses with Suwan1 and derivatives of Suwan1 in the hybrid maize breeding program.

Keywords: Maize, Yield, Population, Combining ability, Heterosis,

Introduction

Broadening genetic base and increasing the yield potential of the maize breeding populations are the prime importance for maize breeders (Eberhart, 1971; Holley and Goodman, 1988; Mungoma and Pollak, 1988). One potential source is the use of exotic germplasm by incorporating exotic sources into adapted sources. In the tropical breeding program, the potential use of exotic germplasm especially from temperate regions was received considerable interest by maize breeders. Its use has been limited primary because of adverse effects of photoperiod mask desirable traits of exotic germplasm. Very early flowering, susceptibility to tropical diseases and severe inbreeding depression are major obstacle. The systematical evaluation and incorporation of exotic germplasm have worked out at Kasetsart University maize breeding program since 1978 in spite of these problems. Several studies have reported the usefulness of incorporating exotic germplasm into a breeding program (Eberhart, 1971; Hallauer and Miranda, 1981, Holley and Goodman, 1988; Iglesias and Hallauer, 1989; Pollak et al,, 1991). Diallel analysis of crosses among populations has been widely used to provide a preliminary analysis to determine the relative potential of population as breeding populations in hybrid breeding program (Hallauer and Miranda, 1981). In general greater genetic diversity is related to greater heterotic values (Hallauer and Eberhart, 1966; Lonnquist and Gardner, 1961; Troyer and Hallauer, 1968). Moreover, Moll et al. (1965) reported that heterosis would increase but only within a limited range of divergence. The objective of this study was to evaluate the breeding potential of ten maize populations for the hybrid breeding program.

Materials and methods

A diallel set was produced among 10 populations in 2007. The populations were represented a range of diverse germplasm developed by maize breeding project of National Corn and Sorghum Research Center (Farm Suwan), Kasetsart University. The 10 populations included in the diallel were:

1. Suwan1(S)C14: A population developed after 14 cycles of S_1 recurrent selection. Suwan1 is a downy mildew resistant version of Thai Composite 1. The resistance was incorporated from Philippine DMR 1 and Philippine DMR 5. Thai Composite 1 included the largest group of materials from several races collected onthe Caribbean Islands, six collections from Mexico representing the elite race Tuxpeno, and

Central America. Five collections were from South America and a few racial complexes represented by Caribbean-Tuxpeno-India-USA.

2. Suwan3(S)C7: A population developed after seven cycles of S_1 recurrent selection. Suwan3 was developed by intermating of 30 S_1 lines of Suwan1(S)C8 and 20 S_1 lines of KC1(ME)C3. Suwan3 contained 20% of sub-tropical germplasm.
3. Suwan5(S)C6: A population developed after six cycles of S_1 recurrent selection. Suwan5 developed by intermating of 60 full sib lines from five elite populations namely, Suwan1(S)C9, Caripeno DMR (S)C5, Thai Composite3(S)C5, Cupurico Flint Compuesto DMR (S)C2, and Amarillo Dentado (F)C5.
4. KS6(S)C4: A population developed after four cycles of S_1 recurrent selection. KS6 was developed by intermating of 40 S_1 lines of Caripeno DMR(S)C4, Amarillo Dentado DMR, Suwan DMR Source 11, and Suwan DMR Source 12.
5. KS23(S)C5: A population developed after five cycles of S_1 recurrent selection. KS23 was a broad base synthetic developed by intermating of 26 inbred lines which combined well with a strain of Suwan1. KS23 contained approximately 35 % of temperate germplasm and was designed to be a counterpart of Suwan1 in hybrid breeding program.
6. KS24(S)C3: A population developed after three cycles of S_1 recurrent selection. KS24 was developed by intermating strains of Suwan1 and Suwan3 which expressed a high combining ability with KS23.
7. KS26(S)C1: A population developed after one cycle of S_1 recurrent selection. KS26 was developed by crossing between collections of Tuxpeno race and improved populations of Corn Belt Dent race. KS26 was synthesized to provide a germplasm source which contained 50% of temperate germplasm.
8. KS27(S)C3: A population developed after three cycles of S_1 recurrent selection. KS27 was developed by intermating strains of Caripeno DMR.
9. KS28(S)C2: A population developed after two cycles of S_1 recurrent selection. KS28 was developed by crossing 16 non-Suwan1 inbred lines with Tuxpeno-1 Selection Sequia DMR.
10. KC15: A population was developed by crossing highland germplasm with temperate germplasm. KC15 was synthesized to provide a germplasm source which contained 100% exotic germplasm.

The 10 populations, 45 population crosses and nine hybrid checks were evaluated in a 8 x 8 triple lattice. The experiments were grown in 2007 at six locations. Plant density was about 66,666 plants per hectare. The plot consists of two rows with 5 m long and 0.75 m between rows. Plots were overplanted and thinned to the desired stand. Conventional fertilization and weed-control practices were applied as the local recommended at all locations for optimum maize production. Grain yield at 15% moisture was estimated and expressed as Mg ha^{-1}. The efficiency of the lattice analysis relative to a randomized complete block analysis was minimal. Therefore, combined analysis of variance was computed with the un-adjusted entry means from each location. The genetic information on the populations was obtained by using Model III suggested by Gardner and Eberhart (1966).

Results and discussion

The combined analysis of the mean data showed significant differences among entries and significant entry by location interaction (Table 1). The population means per se ranged from 2.42 Mg ha^{-1} for KC15 to 6.59 Mg ha^{-1} for KS23(S)C5 (Table 2). The lowest-yielding population was the material which contained 100 % exotic germplasm and also had undergone the least selection for improvement. KC15 and KS26(S)C1 had negative significant variety effect, indicating that their yields were lower than the average of all the populations. The significantly positive values of variety effects demonstrated by KS23(S)C5, KS24(S)C3, and Suwan5(S)C6 indicated that they were promising breeding materials for selecting desirable genotypes by recurrent selection. KS23(S)C5 had the largest GCA effect (0.84**), indicating a high frequency of favorable alleles. The other populations with positive significant GCA effects were KS24(S)C3 (0.12*), Suwan3(S)C7 (0.15**), and Suwan5(S)C6 (0.21**), respectively. KS6(S)C4, KS26(S)C1 and KC15 had negative

Table 1. Mean squares from Gardner and Eberhart (1966) population cross diallel analysis of yield from ten Populations and their 45 diallel crosses tested at six locations in 2007.

Source of Variation	df	Yield (t/ha)
Locations	5	443.96**
Locations × Rep.	12	8.60**
Entries	54	35.85**
Populations	9	11.36**
Populations VS Crosses	1	4.51**
Crosses	44	19.98**
General (GCA)	9	17.2**
Specific (SCA)	35	2.78**
Locations × Entries	270	0.84**
Residual	648	15.77
Total	989	3.45
C.V. (%)		15.5

*,** significant at P = 0.05 and 0.01, respectively.

Table 2. Yield (above diagonal) and specific combining ability effects (below diagonal) for the 45 population crosses, and yield (on diagonal), general combining ability, and variety effects for each of the ten populations per se tested at six locations in 2007.

Populations	SW 1 (S) C14	SW 3 (S) C7	SW 5 (S) C6	KS 6 (S) C4	KS 23 (S) C5	KS 24 (S) C3	KS 26 (S) C1	KS 27 (S) C3	KS 28 (S) C2	KC 15	GCA effects (g_i)	Variety effects (v_i)
	Mg ha^{-1}											
SW 1 (S) C14	5.21	5.96	6.1	6.44	6.86	5.85	5.72	5.95	6.17	5.39	0.25	0.13
SW 3 (S) C7	-0.26*	5.38	6.01	6	6.94	5.68	6.21	5.7	6.27	4.83	0.15**	0.29
SW 5 (S) C6	-0.18	-0.17	5.8	5.54	7.11	6.46	5.63	6.13	5.86	5.25	0.21**	0.72**
KS 6 (S) C4	0.49**	0.15	-0.37**	5.25	6.57	5.67	5.41	5.52	5.72	4.58	-0.12*	0.17
KS 23 (S) C5	-0.05	0.13	0.24*	0.03	6.59	6.92	6.39	6.79	6.11	5.44	0.84**	1.51**
KS 24 (S) C3	-0.35**	-0.41**	0.31*	-0.15	0.14	5.86	5.93	5.96	6.17	4.76	0.12*	0.78**
KS 26 (S) C1	-0.08	0.52**	-0.12	-0.01	0.01	0.26*	4.23	5.79	5.12	3.99	-0.28**	-0.85**
KS 27 (S) C3	-0.15	-0.29*	0.08	-0.2	0.11	-0.01	0.23*	4.96	5.87	4.87	0.02	-0.12
KS 28 (S) C2	0.17	0.38**	-0.09	0.09	-0.48**	0.30*	-0.35**	0.1	5.14	4.53	-0.08	0.06
KC 15	0.41**	-0.04	0.32*	-0.02	-0.12	-0.09	-0.46**	0.13	-0.12	2.42	-1.09**	-2.66**

*, ** significant at P = 0.05 and 0.01, respectively. 0.05

LSD (0.05) for population cross yields is 1.41 Mg ha^{-1}. SE (S_{ij}) =

significant GCA effects of –0.12*, –0.28**, and –1.09**, respectively.

The individual cross yields ranged from 3.99 Mg ha^{-1} for KS26(S)C1 x KC15 to 7.11 Mg ha^{-1} for Suwan5(S)C6 x KS23(S)C5 (Table 2). The greater individual cross yields also included Suwan3(S)C7 x KS23(S)C5 (6.94 Mg ha^{-1}), KS23(S)C5 x KS24(S)C3 (6.92 Mg ha^{-1}), Suwan1(S)C14 x KS23(S)C5 (6.86 Mg ha^{-1}), and KS23(S)C5 x KS27(S)C3 (6.79 Mg ha^{-1}). There were no significant differences among the four top-yielding crosses. The four populations with positive GCA effects were included as parents of top-yielding crosses. When comparing among introgressed exotic germplasm populations (KS23(S)C5, KS26(S)C1, and KC15) in population crosses with Suwan1(100% tropical) which was a widely adapted tropical variety, it indicated that KC15 (100% exotic) gave the lowest yielding cross. On the other hand, KS23(S)C5 (35% exotic) gave the highest yielding cross. The results might indicate that the proportion of exotic germplasm to be incorporated into adapted germplasm was crucial for increasing genetic diversity in the hybrid breeding program. Bridges and Gardner (1987) indicated that the optimum proportion of exotic germplasm to be incorporated differs from short-term and long-term selection goals, and also depends on the performance of the adapted and exotic germplasm. Ten crosses had positive significant SCA effects: Suwan3(S)C7 x KS26(S)C1 (0.52**), Suwan1(S)C14 x KS6(S)C4 (0.49**), Suwan1(S)C14 x KC15 (0.41**), Suwan3(S)C7 x KS28(S)C2 (0.38**), Suwan5(S)C6 x KC15 (0.32*), Suwan5(S)C6 x KS24(S)C3 (0.31*), KS24(S)C3 x KS28(S)C2 (0.30*), KS24(S)C3 x KS26(S)C1 (0.26*), Suwan5(S)C6 x KS23(S)C5 (0.24*), and KS26(S)C1 x KS27(S)C3 (0.23*), respectively.

Suwan1(S)C14 x KS6(S)C6 had the highest high-parent heterosis (22.67%) and also had a positive significant SCA effect (Table2). The top four high-yielding crosses had the high-parent heterosis estimates are shown in parentheses: Suwan1(S)C14 x KS23(S)C5 (4.10%), KS23(S)C5 x KS24(S)C3 (5.01%), Suwan3(S)C7 x KS23(S)C5 (5.31%), and Suwan5(S)C6 x KS23(S)C5 (7.89%), respectively. This study showed that the exotic germplasm especially from temperate region can be incorporated into adapted tropical germplasm to increase genetic diversity relating to increment of yield potential of tropical maize. The research indicated that KS23 was superior to the other exotic maize populations and it seemed that KS23 was a new potential source in crosses with Suwan1 and derivatives of Suwan1 in the hybrid maize breeding program.

Acknowledgments

The authors recognize the following cooperators for their efforts in contributing tested locations for this study: Syngenta Seeds R&D Station Tak Fah; Sweet Seeds Co., Ltd.; Bangkok Seeds Industry Co., Ltd.; Nakhon Sawan Field Crops Research Center, Department of Agriculture; Lopburi Research Station, Kasetsart University; and National Corn and Sorghum Research Center (Farm Suwan), Kasetsart University, Thailand.

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Troyer, A.F., and A.R. Hallauer. 1968. Analysis of a set of early flint varieties of maize. Crop Sci. 8:581-584.Table 1. Mean squares from Gardner and Eberhart (1966) population cross diallel analysis of yield from ten Populations and their 45 diallel crosses tested at six locations in 2007.

The intra-population improvement for QPM (Quality Protein Maize) in ICERI Indonesia

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Abstract. In the world health program FAO wanted to attack the underlying causes of the quality food crisis and not just the symptoms. QPM is a function of heredity and malnutrition. Indonesian Cereals Research Institute (ICERI) has released two opv of yellow and white grain, and shown that distributed of varieties could be developed around 35 thousand ha The grain quality is a progress which is receiving attention in breeding program by generated some population, genetic variability of QPM to increase by intra population improvement which is under one population to selected, evaluated and recombination to found candidate opv. Population Maros synthetic qpm yellow 1 (MSQK1 to increase of two cycles by recurrent selection of families S1. High generation of inbred line (>S4) has been develop as female in hybrid program. Tester as male was develop by back cross procedure to founded BC3F2. Donor from CIMMYT line CLM165 and recurrent parent was Mr14. The result content of lysine and tryptophane was 0,388% and 0,079%. The single cros founded combination MSQ.K1C0.15-2-1-1xMR14Q was found yielded 9.09 t/ha in rice field Palu Central Sulawesi.

Introduction

The area under maize in Indonesia on 2006 were 3,6 million ha with average yielded 3.45 t/ha but in Maluku and Papua the productivity only 1.85 t/ha. (BPA, 2006). There are 29 opv and 87 of hybrids has released untill 2007, and two ware released as QPM Srikandi yellow-1 and Srikandi white-1 (ICERI, 2007). Discovery of the QPM on 1963 by Linn Bates was fouded in the endosperm gen opaque-2 mutant, and endosperm contains a much higher level of tryptophane (Mertz, 1992). The content of lysine and tryptophane of QPM like Srikandi yellow-1 was 0.477% and 0.093% compare to normal maize Bisi-2 content of lysine 0.350 and tryptophane 0.080% (Yasin *et al*., 2005). Nutritional effect of opaque-2 gene was reported that as feeding test four time faster than those normal maize.on young rats, with the same result also on pigs. Young children who had developed a severa protein deficiency disease (kwashiorhor) on a diet of normal maize. There was great hope during the late 1960s that opaque-2 would improve the health of millions people in the countries where QPM was a najor atable in the diet (Bourlaug, 1992; Cordova. and Pandey. 2002; Vasal .2000) QPM is a function of heredity and malnutrition. ICERI QPM was distributed breeders seeds (BS) since two years (2004-2005) were 4.100 kg, the BS has increased in Peovince of NAD, NTB, NTT, and in Central Sulawesi, and there were predicted of 35,000 ha QPM has planted in the farmers field.

Population Maros Synthetic QPM yellow (MSQ.K1) was gererate by intra population improvement, the lines from CIMMYT CML160-CML172 was intercrossed C(n,2) and generation F2 seeds were buk to found of new ICERI QPM population. ICERI was improve QPM population by selected on MSQ.K1 for grain yellow and MSQ.P1 by white grain. There are three kinds of family selection were conducted in ICERI half sib, frll sib and S1 selection. Families evaluated in two-three locations and families selected to reccombine for increase of cycle of population. The status QPM population by intra selection procedure was generate three cycles in four to five years. The objective of the paper was describe of QPM breeding to generate hybrids candidate by intra population improvement in ICERI Maros.

Generate Tester

Tester as male for hybrid QPM program has been develop by back cross procedures (Hallauer, and Miranda, 1988; Cordova,, 2001). The parent lines was selected from male of inbred Bima-1 (Mr14). In the begining of 2003 (wet season) F1 generation founded by CML165xMr14, CML165 was donor for recurrent parent Mr14, F1 material to selected opaque seeds on the light table and back cross to Mr14 for generation BC1F1. The last back cross was selfed two times for new generation of BC3F3 on wet season 2007 (Yasin et al., 2007). There are five years to conducted of back cross to found MR14Q as QPM tester in hybrid program. The back cross also to conducted with same procedure for female of Bima-1 (Mr4) as recurrrent parent and donor by selected CML161. The characters of inbred in the back cross steps could be shown in table 1. Hybrid F1 for two crossed of inbred could be explained that The content of lysine and tryptophane include protein of the two inbred lines was to analyzed in CIMMYT and the result shown in table 1 In table 1 shown than back cross was succesfull to converted opaque-2 gene from donor CML161 and CML165 to parent of Bima-1 (Mr4 dan Mr14).

Plant charakters of donors and recurrent parent shown in table 2 that there are sincronice flowering time between two F1 (CML161xMr4) and (CML165xMR14). Seeds weight vary 30-45 gr/plant. In table 3 that single cross formation founded plant height 180-190 cm and ear height 90-95 cm, Compare with two parent material were 90-110 cm for plant height and 40-65 ear height, so there are significant increase of F1 characters tha the two parent material. The reaction

Table 1. The content of amino acid of Mr4Q and MR14Q by bsck cross procedure (Maros 2003-2007)

Entries	Protein	Lysine	Tryptophane
Parent of hybrid normal maize Bima-1			
Mr4 (female)	12,45	0,335	0,064
Mr14 (male)	12,70	0,388	0,079
Mr4 x Mr14 (F1:Bima-1)	12,31	0,291	0,058
QPM by back cross untill BC3F2			
Mr4Q (female)	11,03	0,495	0,104
Mr14Q (male)	11,47	0,417	0,085
Mr4Q x Mr14Q (F1:Bima-1Q)	11,05	0,524	0,110

(CIMMYT, 2007)

Table 2. Characters of Parent Material (Recurrent and Donor) in Back Cross Procedure. Maros 2003.

Characters	Donor		Recurrent parent	
	CML161	CML165	Mr4	Mr14
Vigor of Seeds, %	63	70	80	85
Days of tassel, days	52	49	50	54
Days of Silk, days	55	53	53	58
Plant height, cm	90	110	90	110
Ear height, cm	40	60	60	65
Stemb	middle	middle	strong	strong
Leaves	long-narrow	long-narrow	long	long-middle
Rooth system	middle	middle	deep	deep
Uniformly of plant	uniform	uniform	uniform	uniform
Plan aspect, score	2	2	2	1
Husc cover, score	2	2	1	1
Ear aspect, score	2	1	1	1
Seeds color	yellow	yellow	yellow	yellow
Tassel color	red	red	red	red
Silk color	red	red	red	red
Seeds texture	flint	flint	Flint	flint
Root lodging	strong	strong	strong	srong
Stem lodging	strong	strong	miiddle	strong
Reaction on pest and disease				
- Maydis leaf blight	middle	middle	tolerance	tolerance
- Rust	middle	middle	tolerance	tolerance
- Downy mildews	susceptible	middle	middle	middle
Tryptophane in protein, %*)	0,82	0,83	-	-
Protein in endosperm, %*)	11,2	11,1	-	-
Seeds weight per plant, g	45	46	30	45

Table 3. Characters F1 of Generation BC1F1, Maros 2004

Characters	CML161xMr14	CML165xMr4
Vigor of Seeds, %	90	95
Days of tassel, days	54	52
Days of Silk, days	58	55
Plant height, cm	180	190
Ear height, cm	95	90
Stemb	strong	strong
Leaves	widht	narrow
Rooth system	deep	deep
Uniformly of plant	1,5	1,5
Plan aspect, score	1	2
Husc cover, score	1	1
Ear aspect, score	yellow	yellow
Seeds color	yellow	red
Tassel color	red	red
Silk color	red	red
Seeds texture	flint	semi-flint
Root lodging	strong	strong
Stem lodging	strong	strong
Reaction on pest and disease		
- Maydis leaf blight	middle	middle
- Rust	middle	middle
- Downy mildews	susceptible	middle
Seeds weight per plant, g	105	80

Table 4. Characters of S2 by Generation BC3F2 Maros, 2005

Characters	Generasi F2 (kawin diri dari F1)	
	CML161xMr14	CML165xMr4
Vigor of Seeds, %	95	95
Days of tassel, days	55	54
Days of Silk, days	59	56
Plant height, cm	160	165
Ear height, cm	80	75
Stemb	strong	strong
Leaves	widht	middle
Rooth system	deep	middle
Uniformly of plant	1	1
Plan aspect, score	1	1
Husc cover, score	1	1
Ear aspect, score	yellow	yellow
Seeds color	red	red
Tassel color	red	red
Silk color	red	red
Seeds texture	flint	flint
Root lodging	strong	middle
Stem lodging	middle	strong
Reaction on pest and disease		
- Maydis leaf blight	middle	middle
- Rust	middle	middle
- Downy mildews	middle	susceptible
Seeds weight per plant, g	45	39

of plant under major pest and disease (maydis leaf blight, rust, and downy mildews) to observed was middle to tolerance. The last back crossed (BC3) has be continued to self in two generation (S2) to found BC3F2, in table 4 that seeds weight per plant founded 39-45 gr/plant (Yasin Arifuddin.,and Mejaya., 2005[a]).

Hybrid's candidate

The single cross generation founded by female from population MSQ.K1C0 in generation S3-S4, inbred was selected after evaluation yield in two sites of Maros experimental farm commonly in Maros and Bajeng. The male as tester was selected from generation BC3F2 which is high content of two amino acids. Evaluation variety trial was conducted in five location by completely randomized design in four replications, each entries was planted in four rows by spacing 75x20 cm. The yield potential of QPM hyrids candidate included control narmal maize shown in table 5. In table 5 shown the single cross of MSQ.K1C0.15-2-1-1xMR14Q was found yielded 9.09 t/ha in rice field Palu Central Sulawesi. The content of lysine, protein and tryptophane of candidate hybrid QPM shown in table 6.

Table 5. Yield Potential of Hybrids Candidate QPM Under Five Location of Central Maize Producrion. 2006/07

QPM Genotypes	Maros, South Sulawesi	Sinjai, South Sulawesi	Palu, Rice field, Central Sulawesi	Palu, dry land, Central Sulawesi	Kupang-Naibonat, NTT
MSQ.K1C0.3-1-1xMR14Q	9,34	4,22	7,30	6,67	5,23
MSQ.K1C0.8-1-1-1xMR14Q	7,70	4,62	8,01	7,31	4,18
MSQ.K1C0.15-2-1-1xMR14Q	7,52	5,46	9,09	6,80	4,96
MR4Q x MR14Q (Bima-1Q)	9,23	4,42	7,67	5,03	3,40
MSQ.K1C0.14-4-2-1xMR14Q	9,98	4,20	7,91	7,00	5,46
MSQ.K1C0.22-1-1xMR14Q	9,26	4,07	7,56	7,24	5,61
MSQ.K1C0.24-3-1-1xMR14Q	6,84	4,46	8,10	5,87	4,39
MSQ.K1C0.6-1-4xMR14Q	7,26	4,37	8,94	7,06	5,70
MSQ.K1C0.61-1-1xMR14Q	7,55	4,64	7,14	7,27	4,33
MSQ.K1C0.153-1-1xMR14Q	8,49	4,76	8,12	6,69	5,01
CML161 x CML165	8,52	5,61	6,33	7,14	5,75
CML141 x CML151	6,74	4,53	8,84	7,04	5,01
OPV QPM					
Srikandi yellow 1	8,57	4,67	7,97	7,08	4,71
Srikandi white 1	9,44	4,44	6,23	6,54	5,20
Normal maize					
Bima 1	9,48	5,43	8,51	7,19	6,03
Bisi 2	10,85	4,24	5,84	6,94	3,88
C/V, %	16,27	18,34	15,10	16,73	23,43
LSD5%	1,40	0,86	1,18	1,15	1,17
LSD1%	1,88	1,15	1,58	1,54	1,56

Table 6. The content of lysine, tryptophane and protein of QPM hybrids compare with normal maize.. Maros. 2007

Entri (materi uml, 2007)	Lysine (%)	Increase to control (%)	Tryptophane (%)	Increase to control (%)	Protein (%)
MSQ.K1C0.3-1-1xMR14Q	0,47	62,06	0,10	100,0	10,92
MSQ.K1C0.8-1-1-1xMR14Q	0,39	34,48	0,09	80,0	8,82
MSQ.K1C0.15-2-1-1xMR14Q	0,50	72,41	0,09	80,0	10,13
MR4Q x MR14Q (Bima-1Q)	0,52	79,31	0,11	120,0	11,06
MSQ.K1C0.14-4-2-1xMR14Q	0,51	75,86	0,12	140,0	11,76
MSQ.K1C0.22-1-1xMR14Q	0,44	51,72	0,08	60,0	8,44
MSQ.K1C0.24-3-1-1xMR14Q	0,47	62,06	0,11	120,0	10,72
MSQ.K1C0.6-1-4xMR14Q	0,50	72,41	0,12	140,0	10,50
MSQ.K1C0.61-1-1xMR14Q	0,54	86,20	0,11	120,0	12,37
MSQ.K1C0.153-1-1xMR14Q	0,49	68,96	0,09	80,0	8,85
CML161 x CML165	0,46	58,62	0,09	80,0	9,93
Srikandi yellow 1	0,46	58,62	0,06	20,0	10,26
Srikandi white-1	0,36	24,13	0,07	40,0	7,81
Bima 1 (control)	0,29	-	0,05	-	12,31
Bisi 2 (control)	0,35	-	0,08	-	11,76

Im table could be concluded that lysine were increased 34-88% and tryptophane 60-140% compare with normal maize as control

Conclusion

Grain yellow of MSQ.K1C0 was generate by intra population improvement with S1 selection. Program of QPM hybrid in ICERI was selected tester by back cross procedure to founded inbred line Mr14Q as recurrent parent which donor was CML165. The analyzed of lysine were 0,388% and tryptophane 0,079% The highest potential of hyrids candidate from evt was MSQ.K1C0.15-2-1-1xMR14Q could be found 9,.09 t/ha in rice field Palu Central Sulawesi Single cros of QPM were increase of lysine 34-88% and tryptophane 60-140% compare with normal maize as control

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Initial results of waxy maize hybrid development in Vietnam

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Abstract. In recent years, the demand of vegetable maize (waxy, sweet and baby corn) has increased rapidly in Vietnam. Waxy corn accounts for 10% of the total 1 million ha over the country. Majority of waxy maize cultivars is OPV, nonconventional hybrids and some foreign hybrids which are being commercialized with high price – over USD 10 kg^{-1} of seed. Vietnam has rather diversified waxy maize germplasm in terms of grain color, quality, maturity, abiotic and biotic stress tolerance which scattered around the country. Based on local and exotic waxy maize germplasm, over 100 lines were developed by conventional methods. 30 lines were genetic diversity analysis using 22 SSR primers. Results revealed that all lines were genetically pure; 10 lines with H of 0%, 10 lines with H of 4.3% and only a line having H of 30.43%. 30 lines on the dendogram were divided in to some genetically distant groups. Results of evaluation of agronomical traits for 18 lines showed that all lines have very early maturity: 105 days in Spring and 88 days in Autunm crop; plant height around 100 cm, ear height of 42.5 cm, 1000 seed weight of 166g (about 60% of field corn), seed yield of 1,300 kg ha^{-1}. Several lines are involved in promising hybrids thanks to good combining ability and agronomical traits. Some hybrids, under national testing network, are going to be released for production this year. Among them, Waxy corn hybrid number one showed the best uniformity, early maturity, good quality and yield potential was higher than checks (OPVs and non-conventional hybrids), that were selected by producers.

Key-words: Waxy corn, grain yield, ear yield, genetic diversity, combining ability, SSR marker

Introduction

Waxy and flint corn are two subspecies, which are the most popular in Vietnam. Total 357 local varieties are maintaining and conserving in National Maize Research Institute of Vietnam (NMRI) including 157 waxy corn (123 white, 17 yellow and 17 violet). In recent years, the demand of vegetable corn (waxy, sweet and baby corn) has increased rapidly in Vietnam. Waxy corn accounts for 10% of the total 1 million ha over the country. Majority of waxy corn cultivars is OPVs, nonconventional hybrids and some foreign hybrids which are being commercialized with high price – over USD 10 kg^{-1} of seed. From 2001 up to now, Vietnam has conducted a program for waxy corn development. Based on local and exotic waxy maize germplasm, over 100 lines were developed by conventional methods. Many waxy crosses are tested every year. Some of hybrids are evaluated in National Testing and Evaluating network. Some of good hybrids are planted in some provinces in recent seasons. The initial results showed that: waxy corn hybrids, which are developed by NMRI, will be satisfied demand of producers.

Materials and methods

Materials

Base on local and exotic germplasms from China, Thailand, over 100 inbred lines with differrent generation were developed. 30 lines were genetic diversity analysis, 18 lines were evaluated agronomical and biological traits, lines which have good morphological and high resistance to biotic and abiotic stresses such as diseases, pest, drought, cold,... were evaluated of specific and general combining abilities.

Two testers, HN1 was developed from VN2 (an OPV of Vietnam) and HN9 was developed from DN31 (Chinese Hybrid).

Checks were OPVs (VN2, VN6) and nonconventional hybrids (MX2, MX4).

Methods

- Developing of inbred lines by self pollination and sister cross.
- Evaluating of combining ability by top cross and diallel cross.
- Evaluating of uniformity, diversity and divising heterosis group follow. AMBIONET with 22 SSR primers at Biotechnology Department of NMRI.
- Field experiments were conducted follow CIMMYT and NMRI.
- Analysing of data follow MSTATC and NMRI programs.

Results and discussion

Agronomical-Biological traits of inbred lines

18 lines were evaluated about duration, morphological, resistance to biotic and abiotic stresses (diseases, pest, drought, cold…) and productivity potential in Spring and Autunm of 2005 (table 1). The resuls showed that, all lines have very early maturity: 105 days in Spring and 88 days in Autunm crop; plant height around 100 cm, ear height of 42.5 cm, rather clean to leaf diseases; Some lines were good lodging resistance (HN1, HN5, HN9, HN10), 1000 seed weight of 166g (about 60%- 70% of field corn), average of seed yield of 1,300 kg ha^{-1} (10.06- 18.30 qt/ha).

Uniformity and diversity of lines

30 lines were genetic diversity analysis using 22 SSR primers at Biotechnology Department of NMRI. Results in table 2 showed that: all lines were genetically pure; 15 lines with H of 0%, 10 lines with H of 4.35%, 3 lines with H of 8.70% and only a line having H of 30.43% (WX22 with S_{-3} generation).

Figure 1 showed that: lines were divised to many heterosis groups in dendogram, both of local and exotic germplasms. Crosses between HN2 (N19) (it is developed from local variety- Pleicu) and most of other lines recorded high yeild, it made separately heterosis group on dendogram

Combining ability

11 lines, which have good agronomical traits were top crossed for testing of combining ability with 2 testers (HN1 namely T1 and HN9 namely T2).

Data in table 3 showed that: The lines that have high general combining ability were HN6, HN5, HN2 and HN8; for 2 testers: HN9 has general combining ability higher than HN1.

So, lines with the name of HN2, HN5, HN6 and HN8 were not only have good agronomical traits, but also have high general combining ability.

Result of testing waxy corn hybrid No.1 (NL1)

Following tested and evaluated combining ability at NMRI, some promissing crosses were selected, one of them was Waxy hybrid No.1 (HN6 x HN1). It was tested in National Evaluating and Testing network in 3 crops: Winter 2006, Spring and Winter 2007.

Table 1. Duration, plant height, ear height, resistances, P1000 kernels and grain yeild of 18 lines

No	Line	Origin	Date from sowing to (cm)				Plant height (cm)		Ear height (cm)		Resis to		P 1000 (g)	Yield (kg/ha)
			Silking		Maturity									
			Spr	Aut	Spr	Aut	Ave.	CV%	Ave.	CV%	Leaf disea	Lod (%)		
1	HN9	DN31 China	65	42	100	80	83.6	4.8	32.8	5.6	2	0	188	1420
2	HN10	NN48 China	69	48	106	87	91.5	4.9	35.2	6.5	1	0	156	1355
3	HN1	VN2 OPV	73	47	105	88	110.4	6.0	43.5	6.3	2	0	170	1540
4	HN12	VN2/NN48	76	52	107	90	110.8	6.7	47.3	6.8	2	5	160	1457
5	HN22	VN2/5640	72	48	104	89	115.7	7.8	45.6	7.2	2	10	162	1120
6	HN11	DN31/NN48	69	47	103	87	93.6	6.4	44.7	6.1	1	5	165	1140
7	HN20	N5640 Loc	71	47	105	86	112.3	6.3	38.5	10.1	2	10	163	1300
8	HN4	VTMB Loc	77	52	107	95	128.4	10.3	63.4	9.3	2	20	175	1330
9	HN2	V.Plei Loc	76	52	108	95	125.5	9.2	56.0	10.2	2	15	183	1425
10	HN21	TMN Loc	78	55	112	97	128.0	9.4	57.2	8.4	2	25	157	1067
11	HN5	Hyb1China	75	50	105	89	106.2	6.9	48.7	7.4	1	0	192	1775
12	HN6	Hyb2 China	73	49	106	90	92.0	6.6	36.7	6.0	1	20	185	1830
13	HN19	IAS2 Loc	68	46	102	84	103.6	5.8	43.4	7.2	2	10	148	1120
14	HN18	IAS3 Loc	71	47	105	86	93.0	5.9	41.2	6.6	2	5	165	1140
15	HN8	IAS8 Loc	74	48	107	87	97.0	7.5	44.7	6.0	2	5	142	1285
16	HN13	IAS14 Loc	69	45	101	85	65.5	7.4	25.4	6.3	2	10	160	1110
17	HN14	IAS15 Loc	72	46	103	89	73.2	6.5	34.1	6.2	2	5	161	1255
18	HN15	IAS16 Loc	72	47	105	89	70.6	7.7	30.0	7.2	3	5	160	1006
Ev			72	48	105	88	100.0	7.0	42.7	7.2			166	1315

* For Yield: $LSD_{0.05}$ = 2.14; CV% = 12

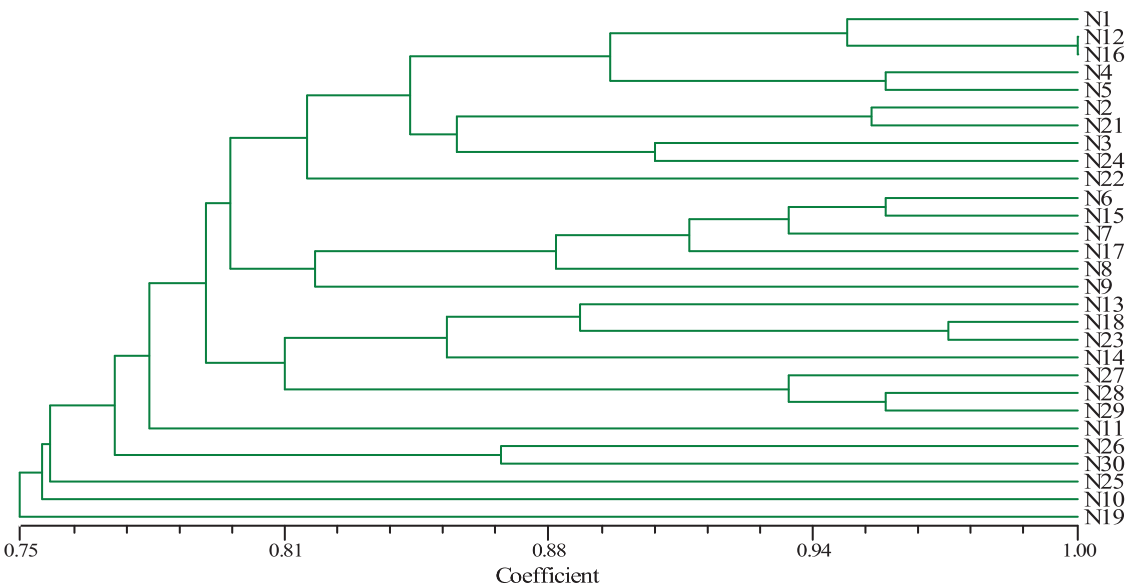
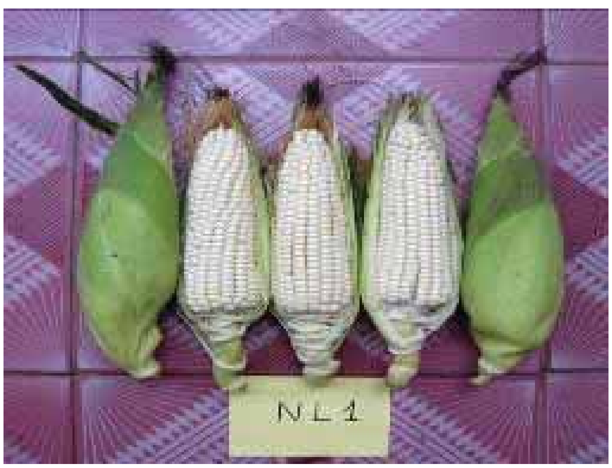


Figure 1: Dendogram of genetic diversity of 30 waxy lines

Table 2. Percentage of Heterozygote (H%) and missed data (M%) of 30 lines

No	Code	Name	Origin	Self	M%	H%
1	N1	HN5	Hybrid1 China	S6	13.04	0
2	N2	HN6	Hybrid2 China	S6	8.70	0
3	N3	HN13	IAS14 Local	S7	0.00	0
4	N4	HN14	IAS15 Local	S7	4.35	0
5	N5	HN15	IAS16 Local	S8	7.39	0
6	N6	HN9	DN31 China	S6	12.74	0
7	N11	HN1	VN2 OPV	S8	0.00	0
8	N8	HN10	NN 48 China	S6	8.09	0
9	N9	HN11	DN 31/NN48	S6	6.09	0
10	N10	HN7	OPV China	S5	13.04	4.35
11	N7	HN16	IAS14 Local	S5	13.04	4.35
12	N12	HN12	VN2-4 OPV	S8	8.70	4.35
13	N13	HN27	VN2-3 OPV	S8	8.70	0
14	N14	HN21	TMN - Local	S8	8.70	8.70
15	N15	HN28	VN2-2 - OPV	S8	11.74	0
16	N16	HN31	N.VuBan - Local	S7	6.09	4.35
17	N17	HN20	N5640- Local	S7	8.70	4.35
18	N18	HN4	VTMB - Local	S8	4.78	0
19	N19	HN2	V.Pleicu - Local	S8	8.70	0
20	N20N220	HN3	VHB - Local	S8	47.83	0
21	N21	HN32	DN104	S7	12.39	0
22	N22	HN19	IAS2- Local	S6	5.39	4.35
23	N23	HN18	IAS3- Local	S6	1.74	4.35
24	N24	HN8	IAS8 - Local	S7	13.04	4.35
25	N25	HN35	OPV -ThaiLand	S6	5.74	4.35
26	N26	HN42	WX-22-Syngenta - Hybrid	S3	4.35	30.43
27	N27	HN41	N.S.Boi Local	S6	3.04	8.70
28	N28	HN21	IAS9 Local	S6	8.70	8.70
29	N29	HN33	IAS10 Local	S6	6.09	4.35
30	N30	HN34	IÁS11 Local	S5	10.74	17.39

The results at 3-4 locations showed that, NL1 has short duration, similar to checks VN2 (OPV) and MX2, MX4 (nonconventional hybrids); plant height was shoter, percentage of grain was higher, the yield of fresh ear (90.24-95.37qt/ha) and grain (49.61- 58.90qt/ha) was significant difference higher than checks (Table 4).



Through tested results, National Center for Plant Testing and Evaluating suggested: " Waxy hybrid No.1 is a very promissed hybrid, suggestion for releasing to production".

Table 3. Value of general combining ability for grain yeild of lines

T T	Lines		Testers	
	Name	g_i	Name	g_j
1	HN10	-4.255	T 1	-0.945
2	HN20	-5.655	T 2	0.945
3	HN4	-0.405	Σ	0.000
4	HN2	4.045*	Ed_i = 0.824	
5	HN5	9.645*	$Ed\ (d_i - d_j)$ = 1.166	
6	HN6	9.895*	$LSD_{0.05}$ line = 1.662	
7	HN19	-3.705	Ec_j = 0.352	
8	HN18	-1.505	$Ed\ (c_k - c_l)$ = 0.497	
9	HN8	0.595	$LSD_{0.05}$ tester = 0.71	
10	HN13	-6.655		
11	HN15	-2.005		
	Σ	0.000		

Table 4. Agronomical traits, resistant abilities and yield of waxy hybrid No.1 at 3 - 4 tested location in 3 crops

Variety	50% Silk	Matu-rity	Height (cm)		Lodging (%)		Disease (Score) Rhiz.	Stem bore (Score)	P1000 (g)	Percen (%)	Yield (kg/ha)	
			Plant	Ear	Root	Stalk					Grain	Fresh ear
Winter 2006												
NL1 48	83	207.3	84.2	5.9	7	4.1	2	241	79.8	58.9	95.73	
VN2 (Ck)	49	84	215.3	98.9	6.3	7.8	2.9	2	222.2	78.5	47.60	89.56
MX2(Ck)	46	82	186.2	72	6.2	7.2	3.9	2	235.8	78.2	47.42	88.60
Spring 2007												
NL1	64	98	198.8	78.4	-	4.3	10.6	2.5	226.9	71.0	49.61	90.37
VN2(Ck)	62	98	198	84.8	-	3.6	12.9	2.6	225.8	71.4	40.16	83.14
Winter 2007												
NL1 56	95	227.3	81.6	-	-	4.6	1.5	214.6	74.9		90.24	
MX4(Ck)	54	93	196.7	67.2	-	-	5.1	2.5	239.9	73.1		83.59
VN2(Ck)	56	96	212.7	90.9	-	-	4.3	1.8	209.2	71.5		81.05

Conclusion

By selfing pollination method from local and exotic germplasm, over 100 inbred lines were developed; these lines are high purity, diversity of genetic, dicision to different heterosic groups. Some of lines have good agronomical traits and high combining ability such as HN1, HN6, HN5, HN2, HN8.

Some of crosses were tested in National Testing and Evaluating, NL1 cross was highly evaluated and producers demand for production because it has short duration, high uniformity, good resistance and high yield potential.

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Studies on four kinds of vitamin contents of sweet-waxy

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Abstract. A new method of breeding sweet-waxy maize was advanced; it was called the cross between allotypic recessive homozygote. The various genes increasing sweet taste and gene wx can be expressed on the same ear. Nine sweet-waxy maize hybridized combinations combined by using the cross between allotypic recessive homozygote and their fifteen parents of recessive homozygote inbred lines were used as materials, and the contents of vitamin E, vitamin B_1, vitamin B_2 and vitamin C of their kernels were measured in the fresh stage. The results showed that there was not any hybridized combination which four kinds of vitamin contents were all the highest. It showed that the sweet-waxy maize hybridized combination of which the four kinds of vitamin contents are all high can only be improved one by one. The wider variation ranges were found in the four kinds of vitamin contents, it laid a foundation for the further select and improvement for the four kinds of vitamin contents. Four kinds of vitamin contents of the color sweet-waxy maize were not always the highest in the same type of materials. Four kinds of vitamin contents of the materials in the spring sowing were not the same as in the autumn sowing. Vitamin E and vitamin C contents in the autumn sowing were generally higher than in the spring sowing, but vitamin B_1 and vitamin B_2 contents in the spring sowing were generally higher than in the autumn sowing. This showed that there was the influence from environment.

Key-words: sweet-waxy corn; vitamin content; cross between allotypic recessive homozygote

Introduction

The corn kernel richly contains many kinds of vitamins. Among them the vitamin B_1 can accelerate wriggling of the stomach and intestine, promote secreting of digestive juice, increase appetite, promote digestion. Vitamin B_2 can prevent and control the perleche. Vitamin C can prevent and control the scurvy. Vitamin C can also play a vital role to metabolism of sugar, protein and so on in the human body, and promote the formation of intercellular substance and the collagen fiber. The embryo of the corn includes massive vitamin E, which can strengthen immunity and disease-resistant ability, delay caducity. Therefore, our body growth and the health care can profit by often eating the corn. Eating the fresh corn ears through directly cooking is the most nutritious and healthy edible methods. Nutritionist called the fresh food corn as “the green vegetables”, “the new nutritional health foods”, “longevity food” and so on. The study results of Yang Ruoming (2001) indicated that the nutrition ingredients and the edible value of the fresh food corn were higher than that of the ripe kernels. Many kinds of vitamin contents in the fresh food corn were also higher than that of the dry kernels. The edible value of the fresh food corn was higher than the processing product of dry ripe kernel.

The sweet-waxy corn which were combined by using the cross between allotypic recessive homozygote was the new type of fresh food corn. The study of their nutritional quality was not yet full. In this experiment, the sweet-waxy corns were used as the materials, and the vitamin E, vitamin B_1, vitamin B_2 and vitamin C contents of the kernels were measured in the fresh stage, in order to provide reference for the quality breeding of the fresh food sweet-waxy corn.

Materials and methods

Experiment materials and field experiment design

9 sweet-waxy corn hybridized combinations and their 15 parents of recessive homozygote inbred lines were used as the materials. Those materials were bred by professor Wu Zikai in Guangxi University using his own intellectual property rights (patent number, ZL.99111739.5). And their synthesis agronomic characters and yield characters were good because the preliminary appraising and comparing have gone on for many years.

9 sweet-waxy corn hybridized combinations are as follows:

CTN13-1, TN13-2, TN13-3 are sh_2-13 type; CTN79-1 is bt_1su_1-79 type; TN79-3 is bt_1sh_2-79 type; TN79-5, TN79-6 are sh_2su_1-79 type; TN43-1, TN43-4 are $bt_1sh_2su_1$-43 type.

15 parental inbred lines are as follows:131@&, 132@&, 133@& are wx single recessive homozygote; 131B&, 132B&ÿ793B&ÿ, 133B&ÿ795B&ÿ, 796B&are sh_2wx double recessive homozygote;791@&, 795@&, 796@&are su_1wx double recessive homozygote;431@&, 434@&, 793@&ÿ791B&ÿare bt_1wx double recessive homozygote;431B&, 434B&are sh_2su_1wx three recessive homozygote.

The experiments were arranged on the experimental farm of the Guangxi University Agriculture College, 4 lines in a plot, the line long was 4.2m, the row spacing was 90cm and 40cm respectively, the distance between plants was 30cm, each material was planted as a plot. Those materials were planted in the autumn of 2003 and in the spring of 2004 separately.

The autumn sowing experiment in 2003 (abbreviated autumn sowing):

On July 27, 2003, 9 sweet-waxy corn hybridized combinations and 15 parents of recessive homozygote inbred lines were planted according to the completely random field experiment design. From Sep.13 to Sep.30, the identical plants were selected and self-fertilizations were made. On the 21th day after pollination, 6 ears in the middle two lines of each material were randomly sampled, and the contents of vitamin E, vitamin B_1, vitaminB_2 and vitamin C were measured.

The spring sowing experiment in 2004(abbreviated spring sowing):

On February 8, 2004, 5 sweet-waxy corn hybridized combinations (CTN13-1, TN13-2, TN13-3, TN79-3 and TN43-4) and their 9 corresponding parental inbred lines were planted once more by completely random field experiment design, the repeated experiment were conducted. Those 5 sweet-waxy corn combinations had been examined and approved for varieties or were being arranged in the regional experiment at that time. From May 1 to May 13, the identical plants were selected and self-fertilizations were made. On the 21th day after pollination, 6 ears in the middle two lines of each material were randomly sampled, and the contents of vitamin E, vitamin B_1, vitaminB_2 and vitamin C were measured.

Measurement methods

After the bract of the fresh ears were shucked off, immediately shelled and the kernels of 6 sampling ears were mixed by using the homogenizer, smashed fully until were became homogenate, it would be used to measure 4 kinds of vitamin contents and the moisture content.

4 kinds of vitamin contents were measured by referring to the country standard methods of the People's Republic of China. It was completed in the biochemistry laboratory of Guangxi analysis measuring research center. Vitamin E was measured by using HPLC method (referring to GB12388-90), vitamin B_1 (referring to GB12390-90), vitamin B_2 (referring to GB12391-90) and vitamin C (referring to GB12392-90) were measured by using fluorescence method.

Results

Four kinds of vitamin contents of the sweet-waxy corn hybridized combinations

The measurement results of the 4 kinds of vitamin contents for the sweet-waxy corn combinations on the 21th day after pollination were gathered together in table 1.

From table 1, vitamin E, vitamin B_1, vitamin B_2 and vitamin C were all included in the kernels of the sweet-waxy corn hybridized combinations on the 21th day after pollination. The vitamin E content was 1.18-3.81 mg/100g, vitamin B_1 content was 0.051-0.215mg/100g, vitamin B_2 content was 0.24-0.64mg/100g and vitamin C was 25.79-61.71mg/100g. The results showed that the 4 kinds of vitamin contents all had a wider variation range.

In the autumn sowing and spring sowing, 4 kinds of vitamin contents for 13, 79 and 43 type were compared, no clear regularity was found. There were some differences for the 4 kinds of vitamin contents among the different types of the sweet-waxy corn. Even in the same type, four kinds of vitamin contents for different combinations were also different, the difference between the highest and the lowest was large, it showed that every kind of vitamin content can be selected and improved. The hybridized combination of which four kinds of vitamin contents were all highest was not found. It showed that it would be difficult to enhance the 4 kinds of vitamin contents at the same time. It could be improved one by one.

Four kinds of vitamin contents were different between in the autumn sowing and in the spring sowing. Vitamin E contents of every combination except CTN13-1 in the autumn sowing were higher than in the spring sowing. Vitamin C contents of all combinations in the autumn sowing were higher than in the spring sowing. Vitamin B_1 contents of every combination except CTN13-1 in the spring sowing were higher than in the autumn sowing. Vitamin B_2 contents of all combinations in the spring sowing were higher than in the autumn sowing. It showed that there was the influence from environment.

Table 1 Vitamin contents of the sweet-waxy corn combinations on the 21th day after pollination mg×$100^{-1}g^{-1}$

.	Vitamin E		Vitamin B_1		Vitamin B_2		Vitamin C	
	Genotype	Mean	Genotype	Mean	Genotype	Mean	Genotype	Mean
Autumnsowing	CTN79-1	3.81	TN79-3	0.123	TN79-6	0.64	TN13-3	61.71
	TN43-1	3.57	CTN13-1	0.115	CTN79-1	0.49	TN79-6	46.38
	TN43-4	2.88	TN43-4	0.114	TN43-1	0.33	TN43-4	45.58
	TN13-3	2.77	TN43-1	0.102	TN43-4	0.31	TN79-5	39.46
	TN79-3	2.68	TN79-5	0.097	TN79-5	0.30	CTN13-1	38.85
	TN79-5	2.38	TN13-3	0.094	TN13-3	0.29	TN43-1	36.23
	TN79-6	2.29	TN13-2	0.088	TN13-2	0.28	TN79-3	35.47
	CTN13-1	1.78	TN79-6	0.087	CTN13-1	0.25	TN13-2	35.14
	TN13-2	1.47	CTN79-1	0.051	TN79-3	0.24	CTN79-1	32.48
Springsowing	TN43-4	2.65	TN79-3	0.215	TN79-3	0.48	TN13-3	29.34
	TN13-3	1.97	TN13-2	0.181	TN43-4	0.47	TN13-2	28.47
	CTN13-1	1.95	TN43-4	0.178	TN13-3	0.46	TN79-3	28.22
	TN79-3	1.64	TN13-3	0.137	TN13-2	0.44	CTN13-1	26.32
	TN13-2	1.18	CTN13-1	0.082	CTN13-1	0.29	TN43-4	25.79

Note: The contents of the vitamin E, vitamin B_1, vitamin B_2 and the vitamin C were showed by dry weight content in this experiment. The same as below.

Tale 2. Vitamin contents of the parental lines on the 21th day after pollination mg×$100^{-1}g^{-1}$

	Vitamin E		Vitamin $_1$		Vitamin $_2$		Vitamin C	
	Genotype	Mean	Genotype	Mean	Genotype	Mean	Genotype	Mean
Autumnsowing	795	5.21	431	0.189	793, 791	0.70	796	79.09
	791	5.15	434	0.154	431	0.52	431	71.67
	431	4.70	796	0.153	434	0.50	131	66.30
	431	4.20	133, 795	0.147	795	0.49	434	64.31
	793, 791	3.57	132, 793	0.135	796	0.48	132, 793	54.38
	796	3.01	434	0.127	434	0.46	133, 795	54.26
	131	2.96	132	0.111	132, 793	0.44	133	50.98
	434	2.62	796	0.102	133, 795	0.43	431	49.03
	133	2.21	131	0.094	796	0.38	793, 791	40.67
	796	2.14	791	0.091	131	0.37	434	38.38
	132	1.89	133	0.088	431	0.35	795	37.95
	131	1.54	431	0.082	131	0.29	796	34.69
	132, 793	1.43	131	0.081	132	0.26	132	29.21
	133, 795	1.21	793, 791	0.080	133	0.25	791	28.01
	434	0.95	795	0.051	791	0.22	131	18.46
Springsowing	793	3.35	133	0.220	434	0.94	133	95.47
	132	2.40	434	0.207	434	0.78	133	63.51
	133	2.09	131	0.207	132, 793	0.73	131	54.58
	131	2.03	131	0.204	133	0.53	434	39.84
	434	1.70	132	0.184	131	0.52	434	36.40
	434	1.52	132, 793	0.161	793	0.50	793	34.27
	131	1.38	133	0.143	132	0.40	132, 793	31.57
	133	1.36	793	0.142	133	0.39	132	23.45
	132, 793	1.17	434	0.106	131	0.33	131	15.80

Among the 9 sweet-waxy corn combinations, CTN13-1 and CTN79-1 were part of colored types. Their 4 kinds of vitamin contents were mostly not the highest in the same types of the sweet-waxy corn.

Four kinds of vitamin contents of the parental inbred lines

The measurement results of the 4 kinds of vitamin contents for the parental inbred lines on the 21th day after pollination were gathered together in table 2.

From table 2, the contents of the vitamin E, vitamin B_1, vitamin B_2 and vitamin C were different among the different recessive homozygote. Even in the same type of recessive homozygote, the variation ranges of the 4 kinds of vitamin contents were also large. In those recessive homozygote parental inbred lines, the vitamin E content was 0.95-5.21mg/100g, vitamin B_1 content was 0.051-0.220mg/100g, vitamin B_2 content was 0.22-0.94 mg/100g and the vitamin C content was 15.80-95.47 mg/100g. It showed that the variation ranges of the 4 kinds of vitamin contents for the recessive homozygote were large. The highest content was several times more than the lowest.

From the result of synthesis comparison, 4 kinds of vitamin contents of sh_2su_1wx were relatively higher and that of wx were relatively lower than that of the others. The regularity of the parental inbred lines in the autumn sowing and in the spring sowing was approximately identical with the hybridized combinations. The vitamin E and vitamin C contents in the autumn sowing were mostly higher than in the spring sowing, of them, 3 recessive homozygote in the spring sowing were higher than in the autumn sowing, 6 recessive homozygote in the autumn sowing were higher than in the spring sowing. The vitamin B_1 content of every line except 434 @& and 133 B&, the vitamin B_2 content of every line except 793 @&in the autumn sowing were higher than in the spring sowing. It showed that the influence of environment cannot be neglected.

In those recessive homozygote, 791 @& (su_1wx) was black corn and 131 @& (wx) was purple corn. The vitamin contents of different color corn kernel from the same type of recessive homozygote were compared. The vitamin contents of the black and the purple recessive homozygote corn were mostly not the highest, some were even lower than that of the yellow and white recessive homozygote of the same type.

Comparison of the 4 kinds of vitamin contents between the sweet-waxy corn hybridized combinations and their corresponding parental inbred lines

The contents of the vitamin E, vitamin B_1, vitamin B_2 and vitamin C were compared between the sweet-waxy corn hybridized combinations and their corresponding parental inbred lines on the 21th day after pollination. The results showed that the content of vitamin E for TN13-3 and TN43-4, vitamin B_1 for CTN13-1, vitamin B_2 for TN79-6, vitamin C for TN13-3 in the autumn sowing and vitamin E for TN43-4, the vitamin B_1 for TN79-3 in the spring sowing were higher than their parents, the others were all situated middle or lower than their parents. Only the vitamin E content of TN43-4 was higher than the parents in the two experiments. The identical regularity was not found from others.

Discussion

Through the comparison of the 4 kinds of vitamin contents among the sweet-waxy corn hybridized combinations, the hybridized combination of which the 4 kinds of vitamin contents were all highest were not found. It showed that the sweet-waxy corn hybridized combination of which the 4 kinds of vitamin contents were all high could be improved one by one, it could be difficult to enhance the 4 kinds of vitamin contents at the same time. But according to the consumer demand, the sweet-waxy corn combination of which certain kind of vitamin content is relative high could be bred. For example, the vitamin B_1 content for TN79-3 and the vitamin C content for TN13-3 are high.

There were difference for the 4 kinds of vitamin contents among different sweet-waxy corn hybridized combinations and their recessive homozygote parents, some differences was as high as several times. There were also differences for the 4 kinds of vitamin contents among different materials in the same genotype. It showed that there were wide variations for those materials. It laid a foundation for the further select and improvement of the 4 kinds of vitamin contents. The recurrent selection methods can be applied. The results of the 4 kinds of vitamin contents were different in the autumn sowing and in the spring sowing. The vitamin E and vitamin C contents in the autumn sowing were generally higher than in the spring sowing, but the vitamin B_1 and vitamin B_2 contents in the spring sowing were generally higher than in the autumn sowing. It showed that the influence from environment can not be neglected.

In this experiment, the regularity of the sweet-waxy corn hybridized combinations and their corresponding parents were different for the 4 kinds of vitamin contents. This possibly because those parents were different allotypic recessive homozygote, and those allotypic recessive homozygote were generated by different gene, and the gene interactive effects were different.

The vitamin B_2 content of the sweet-waxy corn in this experiment was higher than the measurement result of Liu Xunjia for the sweet corn Hua Tianyu 1[9].The vitamin B_1 contents in some sweet-waxy corn combinations were also higher than the measurement result of Liu Xunjia for the sweet corn. In this experiment, the vitamin C and vitamin E content of the sweet-waxy corn were also higher than that measurement results by using waxy corn and sweet corn as materials. Therefore, considered from the 4 kinds of vitamin contents angle, the sweet-waxy corn can be used as a new type of high-quality fresh food corn to supply market. But we did not set up super-sweet or waxy corn hybrids as the check, the further experiment is need to be conducted for gaining the specific conclusions.

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Section 3

Biotechnology Tools & Uses

Revisiting the Hetero-Fertilization Phenomenon in Maize Using Molecular Markers

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Abstract. We have developed a seed DNA-based marker-assisted selection (MAS) system using single maize seeds. The accuracy of MAS based on this method may be affected by hetero-fertilization (HF), which may cause seed DNA-based analysis to wrongly predict plant genotypes due to genotyping of endosperm DNA which could be different from that of the embryo and thus the derived plant. To investigate the potential influence of HF on seed DNA-based MAS and the effect of different genetic backgrounds, five F_2 and four three-way segregating populations (each with 534 to 1024 individuals) were used for comparative SSR marker-based analysis of endosperm and embryo. The results indicate that the frequency of HF within these populations ranged from 0.14% to 2.18%, with an average of 1.16%. The highest proportion of HF across both types of population was contributed by the pollen gametes, as reported previously based on morphological marker analyses. This is the first report of HF contributed by maternal gametes in populations from three-way crosses, albeit at very low frequency (0.14%-0.65%). Although single fertilization or haploid tissues were not observed in the tested materials, seed DNA-based analysis combined with specific genetic stocks could be useful for revealing the relationships among various aberrant fertilization phenomena.

Key words: Hetero-fertilization, Seed DNA-based genotyping, SSR markers, Marker-assisted selection, maize

Introduction

We have previously developed a seed DNA-based genotyping system for improving the efficiency and reducing the cost of marker-assisted selection (MAS) (Gao et al. 2008). This has already been use for indirect selection of maize grain quality traits such as provitamin-A and quality protein maize (QPM) at CIMMYT because of its unique advantages. This method is based on the assumption that the embryo genotype of a given maize kernel is not significantly different from its corresponding endosperm genotype. On this basis, the genotype of individual lines can be tested before the decision is made to plant the seed, providing several advantages compared to traditional leaf DNA-based MAS systems (Xu and Crouch 2008). Hetero-fertilization (HF) was firstly reported in maize by Sprague (1929), and potentially threatens the accuracy of seed DNA-based genotyping. Normal double fertilization involves one of two sperm cells from the same pollen grain fusing with an egg cell to produce the embryo while the other sperm cell fuses with the two central cells to form endosperm. In contrast, HF is generally considered to occur when the egg cell and the central cell are fertilized with two different sperm cells that are delivered by two different pollen grains (Sprague 1932). As a result, when HF occurs it may lead seed DNA-based analysis to wrongly predict plant genotypes if the genotype of the endosperm is different from that of the embryo and thus of the resultant plant. Therefore, it is important to evaluate the frequency of HF and the extent of genetic variation it causes across diverse maize germplasm. In this way, it should be possible to estimate the impact of HF on the accuracy of seed DNA-based genotyping systems applied in genetic studies and marker-assisted plant breeding programs.

Previous studies regarding HF in maize have exclusively relied on morphological markers like kernel color in the aleurone and scutellum. In maize, the color of components of the kernel is determined by genes involved in anthocyanin and carotenoid synthesis pathways. Thus, in crosses between male parents heterozygous for one or more of the color genes and female parents that are effectively recessive tester lines, the occurrence of HF can be deduced from a seed with colorless aleurone and colored scutellum (Sprague 1932; Robertson 1984; Kraptchev 2003). However, the color is modified by many background genes as well as influenced by gene dosage and environmental conditions (reviewed by Ford 2000). For this reason assessment of HF frequency using morphological markers is not straightforward, and may suffer similar loss of accuracy as reported for identification of haploids using pigmentation markers (Belicuas et al. 2007).

Molecular marker analysis of seed DNA (extracted from the endosperm) and leaf DNA (extracted from the plant derived from the associated embryo) allows large-scale assessment of HF frequency in any population. The purposes of the study reported here were to generate reliable estimates of HF frequency across diverse lines using molecular markers in order to: (1) determine the scale and extent of HF across different sources of maize germplasm likely to be used in seed DNA-based genotyping in maize; (2) test whether HF can be caused by maternal effects in addition to pollen; and (3) survey other abnormal fertilization phenomena and their potential impact on seed DNA-based genotyping.

Materials and methods

Plant materials

In this study, two types of maize hybrid seeds were used for DNA extraction and marker analysis, including F_2 seeds harvested from the F_1 plants of five single hybrids and four three–way crosses with seed harvested from the plants of two inbred lines that were pollinated by F_1 plants and from the F_1 plants of two single hybrids pollinated with inbreds. The detailed pedigree of parental lines and corresponding pollination model of hybrids are described in Table 1.

DNA extraction and genotyping

Single seed-based sample collection and DNA extraction using a part of endosperm were performed as described by Gao et al. (2008). Sampled endosperm was transferred into individual 1.1 ml tubes in a 96-tube plate (12 rows each with eight linked tubes, Neptune, CA, USA). The remnant cutting seed was subsequently placed in 48-well plates. Two 48-well plates together stored the sampled seeds that corresponded to one 96-tube plate for the endosperm samples and resulting DNA. Leaf tissue of individual plant was collected at 3-leaf phase. Leaf DNA used to represent embryo genotype in each comparative experiment was extracted using a DNA isolation protocol developed for leaf tissue at CIMMYT (CIMMYT Applied Molecular Genetics Laboratory 2003).

To avoid error and improve efficiency, all operations including endosperm sampling, planting of cutting seed, collection of leaf tissue, and DNA extraction and PCR amplification for both endosperm and leaf samples were performed using plate containing 12 rows by 8 tubes as a basic unit. In the meantime, an empty position remained for leaf sample through collection of leaf DNA to genotyping process when a cutting seed fails to germinate. In general, approximately 95% of germination rate was observed for each population.

Detection of HF events based on polymorphic SSR markers

For both F_2 and three-way cross populations, HF events were revealed based on the detection of different genetic constitution between endosperm and embryo (represented by leaf) using one SSR marker that has been screened in advance to show polymorphism among their parents. In theory, one polymorphic SSR marker can detect half of HF events for both F_2 and three-way cross populations. When HF occurs, there are 50% of chance for two sperms to have different alleles at a given locus

Table 1 Segregating populations derived from diverse germplasm for hetero-fertilization detection

Name	Cross model	Germplasm source and description of kernel characteristics
Five F_2 populations		
HP1	Line 1 × Line 2	Line 1: Progeny of P390bcoC3F191 and P73TLC3, white-flint kernel
		Line 2: Progeny of LPSEQC7, white-flint kernel
HP2	Line 3 × Line 4	Line 3: Progeny of CL-RCW46, white-dent kernel
		Line 4: Progeny of CL-RCW84, white-flint kernel
HP3	Line 5 × Line 6	Line 5: Progeny of MIRC5, white-dent kernel
		Line 6: Progeny of CL-RCW45, white-flint kernel
HP4	Line 7 × Line 8	Line 7: Progeny of CML464 and CML175,yellow-flint kernel
		Line 8: Progeny of CML176 and BTZTVC PR93A,yellow-flint kernel
HP5	CML492×CML494	Maize inbreds released by CIMMYT
Four three-way crosses		
HP6	(Line 3×Line 4)×CML494	Described as above
HP7	(CML484×CML312)×CML494	Described as above
HP8	CML461×(Line 7×Line 8)	Described as above
HP9	CML246×(Line 7×Line 8)	Described as above

which lead to different genotypes between endosperm and embryo. In contrast, there are another 50% of chance for the two sperms to have an identical allele at the same locus, which lead to an identical genotype between endosperm and embryo, and thus HF event cannot be detected even if the HF has occurred. The HF event missed by using one marker can be detected by additional markers. Each additional marker will detect 50% of the HF events that have been missed by the previous markers. Therefore, more polymorphic markers are used, the HF frequency closer to real frequency can be detected for a given population. However, the HF events that can be identified by each additional marker will decrease drastically. Therefore, we use one polymorphic SSR marker to detect the HF events and theoretical HF frequency can be obtained by doubling the HF frequency detected by one marker. PCR and genotyping were performed as described by Gao et al (2008).

Results

The HF detected by SSR markers in F_2 seeds from five selfed single hybrids is shown in Fig. 1. Hetero-fertilized seeds were observed in all five F_2 populations, including those with a homozygous endosperm and a heterozygous embryo (AAA/AB or BBB/AB) as well as those with a heterozygous endosperm and a homozygous embryo (AAB/AA or BBA/BB). We have observed that pericarp contamination causes very little interference of the accuracy of HF detection and only some primers are sensitive to this (Gao et al.2008). To eliminate any potential interference caused by pericarp contamination in F_2 populations, we use an additional eight to ten polymorphic SSR markers at different loci until a heterozygous endosperm and corresponding homozygous leaf was found. The frequency of HF in each of the five F_2 populations (shown in Table 2), ranged from 1.02% to 2.18%.

For seeds from the four populations generated from three-way crosses, two populations (HP6 and HP7) were harvested from crossing model (A×B)×C in which a single hybrid was used as the maternal parent and an inbred line was used as the pollen donor. Since the pollen was homozygous, any observed HF could only have resulted from maternal parent effects. Five HF events were observed from 765 pairwise comparisons in HP6 but only one HF event was observed from 716 pairwise comparisons in HP7 (Table 3). To our knowledge, this is the first report in maize that HF can be caused by events in the egg cells.

The other two populations generated from three-way crosses (HP8 and HP9), were harvested from the crossing

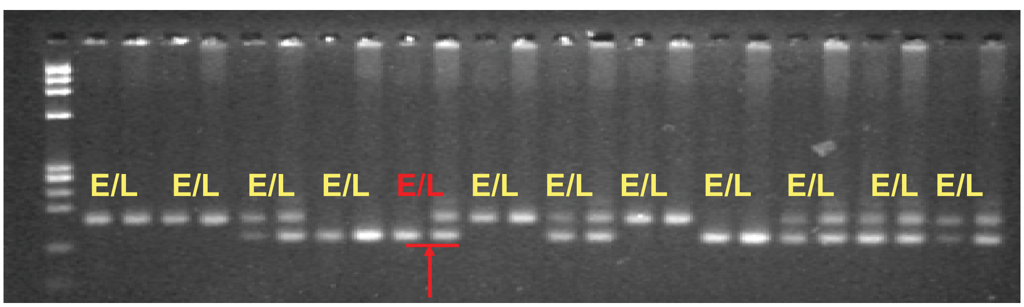

Fig. 1 Hetero-fertilization event detected in F_2 populations (HP4) as shown by the arrow. E: endosperm L: leaf.

Table 2 Hetero-fertilization frequencies detected in five F_2 populations

Population (AxB)	SSR	No. of normal case (E/L)			No. of hetero-fertilization events (E/L)		Total (%)	Ratio
		AAA/AA	BBB/BB	AAB/AB	AAA/AB +BBB/AB	AAB/AA+BBA/ BB		
HP1	Umc1040	142	123	263	1+4	1+0	534	1.12
HP2	Umc1015	94	340	438	2+2	2+3	881	1.02
HP3	Bnlg1270	171	191	311	4+4	4+3	688	2.18
HP4	Umc1008	185	144	332	4+2	2+0	669	1.19
HP5	Umc1071	292	246	468	3+9	1+5	1024	1.76

Note: The pollination model for five F2 populations is A×B, where A is used as female parent and B as male parent. For consistency, the genotypes of SSR allele combination in endosperm (E) and leaf (L) are shown using the same symbol with the pollination model A and B.

Table 3 Hetero-fertilization frequencies detected in four three-way cross populations

Population (A×B)×C	SSR	No. of normal case (E/L) AAC/AC	BBC/BC	No. of hetero-fertilization events (E/L) AAC/BC + BBC/AC	Total	Ratio (%)
HP6	Umc1015	395	365	2+3	765	0.65
HP7	Bnlg1144	369	346	1+0	716	0.14
A×(B×C)		AAB/AB	AAC/AC	AAB/AC+ AAC/AB		
HP8	Umc1805	416	307	2+6	731	1.01
HP9	Bnlg1043	433	432	3+9	877	1.37

Note: The two pollination models for three-way crosses are (A×B×C and A×(B×C). For consistency, the genotypes of SSR allele combination in endosperm (E) and leaf (L) are shown using the same symbol with the pollination model A, B and C.

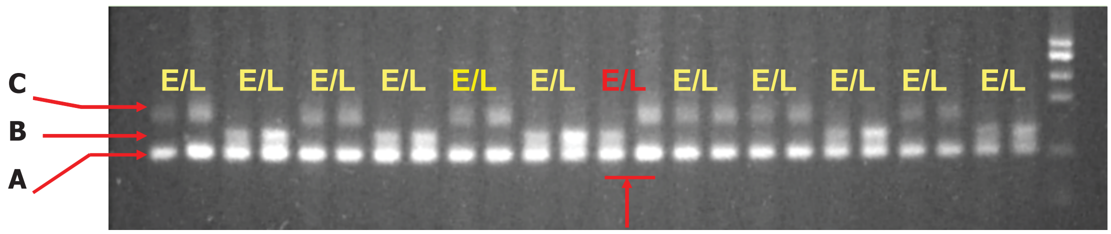

Fig. 2 Hetero-fertilization event detected in three-way cross populations (HP8) as shown by the arrow. E: endosperm L: leaf.

model A×(B×C) in where an inbred line is used as maternal parent and single hybrids were used as the pollen donor (Fig. 2). Eight HF events were identified in HP8 (from 731 pairwise comparisons) and 12 HF events were identified in HP9 (from 877 pairwise comparisons). The frequency of HF in HP8 and HP9 was 1.01% and 1.37%, respectively. In these populations the HF events can only have been contributed by pollen cells.

Discussion

Sprague (1932) proposed that HF occurs when egg and polar nuclei are of different genetic constitution and fuse with identical sperms, or conversely, when the egg and polar nuclei are of the same genotype but at syngamy fuse with sperm cells having different genotypes. Due to confounding factors associated with using morphological markers associated with the color of different components of the kernel, it is hard to design an experiment to test Sprague's original conclusion. Moreover, Sprague's experiment only observed the maternal contribution to HF. By using molecular marker analysis of three-way crosses, it is now possible to precisely test Sprague's hypothesis. In our study reported here, HF events caused by maternal effects were detected in each of the two types of populations tested. The mechanism of HF is still unknown, particular regarding the phenomenon arising from embryo sac events. Although a number of explanations for aberrant fertilization events have been proposed, including mutation and non-disjunction (Sprague 1932; Ohad 1997).

Although HF is a rare phenomenon in maize, it may still affect the accuracy of application of endosperm DNA-based MAS when embryo and endosperm genotypes differ. In some maize genetic stocks, Sprague (1932) found HF to be as high as 25%. At this level, the accuracy of seed DNA-based genotyping would be unacceptably compromised. We surveyed nine populations covering a wide diversity of maize germplasm and observed an average HF frequency of 1.45% in five F_2 populations and a lower frequency in three-way crosses. This is consistent with previous reports suggesting that there was little variation in frequency of HF incidence in most cultivated maize germplasm. At a HF frequency of 1-2%, the resultant increase in genotyping errors is acceptable, particularly if we consider the advantages provided by seed DNA-based genotyping.

Kao (1997) considered that the aberrant fertilization mechanisms underlying haploidy and HF in maize may be associated. Current explanations suggest that haploids are produced when a single haploid sperm from the pollen grain fertilizes its polar nucleus and the unfertilized egg cell develops parthenogenetically into haploid. However, we did not detect any haploids in this study as there were no haploid inducer lines included in the parental genotypes.

For example, “stock6” has an inducer rate of 3% in self-pollinated progeny populations while other stocks may provide a haploid inducer incidence of 0.5-1% (Lashermers and Beckert 1998). The method used in this study, when combined with haploid inducer lines, may be useful for revealing relationships among various types of aberrant fertilization including haploid and apomixis.

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Molecular markers for maize improvement in Asia

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Introduction

Maize (*Zea mays* L.) holds a unique position in world agriculture as a food, feed and industrial crop *par excellence*. Although the developed countries, particularly USA, contribute predominantly to the maize production, demand for maize in developing countries is expected to surpass the demand for both wheat and rice by the year 2020 (Pingali and Pandey, 2001). However, average productivity of maize in several developing countries is still considerably low. About 45 million hectares of maize is grown in the lowland tropics, where a range of climatic, biotic and abiotic constraints severely affect productivity. The challenges are diverse and complex, and there is no single technological solution.

While significant progress has been made in relation to maize improvement worldwide using traditional breeding strategies, considerable scope still exists to further enhance maize productivity. Modern molecular tools and techniques can complement conventional approaches to allow breeders to effectively address priority research areas. The use of DNA-based markers for genetic analysis and improvement of diverse agronomically important traits in crop plants like maize has emerged as one of the most powerful applications of agricultural biotechnology in the recent years. Here, I shall focus on applications of molecular markers in modern maize breeding, with particular emphasis on research efforts in Asia.

Molecular markers, mapping populations and genome mapping

Phenotypically, maize is the most variable of all the food cereals. This variability continues down all the way to the DNA level. In addition, maize has benefited from great research efforts worldwide, thanks to its importance as a food, feed and industrially important crop. Consequently, an array of molecular markers have been developed during the last few decades in maize, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) or microsatellites, single nucleotide polymorphisms (SNPs), etc.(Prasanna and Hoisington, 2003).

Among the different types of PCR-based DNA markers available for diverse applications in genetics and breeding, microsatellite or Simple Sequence Repeat (SSR) markers are often preferred for reasons of cost, simplicity and effectiveness. SSR markers are robust, codominant, hypervariable, abundant, and uniformly dispersed in plant genomes (Powell *et al*., 1996). In maize, several thousand mapped SSR markers are available in the public domain (MaizeGDB; http://www.maizegdb.org).

Mogg *et al.* (1999, 2002) showed that by sequencing the flanking regions of maize microsatellites, a Single Nucleotide Polymorphism (SNP) could be found every 40 bp. Batley *et al.* (2003) developed and validated a computer-based method to identify candidate SNPs and small insertions/deletions from EST data. Given that the maize genome is estimated to be 2.5 x 10^9 bp in size, there is a potential for up to 62 million SNPs in maize. With the recent initiation of a large-scale EST sequencing programme in maize (http://www.zmdb.iastate.edu/zmdb/ EST_project.html), a new and potentially rich source of SNPs and genic SSRs has been uncovered. While both SSRs and SNPs can be reliably applied on a large scale with only small quantities of DNA required for PCR amplification, SNPs are highly amenable for automation, and therefore, offer significant advantages for breeding purposes. SSRs, however, are the preferred choice when codominant, multiallelic information is required, or when the infrastructure and resources are limited. Besides these markers, a number of genes controlling various aspects of plant development, biotic and abiotic stress resistance, and quality characters have been cloned and characterized in maize, which are excellent assets for molecular breeding.

Maize was one of the first major crop species for which a molecular map was developed (Helentjaris *et al*., 1986). Linkage mapping has been carried out ever since in maize by numerous laboratories and large amounts of data are now available in the MaizeGDB database (http://www.maizegdb.org/). Also, the development of an immortal mapping population, 'Recombinant Inbred Lines' (RILs), for molecular mapping was undertaken for the first time in maize (Burr *et al*., 1988; Burr and Burr, 1991).

To help build a high-resolution maize genetic map, Lee *et al.* (2002) developed the intermated B73 x Mo17 (IBM) population by randomly intermating an F_2 population derived from the single cross of the inbreds B73 and Mo17 for several generations prior to extraction of intermated recombinant inbred lines (IRILs). After genotyping these IRILs with 2046 markers, the Maize Mapping Project (MMP) constructed a genetic map (IBM2) that contains 2026 markers (Coe *et al.*, 2002; Cone *et al.*, 2002).

The present reference public map of maize is IBM2 map (see MaizeGDB). The first panel (IBM), derived from B73 x Mo17, is publicly available from the Maize Genetics Cooperation Stock Center. The second panel (LHRF) was developed from F2 x F252 to map loci monomorphic on IBM. Falque *et al.* (2005) built framework maps of 237 loci from the IBM panel and 271 loci from the LHRF panel. Both maps were used to place 1454 loci (1056 on map IBM_Gnp2004 and 398 on map LHRF_Gnp2004) that corresponded to 954 cDNA probes previously unmapped. A new genetic map of maize, ISU–IBM Map4, that integrates 2029 existing markers with 1329 new indel polymorphism (IDP) markers has been developed using intermated recombinant inbred lines (IRILs) from the intermated B73 x Mo17 (IBM) population (Fu *et al*., 2006).

Although a number of mapping populations have been developed in some Asian countries, particularly China and India, for mapping QTLs for various traits, published literature on availability of immortal mapping populations is very limited. In India, two RIL populations have been developed, one using CM139 (downy mildew susceptible) and NAI116 (downy mildew resistant), and another using CA00106 (BLSB-tolerant) and CM140 (BLSB-susceptible), as parental lines. The former has been graphically genotyped, and selected genotypes have been utilized for transcriptome profiling for sorghum downy mildew resistance (see Parul et al., in this volume).

Salient applications of molecular markers for maize improvement

Molecular markers are being utilized by several maize labs in Asia for diverse purposes including: (i) DNA fingerprinting and analysis of genetic diversity in inbred lines, breeding populations and landraces; (ii) determination of Essentially Derived Varieties (EDVs); (iii) assignment of inbred lines to specific heterotic groups; (iv) linkage disequilibrium (LD) analysis; (v) tagging qualitatively inherited genes of interest; (vi) mapping Quantitative Trait Loci (QTL) for diverse traits; and (vii) molecular marker-assisted selection (MAS) in breeding programmes.

DNA fingerprinting and genetic diversity analysis of maize in Asia

DNA fingerprinting and genetic diversity analysis using molecular markers is of significant utility in effective management of germplasm collections. Increasing emphasis is also being placed on comprehensive analysis of genetic diversity in breeding materials of major crops, including maize. Accurate assessment of the levels and patterns of genetic diversity using molecular markers is particularly helpful in maize breeding for (i) maintenance and broadening of the genetic base of the elite germplasm; (ii) assignment of lines to heterotic groups; (iii) selection of appropriate parental lines for hybrid combinations; and (iv) generation of segregating progenies with maximum genetic variability for further selection (Mohammadi and Prasanna, 2003).

The impetus for application of molecular markers for maize improvement in Asia was provided by the Asian maize Biotechnology Network (AMBIONET), initiated by CIMMYT in 1998 in six Asian countries (China, India, Indonesia, Philippines, Thailand and Vietnam). Under AMBIONET, microsatellite/SSR markers have been successfully utilized for DNA fingerprinting and analysis of genetic diversity in each of the six countries (George *et al*., 2004a). In addition, using the diverse Asian maize inbred lines, besides six reference genotypes from CIMMYT, 'standard allele kits' for ~80 SSR loci have been developed and placed in public domain. Molecular characterization of the Asian maize inbred lines revealed a narrow genetic base for the CIMMYT lines developed for the Asian region. In contrast to the case in southern China where 95% of the lines clustered separately from the CIMMYT lines, lines in the Indonesian breeding program show a closer relationship with the CIMMYT lines, reflecting a long history of germplasm exchange (George *et al*., 2004a). Analysis of the SSR diversity in 102 Asian inbred lines revealed the effect of selection for downy mildew resistance (George *et al*., 2004b), besides indicating that maize breeding activity in Asia had not caused a decline in the overall amount of diversity in the region.

SSR markers have also been deployed for analysis of genetic diversity of maize inbred lines in China (e.g., Yuan *et al*., 2000; Li *et al*., 2002; Xie *et al*., 2007), India (e.g., Pushpavalli *et al*., 2002; Mohammadi *et al*., 2002, 2008), Japan (Enoki *et al*., 2002), Iran (Choukan *et al*., 2006), and Thailand (e.g., Phumichai et al., 2008). The utility of SSRs in assigning lines to heterotic groups and relating the SSR-based genetic distance with hybrid yield or heterotic performance in maize were also explored by a few research groups in Asia (e.g., Yuan *et al*., 2001; Xu *et al*., 2005; Mohammadi *et al*., 2002, 2008; Prasanna and Hoisington, 2003; Choukan *et al*., 2006). These studies clearly revealed that broad based maize populations can be formed using combined criteria of SSR-based genetic distance, *per se* performance of lines, GCA and SCA. Besides analyses of maize lines commonly used in breeding programmes, SSR markers have also been utilized for analysis of genetic diversity in the QPM (Quality Protein Maize) lines developed in India vis-à-vis CIMMYT QPM lines (Bantte and Prasanna, 2003).

Besides aiding in the maintenance of genetic purity of inbred lines, molecular markers also play an important role in plant varietal protection, specifically as tools to distinguish an EDV (Essentially Derived Variety) from an initial (protected) variety, as these markers allow tracing of chromosomal segments from the parent to their progeny and make possible empirical evaluation of genetic relatedness. A series of experiments undertaken at University of Hohenheim, Germany, clearly demonstrated that SSRs are highly suitable in differentiating an EDV from an initial variety in maize (Heckenberger *et al*., 2002, 2003, 2005). Studies are also being undertaken at IARI, New Delhi, for developing protocols for ascertaining the EDV status using the Indian maize germplasm.

Maize has an unparalleled genetic richness as is evident from the existence of an array of diverse landraces in several countries worldwide, including India. SSR markers also proved effective in differentiating accessions of Oriental Maydeae from the American Maydeae, and revealed high genetic divergence among the NEH (North-eastern Himalayan) landraces in India (Prasanna *et al*., 2006). Molecular marker analysis is also now aiding in unraveling the genetic structure of global maize populations. SSR analysis of nearly 880 populations, including landraces/locals/OPVs from South America, North America, Europe, Africa and Asia, has been recently completed under a GCP (Generation Challenge Program) Project (GCP14). With the availability of high throughput microsatellite/SSR genotyping technologies and statistical software, such as 'FREQS-R' that aid in computation of allele frequencies based on genotyping of 'DNA bulks' of heterogeneous populations (Dubreuil et al., 2006), DNA fingerprinting and analysis of molecular diversity has been efficiently extended to landraces, populations, open-pollinated varieties, and germplasm accessions. Analysis of this dataset is in progress which could not only lead to a worldwide picture of diversity in maize populations but also could aid in tracking the maize migration routes from the centre of origin.

Maize in India is interesting due to the presence of some unique, genetically isolated populations cultivated locally, sometimes for over a century, especially in the NEH region (Prasanna and Sharma, 2005). Recent studies at IARI (India), including multi-location evaluation of agronomic performance of ~150 selected landrace accessions, and SSR-based characterization using the population DNA bulks, revealed significant intra-population and inter-population diversity as well as the genetic distinctness of 'Sikkim Primitives' (a landrace with high prolificacy) from the rest of the accessions (see Sharma *et al*., in this volume). The study also led to the identification of some highly promising landrace accessions from the NEH and other regions in India.

Association mapping or linkage disequilibrium (LD) analysis

'Association genetic analysis' or Association mapping' makes use of genomic surveys of *linkage disequilibrium* (LD). Originally developed for human genetics, statistical methods and their derivatives for detection of LD are now increasingly being applied to crop plants like maize, leading to analyses of population genetic structure and QTL detection. The candidate gene approach, using a Bayesian model-based probabilistic clustering, implemented through the STRUCTURE software, was first utilized for associating *Dwarf8* polymorphisms with flowering time variation in maize (Thornsberry et al., 2001).

Hierarchical clustering and factorial analyses have been extensively employed to analyze genetic relationships and quantitative positioning of accessions. In the recent years, several studies have been undertaken using the powerful population admixture model implemented through the software STRUCTURE. For instance, Wang *et al.* (2008) used 145 genome-wide SSR markers to assess the genetic diversity, population structure, and LD of a mini core set of 95 maize inbred lines which represented the Chinese maize inbred lines. Xie et al. (2008) analyzed a set of 187 commonly used maize inbred lines in China and inferred six subpopulations based on the ADMIXTURE model in STRUCTURE; these include PA, BSSS (includes Reid), PB, Lan (Lancaster Sure Crop), LRC (Luda Reb Cob, a Chinese landrace, and its derivatives), and SPT (Si-ping-tou, a Chinese landrace and its derivatives).

Empirical analyses of LD patterns have been undertaken for diverse genes in maize, including *Dwarf8* (*D8*), *Yellow1* (*Y1*), *Teosinte Branched 1* (*Tb1*) and most recently, for the *Lycopene epsilon cyclase* (*LcyE*) gene. These studies not only highlighted the importance of haplotype modeling, but also indicate the tremendous potential for maize improvement through identification of favourable alleles/haplotypes of interest (e.g., Harjes et al., 2008).

The genetic and statistical properties of the nested association mapping (NAM) design, implemented in maize (26 diverse founders; 5000 distinct immortal genotypes) to dissect the genetic basis of complex quantitative traits, were elucidated by Yu et al. (2008). The NAM design simultaneously exploits the advantages of both linkage analysis and association mapping. By integrating genetic design, natural diversity, and genomics technologies, this novel strategy is expected to aid in linking molecular variation with phenotypic variation for various complex traits.

Mapping QTLs for important traits of maize in Asia

Following the first report of a QTL in maize (Stuber *et al*., 1987), maize researchers worldwide have developed several detailed QTL and single gene maps for traits that are of considerable agronomic importance. Detailed information of molecular markers tagging genes/QTLs are now available for: (i) fungal disease resistance (e.g., downy mildews, Turcicum leaf blight, common rust, Polysora rust, Fusarium ear rot, Gibberella stalk rot, grey leaf spot, anthracnose stalk rot etc.); (ii) viral disease resistance (e.g., maize streak virus, maize dwarf mosaic virus, wheat streak mosaic virus etc.); (iii) insect pest resistance (e.g., European corn borer, Southwestern corn borer, corn ear worm, sugarcane borer etc.); (iv) abiotic stress tolerance (e.g., drought, heat, waterlogging, aluminum toxicity, etc.); (v) agronomic and morphological factors, including grain yield, forage yield, plant height, plant architecture, earliness, anthesis, silking, root traits etc.; (vi) grain quality (e.g., protein content, oil content, starch content, protein quality, fatty acid composition etc.); and (vii) specialty characters (e.g., sweetness, anther culture response, pollen-style interactions, pollen germination and growth etc.). QTL analyses has certainly aided in greater understanding of the genetic architecture of various traits, including disease resistance (Wisser *et al*., 2006) and drought tolerance (Tuberosa *et al*., 2007), in crops like maize.

Under AMBIONET, major QTLs for downy mildew resistance (George *et al*., 2003; Nair *et al*., 2005) were identified and validated, leading to the development of QTL-NILs in India. These QTL-NILs are not only being used for developing downy mildew resistant maize hybrids, but also for undertaking transcriptome profiling for downy mildew resistance (see Parul *et al*., in this volume).

QTLs for several important traits have been mapped by the Asian institutions, particularly in China and India. The traits include plant height (Wang *et al*., 2006; Zhang *et al*., 2007), downy mildew resistance (Agrama *et al*., 1999; George *et al*., 2003; Nair *et al*., 2005; Sabry *et al*., 2006), SCMV resistance (Zhang *et al*., 2003), common smut resistance (Ding *et al*., 2008), head smut resistance (Li *et al*., 2008), *Fusarium moniliforme* ear rot resistance (Zhang *et al*., 2006), Banded leaf and sheath blight (BLSB) resistance (Zhao *et al*., 2006), yield under drought stress at flowering time (Prasanna *et al*., 2004, 2008; Xiao *et al*., 2005; Lu *et al*., 2006), nutrient components under low nitrogen stress (Liu *et al*., 2008), high-oil (Song *et al*., 2004), popping ability (Babu *et al*., 2006; Li *et al*., 2006), and CMS-S (Tie *et al*., 2006).

Powerful analytical techniques are now available to scan the genome for significant marker-trait associations and estimate QTL positions and effects. It is also now possible to estimate epistatic effects of QTLs as well as QTL x environment interactions, as was demonstrated in case of traits like plant height (Zhang *et al*., 2007), common smut resistance (Ding *et al*., 2008), drought tolerance (Prasanna *et al*., 2008), and BLSB resistance (see Garg *et al*., in this volume).

Integration of multiple results from QTL studies is a key point to understand the genetic determinism of complex traits. Meta-analysis has multiple applications including QTL localization across populations, gene cloning and marker-assisted selection. Wang *et al*. (2006) constructed an integrated QTL map, based on 1201 published maize QTLs conferring 68 traits. The map showed that maize QTLs for various traits usually clustered in all chromosomes. Twenty-two plant height QTLs of maize were co-linear with 64 plant height QTLs of rice, and 43 grain yield QTLs of maize were co-linear with 7 grain yield QTLs of rice. A substantial number of candidate quantitative trait genes for plant height of maize were also found.

Veyrieras *et al*. (2007) presented a new computational and statistical package, called 'MetaQTL' (http://bioinformatics.org/mqtl), for carrying out whole-genome meta-analysis of QTL mapping experiments. MetaQTL offers a complete statistical process to establish a consensus model for both the marker and the QTL positions on the whole genome.

MAS for maize breeding in Asia

Significant progress has been made worldwide in optimizing MAS for improvement of both qualitatively and quantitatively inherited traits using maize as a model system. One of the successful examples of MAS for maize improvement is the utilization of *opaque2* specific SSR markers in conversion of the normal maize lines into quality protein maize (QPM) lines with enhanced nutritional quality

(Prasanna *et al*., 2001; Morris *et al*., 2003; Babu *et al*., 2004, 2005). MAS-derived QPM (Quality Protein Maize) hybrid, 'Vivek QPM Hybrid 9' has been recently released by VPKAS (Almora). This QPM hybrid was developed through transfer of *o2* gene and endosperm modifiers in the two parental lines (CM145 and CM212) of Vivek Hybrid 9. Under the ICAR Network Project on Molecular Breeding, the Maize Genetics Unit at IARI, New Delhi, has converted five inbred lines (CM137, CM138, CM139, CM140 & CM212) into their QPM versions (see Khanduri *et al*., in this volume). We have also recently pyramided major genes conferring resistance to Turcicum leaf blight and Polysora rust in five elite, but susceptible, parental lines (CM137, CM138, CM139, CM140 and CM212) (see Prasanna *et al*., in this volume). Similar efforts are being made in China, particularly with respect to QPM and SCMV resistance, and MAS products are in pipeline (S. Zhang, personal communication).

Unlike the case of simply inherited traits that are controlled by one or few major genes, improvement of polygenic traits through MAS is a complex endeavour. The difficulty in manipulating quantitative traits is related to their genetic complexity - principally the number of genes involved in their expression and epistatic interactions. Despite these limitations, there have been some successful examples of MAS for improving polygenic traits in maize. Researchers at CIMMYT (Mexico) dissected the genetic components of maize drought tolerance, identified major QTLs for component traits, and carried out MAS experiments for the improvement of both lines and populations (Ribaut and Ragot, 2006).

Prior knowledge of QTL locations can be exploited in marker-assisted recurrent selection (MARS). MARS refers to the improvement of an F_2 population by one cycle of marker-assisted selection (i.e., based on phenotypic data and marker scores) followed by three cycles of marker-based selection (i.e., based on marker scores only). Bernardo and Charcosset (2006) examined the usefulness of having prior knowledge of QTLs under genetic models that included different numbers of QTLs, different levels of heritability, unequal gene effects, linkage, and epistasis, and concluded that with known QTL, MARS is most beneficial for traits controlled by a moderately large number of QTL (e.g., 40). Bernardo and Yu (2007) further analyzed the prospects for genome-wide selection for improving quantitative traits in maize, and concluded that this approach is superior to MARS as the former effectively avoids issues pertaining to the number of QTL controlling a trait, the distribution of effects of QTL alleles, and epistatic effects due to genetic background.

Maize seed companies have successfully exploited marker-QTL associations in population improvement and cultivar development (Johnson, 2001, 2004; Koebner, 2003; Eathington *et al*., 2007). Some of the important factors that contributed toward effective utilization of MAS schemes in maize breeding, specifically by the private sector, have been the use of year-round nurseries or continuous nurseries, high throughput genotyping and phenotyping, and integration of both the rapid datasets for decision making (Ragot and Lee, 2007; Eathington et al., 2007).

Prospects

Understanding the complex web of interactions between genes and environmental factors, and effective application of this information in breeding strategies is a challenging endeavour. To obtain relevant information, it is imperative to exploit the tools of both classical and molecular genetics. The developments in the recent years in relation to molecular marker technology and QTL analysis have allowed identification of genomic regions involved in an array of agronomically important traits in diverse crop species, including maize. Such information is not only helpful in MAS, but is also providing clues to better understand genome organization and genetic dissection of complex traits.

The literature on maize is replete with reports of QTLs and DNA markers for every conceivable character. However, very few institutions worldwide, including those in Asia, have validated the marker-trait associations and effectively used this information toward cultivar development. It must also be noted that expression of some traits can be greatly affected by genotype-by-environment interaction and epistasis which can complicate the application of MAS. Therefore, it is extremely important to verify or validate the selective power of markers in a range of populations representing the diversity of current breeding populations.

Besides some encouraging developments in molecular breeding, structural and functional genomics research in maize is progressing at a healthy pace. From a time when the maize genome was considered to be too complex to consider large-scale physical mapping and sequencing, we have now reached a point where the maize draft genome sequence would be available soon. It is also important that institutions in Asia start developing proper genetic/genomic resources and tools for undertaking functional genomics and to characterize genes relevant for molecular breeding and genetic improvement.

With the development and access to abundant and reliable PCR-based markers, such as SSRs and SNPs, in crop plants like maize, efficiency of genotyping large populations or breeding materials has increased tremendously. However, accurate determination of phenotypes remains a significant challenge, especially for complex, highly environmentally influenced traits. It is the access to accurate phenotyping information, and not genotyping that may limit our ability to utilize powerful approaches such as association analysis.

The NARS in some of the Asian countries, particularly China and India, have demonstrated the capacity to effectively apply modern biotechnology, particularly molecular markers, for maize improvement. However, cost-effective application of molecular marker technology to agriculturally important problems cannot be done in isolation. Networking can facilitate development of an integrated system for efficient application of molecular tools and techniques, including QTL mapping and MAS. An USAID study highlighted the impact of such a network (AMBIONET) on maize improvement in Asian countries like India, China and Indonesia (Pray, 2006). A major investment in MAS infrastructure, including year-round nurseries, high throughput phenotyping and genotyping facilities (similar to those established by some multinational concerns in USA, Europe and Australia) and dedicated personnel are now required in the Asian institutions to effectively deploy MAS, maximize the gains and minimize the operational expenses and time required for cultivar development.

Acknowledgements

My sincere thanks to: the Indian Council of Agricultural Research (ICAR) for National Fellowship, and for funding various research projects on maize molecular breeding; CIMMYT and other Asian partners in AMBIONET (1998-2005); GCP14 partner institutions; collaborators at different institutions across India, including IARI (New Delhi), DMR (New Delhi), GBPUAT (Pantnagar), MPUAT (Udaipur), VPKAS (Almora), UAS-ZRS (Mandya), UAS-ARS (Naganahalli), HPKV-RRS (Bajaura), ICAR-NEH Centre (Tadong); and my dedicated research team at Maize Genetics Unit, IARI, New Delhi.

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Genetic Analysis and Mapping of QTLs for Resistance to Banded Leaf and Sheath Blight (*Rhizoctonia solani* f. sp. *sasakii*) in Maize

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Abstract. To identify genetic variability for resistance to banded leaf and sheath blight (BLSB) in India, 29 selected inbred lines were evaluated under artificial inoculation at three disease hot spots (Udaipur, Pantnagar and New Delhi, India) during the kharif (monsoon) seasons of 2002-2004. The study indicated high vulnerability of the maize lines to the BLSB isolates; only one inbred line (CA00106) offered moderate resistance to BLSB at all three locations, while some (eg, CM104 and LM6) showed moderate resistance at specific locations. To analyze the genetic basis of resistance to BLSB, a half-diallel set was generated using nine inbred lines (CA00106, CA00310, CM104, CM105, CM300, CM139, CM140, LM5 and LM6) differing in their responses to BLSB. Evaluation of this set at Delhi, Pantnagar and Udaipur revealed larger SCA effects as compared to GCA effects, and a significant contribution of nonadditive gene action. The combining ability analyses indicated that BLSB resistance was both generally and specifically transferable among crosses. An $F_{2:3}$ mapping population comprising 193 individuals was generated using CA00106 as the resistant parent and CM140 as the susceptible parent. Genotyping of this mapping population was undertaken using 127 polymorphic SSR (single sequence repeat) markers covering the maize genome. Linkage mapping was performed with the MULTIPOINT software using genotypic data from 108 SSR markers. The map had a total length of 2001.3 cM, with an average marker interval of 19.53 cM. Phenotyping of 192 F_3 families, along with the parental lines, was undertaken in 2005 at the three locations. Quantitative Trait Loci (QTL) mapping using QTL Cartographer revealed location-specific QTLs for BLSB resistance, with most of the favorable QTL alleles contributed by the resistant parent CA00106. Analysis using QTL Network V2.0 led to the identification of three QTLs (on chr. 6, 8 and 9) with significant epistatic interactions.

Key words: Maize, BLSB, resistance, genetic variability, QTL mapping

Introduction

Banded leaf and sheath blight (BLSB), caused by *Rhizoctonia solani* f.sp. sasakii Exner (teleomorph) *Thanatephorus sasakii* is considered one of the most important diseases of maize in South and Southeast Asia (Sharma and Saxena 2002). It has the potential to cause significant reduction in and fodder quality in maize. Various methods – mechanical as well as chemical – have been suggested to control BLSB. With increasing appreciation of the need for cost-efficient and environmentally sound means of disease management, and given the availability of powerful tools and techniques to genetically analyze disease resistance, there is now renewed emphasis on host-controlled resistance for economically important diseases. However, very limited information is available regarding the genetic basis of resistance to BLSB and the QTLs governing BLSB resistance (Zhao *et al.* 2006).

This study was undertaken to analyze the genetic variability for resistance to BLSB in maize, to study the mode of inheritance of resistance to BLSB, and to undertake molecular marker analyses of BLSB resistance.

Materials and Methods

To generate multilocation phenotypic data on genetic variability for BLSB resistance, experiments were carried out at three BSLB hot spots (Udaipur in western India; Pantnagar in northern India; and New Delhi in northern India) during the *kharif* (monsoon) season of three consecutive years (2002-2004). The response of 29 selected inbred lines, comprising 20 lines from the CIMMYT-Asian Regional Maize Program (CIMMYT-ARMP), 7 CM (Coordinated Maize) lines, and two LM (Ludhiana Maize) lines, against BLSB infection wa evaluated by artificial inoculation (barley grain culture method).

To analyze the genetic basis of resistance to BLSB, a half-diallel set was generated using nine inbred lines (CA00106, CA00310, CM104, CM105, CM300, CM139, CM140, LM5 and LM6) differing in their responses to BLSB. The experimental crosses, along with the parental lines, were evaluated for their response against BLSB infection during the monsoon season of 2004 at Pantnagar, Udaipur and Delhi.

Air-dried barley grain with fungal growth were used for field inoculation, as outlined by Ahuja and Payak (1978), on 30-60-days-old plants (depending on the maturity group of the maize genotypes). High humidity (>90%) was maintained throughout the disease development period by frequent irrigation. Observations on disease rating were recorded 30-40 days after inoculation on a standard 1-5 disease rating scale, as suggested by Ahuja and Payak (1983) and Vimala and Mukherjee (1987). We rated at least 10 plants in each replication for each entry. Disease reaction was assessed on 40 days old plants after inoculation, and utilized these disease ratings to estimate the disease index (DI), as suggested by Wang and Dai (2001): DI = [Ó (severity class or disease score × number of plants in this class) / the highest possible severity class or disease score × total number of investigated plants] × 100. The responses of the genotypes were rated on the basis of the DI values, as per the following scale: 0-30 – resistant; >30-60 – moderately resistant; >60-90 – susceptible; and >90-100 – highly susceptible.

An $F_{2:3}$ population was derived using CM140 and CA00106 as the parents. The DNA was extracted from 193 F_2 individuals using the CTAB procedure with suitable modifications. SSR analysis was undertaken as per protocols optimized at the Maize Genetics Unit of the Indian Agricultural Research Institute (IARI). A total of 470 SSR loci uniformly spaced in the genome were assessed for SSR polymorphism in the parental lines of the mapping population. Of these, 127 were found to be polymorphic based on high-resolution agarose gel electrophoresis. These polymorphic SSR markers were used for genotyping the 193 F_2 individuals. The standard $÷^2$ test was used to test the segregation pattern at each marker locus for deviations from the expected Mendelian segregation ratio of 1:2:1 (for an F_2 derived F_3 mapping population). Linkage mapping was carried out using MULTIPOINT Version 1.2 (Korol *et al.* 2006), with an LOD score threshold of 3.0 and Kosambi mapping function.

The F_3 families, along with the parental lines, were evaluated using a randomized block design (three replications each) in disease screening nurseries at Udaipur, Pantnagar and Delhi. Analyses of phenotypic data were conducted separately for each location, considering all effects in the statistical model as random. The genotypic data consisted of 108 SSR marker loci, and the phenotypic data comprised the BLSB Disease Index for the three study locations. The method of composite interval mapping (CIM; Zeng 1994) as implemented in QTL Cartographer V2.5 (Wang *et al.* 2005) was used to map QTLs and their genetic effects. Model 6 of Zmapqtl procedure was used stipulating an F_2 population, scanning the genome every 2 cM, and specifying cofactors identified by forward and backward regressions that explained most of the variation for a given trait. We also estimated the epistatic interactions among the QTLs as well the QTLs with the environment by using QTL Network software.

Results and Discussion

Evaluation of the 29 inbred lines at the three locations revealed a few sources of resistance like CA00106, CM104 and LM6 at different hot spots. Only CA00106 recorded moderate resistance to BLSB at all the three locations (Table 1). Several elite maize inbred lines (CM/LM lines) that are frequently used in maize breeding programs in India showed a high level of susceptibility to the BLSB isolates. CM139 and CM140, the parental lines of a popular maize hybrid Parkash, were found to be susceptible to BLSB at both Udaipur and Delhi. The lack of adequate sources of resistance to BLSB in elite tropical maize germplasm and the high levels of susceptibility of inbred lines developed at various institutions, including CIMMYT, were highlighted by this analysis.

Analysis of the half-diallel data set clearly indicated that there were significant genetic differences among the parents chosen and their prepotency. The experiments demonstrated (i) the polygenic nature of BLSB resistance; (ii) larger SCA effects as compared to GCA effects; (iii) the significant contribution of nonadditive gene action; (iv) the possible occurrence of a few favorable resistant alleles in some of the BLSB-susceptible lines like CA00310 and CM140, contributing to reduced disease index in cross combinations. The combining ability analyses indicated that BLSB resistance was both generally and specifically transferable among crosses. Crosses among moderately resistant lines (CA00106, CA00310, CM104 and LM6) expressed different levels of resistance, but all have potential in developing BLSB resistant germplasm. LM6, in particular, showed significant negative GCA effects, indicating contribution to resistance of the experimental crosses at all the three locations. The promising inbred lines and experimental hybrids identified through this study can be potentially utilized in BLSB resistance breeding programs in India.

Intensive analysis of molecular polymorphisms in a subset of six inbred lines, using 106 SSR markers, aided in identification of CM140 as the genetically most divergent line that could be used in combination with CA00106 for generation of the $F_{2:3}$ mapping population. Intensive analysis of SSR polymorphisms was undertaken using a total of 584 SSR markers covering every bin location in the maize genome. This polymorphism survey facilitated identification of 127 polymorphic SSR markers that could be utilized for genotyping of the mapping population. Genotyping of the F_2 mapping population, comprising 193 individuals, was undertaken using 127 SSR markers that were found polymorphic between the parental lines (CM140 and CA00106). The Chi-square test was carried out to ascertain the goodness-of-fit of the segregation pattern for each locus. Linkage mapping was performed using the MULTIPOINT software using genotypic data from 108 SSR markers (after removing 12 SSR loci showing segregation distortion and 7 SSR loci causing neighborhood distortion). The Kosambi mapping function was used to convert recombination frequencies into map distances (cM). The final linkage map had a total length of 2001.3 cM, with an average marker interval of 19.53 cM.

Phenotyping of the 192 F_3 families, along with the parental lines, was undertaken for recording responses to BLSB under artificial inoculation conditions, during the rainy season of 2005 at the three locations. ANOVA indicated significant genetic variation for BLSB resistance in the F_3 families at all the three locations. Heritability values, estimated using variance components, were found to be higher at Delhi and Pantnagar (67% and 63%, respectively), but at Udaipur, the heritability was quite low (19%).

Quantitative Trait Loci (QTL) mapping was carried out using the phenotypic and genotypic data sets for BLSB, by employing composite interval mapping (CIM) using QTL Cartographer Version 2.5. The threshold levels employed for declaring significant QTLs were mostly LOD>2.5 or LOD>3.0. A total of eight QTLs influencing resistance to BLSB at different locations in India were identified in our study. Three QTLs for BLSB resistance were located at Delhi; one each on chr. 4 (*bnlg252-bnlg1621*), chr. 8 (*umc2146-umc1172*) and chr. 9 (*phi108411-umc2346*). Three QTLs influencing BLSB resistance at Udaipur were mapped, one each on chr. 2 (*umc2363-umc1622*), chr. 3 (*umc2101-umc1892*), chr. 6 (*umc1127*) and chr. 10 (*bnlg1518-bnlg1526*). Only one QTL influencing BLSB resistance at Pantnagar could be detected on chr. 7 (*umc1066-bnlg1792*) (Fig.1).

Most of the favorable QTL alleles were contributed by the resistant parent CA00106, but at Udaipur even the susceptible parent CM140 contributed a QTL for BLSB resistance. The QTL for BLSB resistance detected on chr. 7 had the largest effect at Pantnagar (accounting for 11.5% of phenotypic variation), while the one on chr. 3 contributed 4.9% of phenotypic variance at Udaipur. Only two QTLs showed dominant gene action (one each at Delhi and

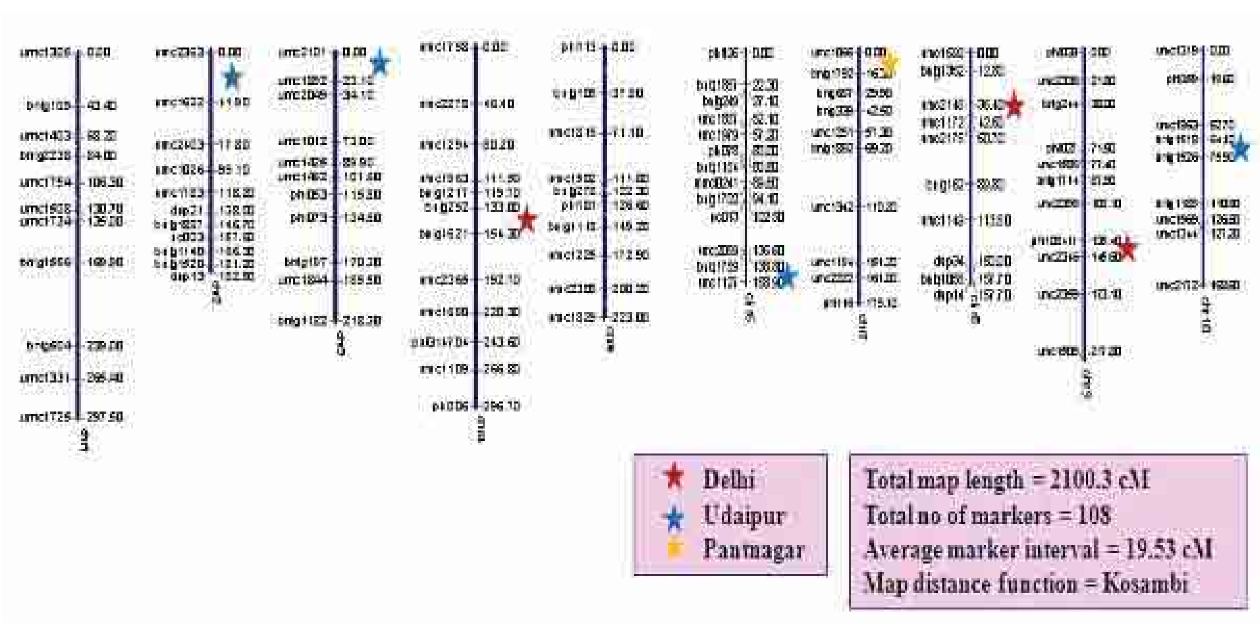

Figure 1. Linkage map of maize based on an SSR analysis of F_2 mapping population derived from a cross involving the parental lines CA00106 and CM140. The positions of QTLs for BLSB resistance identified at different locations are indicated on the map.

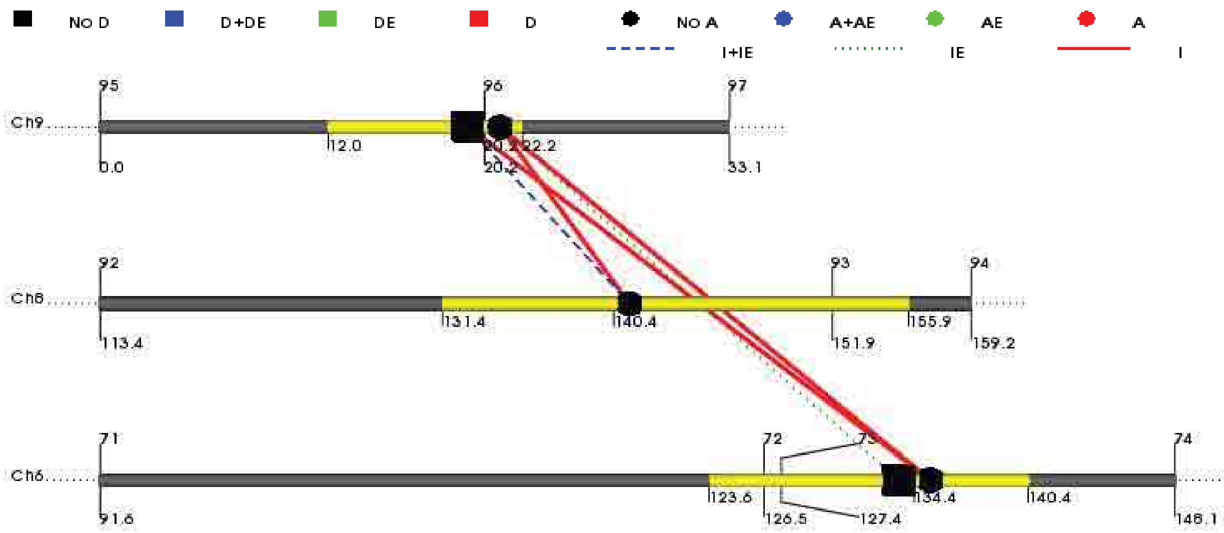

Figure 2. Depiction of epistatic QTL interactions influencing BLSB resistance in maize.

Udaipur), while the rest revealed overdominant gene action at the three locations. Three significant epistatic QTLs we identified on Chr. 6, 8 and 9 (Fig.2), using QTL Network software. These interactions include those with only epistatic main effects (I), with only epistasis × environment interaction (IE) effect, as well as with both effects (IE). These results suggest that the inheritance of BLSB could be more complex than expected**.**

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Relationship between Genetic Distance-based SSR Markers and Phenotypic Performance of Single-Cross Hybrid Maize

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Abstract. Genetic diversity (GD) is one of the criteria for selection of parents for hybrid breeding. The objective of this study was to evaluate the relationship between genetic distance and phenotypic performance, especially seed weight per plant of testcross and diallel cross-derived hybrids. Thirty-four inbred lines including two testers (Mr4 and Mr14) were characterized by using 36 microsatellite loci. Evaluation of single-cross hybrids in two steps followed the hybrid breeding program. The first step was evaluation of single crosses derived from testcrossing with Mr4 and Mr14. The second step was evaluation of single-cross hybrids derived from diallel crossing (7×7) that were selected on the basis of genetic distance. The results showed that the testcross-derived single-cross hybrids had a moderate value of correlation between microsatellite marker-based GD and seed weight per plant, ie, 0.81 and 0.76, respectively. For the diallel-cross-derived single-cross hybrids, the correlation values between GD and seed weight per plant, specific combining ability (SCA), high parent heterosis (HPH) and mid-parent heterosis (MPH) were 0.72, 0.62, 0.60 and 0.68, respectively. From the diallel cross method, six single-cross hybrids showed potential to be developed as new hybrid varieties with seed weight ranging from 176.05 g per plant to 181.24 g per plant. The results of this study indicate that both steps had moderate correlation between GD and morphological traits. It means that we need only use one of the two steps in breeding programs to select parents of hybrid with high heterosis, thus allowng us to cut one or two seasons in hybrid maize breeding programs.

Key words: Genetic distance, test cross, diallel, heterotic group

Introduction

Identifying parental combinations that can produce hybrids of superior yield is the most important step in a breeding program. However, it is a costly and time-consuming process as it involves crossing the available inbred lines and evaluating the hybrids in extensive yield trials (Bernardo 1992). Parents with high general combining ability and large genetic distance have been reported to produce hybrids with better yield performance (Cox and Murphy 1990; Diers *et al*. 1996). Further, advances in genome research have generated interest in using molecular markers to predict hybrid performance in breeding programs.

Morphological and molecular marker-based genetic diversity studies in relation to hybrid performance have been undertaken in several crops. Positive association between morphological genetic distance and heterosis has been reported for crosses in wheat (Cox and Murphy 1990) and alfalfa (Riday *et al*. 2003). Investigations in maize, rice and rape seed have shown that the molecular genetic diversity of parents is significantly correlated with hybrid performance, and that yield heterosis could be predicted using molecular markers (Diers *et al.* 1996; Riaz *et al.* 2001; Betran *et al.* 2003). In addition to the identification of potentially high-yielding hybrids, genetic distance measurements help us assign new pure lines to heterotic groups. The objectives of our study were to correlate between microsatellite marker-based genetic distance (GD) and the seed weight per plant of (a) testcrosses; and (b) diallel-cross-derived single-cross hybrids.

Materials and Method

The DNA from ten-day-old seedlings of 34 inbred lines including two testers, Mr4 and Mr14, was extracted according to the modified CTAB method (Kant *et al*. 2004). Single sequence repeat (SSR) analysis was performed as described by George *et al*. (2004). Thirty-six SSR markers were developed to create the molecular marker data. Genetic distances were determined and a dendrogram compiled via the unweighted pair group method of arithmetic average (UPGMA) using NTSYS-pc 2.1 (Rohlf 2000).

We first evaluated the field performance of the two sets of single-cross hybrids obtained by the testcross method with the two testers (Mr4 and Mr14) at the Bajeng Experimental Station. Each set consisted of 32 single-cross hybrids plus three commercial hybrids. They were evaluated in a simple lattice 7 × 6 with two replications. The unit trial consisted of double-row plots 5 m long, with 75 cm spacing between rows, and 20 cm within a row. The recommended doses of fertilizer were used.

Then we also evaluated the performance of 21 single-cross hybrids obtained by the diallel cross method 2 (Griffing 1956). Seven inbreds from different groups having low to high average GD, and from different source populations were selected for diallel hybrid evaluation. Twenty-one F_1 hybrids plus four commercial hybrids were evaluated in a randomized block design (RBD) with four replications at two locations in a single-row plot 5 m long with 75 cm spacing between rows. The recommended doses of fertilizer were used.

Griffing's Method 2 Model 1 (fixed effect) diallel analysis was used to estimate seed weight per plant, general combining ability (GCA) for parents and specific combining ability (SCA) for hybrids (Griffing 1956). Heterosis was determined as follows:

1. Mid-Parent Heterosis (MPH)(%)

$$h = \left[\frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \right] \times 100$$

2. High-parent heterosis (HPH)(%)

$$h = \left[\frac{\overline{F_1} - \overline{HP}}{\overline{HP}} \right] \times 100$$

where F_1 is the F_1 performance; MP = (P1+P2)/2 in which P1 and P2 are the performances of inbred parents, respectively, and HP is the high parent value.

Results and Discussion

Microsatellite or SSR markers are the most polymorphic markers based on differences in simple repetitive sequences that are flanked by a concerved border. They are distributed all over the genome, making them the most suitable for paternity studies. With DNA sequence information becoming increasingly available, an automated process to identify and design PCR primers for amplification of SSR loci would be a useful tool in plant breeding programs. In the simple laboratory, SSR markers are easy to handle.

We observed the genetic diversity of 34 inbred lines originating from various source populations. The results revealed high levels of genetic diversity between inbred lines, indicated by the average level of polymorphism (0.61). These genotypic data also showed that the dendrogram construction based on UPGMA could differentiate among the inbreds. We clustered the inbreds into five groups based on genetic similarities and consistency with pedigree data. However, the limitation of using pedigree data showed up in this study. Four inbreds having the same initisal (SP007) were scattered in three groups or had no clear pedigree data. Using microsatellite markers is effective in selecting hybrid parents and can be a rational strategy in hybrid breeding programs (Fig. 1).

The genetic distance estimates for the single-cross hybrids derived from testcrosses with Mr4 and Mr14 ranged from 0.62 to 0.89 and from 0.52 to 0.87, respectively. Hybrids obtained from very closely related parents (P5/GM26-22 × Mr4) expressed the lowest seed weight per plant. The highest seed yield per plant was obtained from hybrids with moderate divergence between parents and not from distantly related parents. Two F_1 single crosses were significantly different compared to the control Bima1: P5/GM26-9 × Mr4 and Bisma-3-1 × Mr14. They had seed weight of 179.10 g per plant and 178.52 g per plant respectively. The GD values of these two hybrids were 0.82 and 0.84 respectively, compared to 0.65 for the control Bima1. The correlation value between genetic distance and seed weight per plant of the two sets of F_1 maize testcrosses (Mr4 and Mr14 being the testers) were of a medium level, ie, 0.81 and 0.76 respectively. It is worth considering more inbreds that have more potential than Mr4 and Mr14 to arrive at the best heterotic pattern in Indonesia by using molecular markers as tools (Table 1).

Single-cross hybrids derived from diallel crosses showed that the intergroup crossing on the basis of genotypic grouping in general produces hybrids with greater yield potential than intragroup crossing. It was concluded that the use of microsatellite markers was efficient in placing the inbreds in different heterotic groups. The values of correlation between GD and morphological traits (seed weight per plant, specific combining ability (SCA), as well as MPH and HPH) of single-cross hybrids were are on a medium level: 0.67, 0.59, 0.55 and 0.64 respectively (Table 2). However, there is a need to add more primers to obtain the best correlation between GD and seed weight per plant. From this experiment, six single-cross hybrids seemed to have potential for development

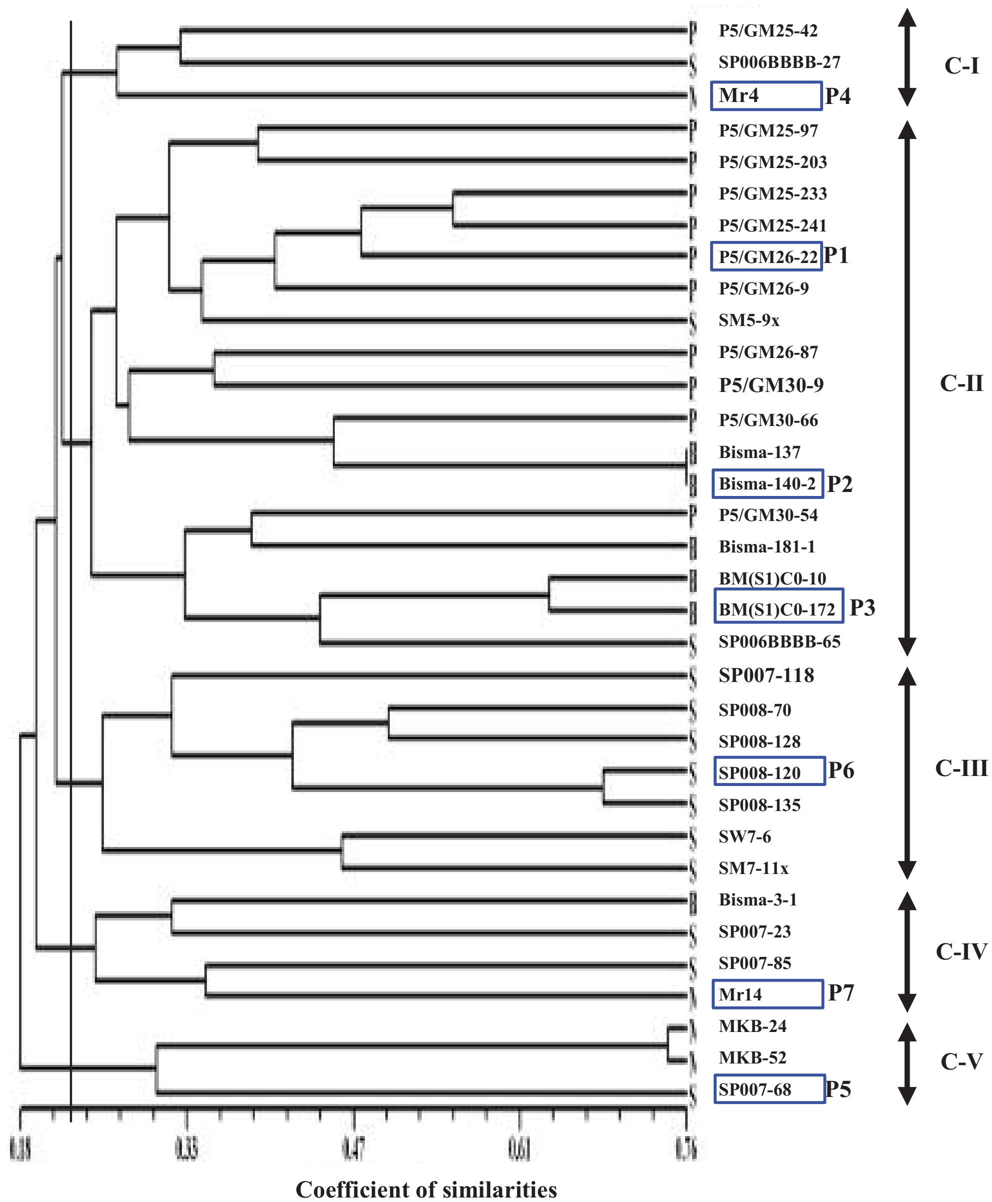

Figure 1. Dendrogram of 34 inbred lines clustered on the basis of SSR marker-based genetic distance estimates. P1, P2, P3, P4, P5, P6, P7 are inbred lines that were selected as candidate parents of hybrids.

Table 1. Average value of genetic distance, 1000-seed weight (g) and correlation value between genetic distance and average seed weight per plant (g) of single-cross hybrids derived from testcrosses.

Genotipe hasil silang uji	Single-cross hybridswith tester Mr4	Single-cross hybrids with tester Mr14
Average genetic distance	0.75	0.74
Average seed weight per plant (g)	146.94	143.69
Correlation value (r)	0.81	0.76

Tabel 2. Value of genetic distance and seed weight per plant of single-cross hybrids[1].

	P5/GM26-22	Bisma-140-2	BM(S1)C0-172	Mr4	SP007-68	SP008-120	Mr14
P5/GM26-22		0.75	0.67	0.66	0.65	0.74	0.61
Bisma-140-2	292.17		**0.54**	0.77	0.82	0.72	0.70
BM(S1)C0-172	272.16	232.49		0.73	0.72	0.71	0.73
Mr4	283.03	308.47	300.65		**0.86**	0.75	0.70
SP007-68	292.20	298.94	278.92	287.90		0.76	0.74
SP008-120	323.65	288.80	276.23	287.66	293.19		0.73
Mr14	295.04	**354.87**	340.98	362.15	328.02	274.39	

[1.] Above diagonal: Genetic distance (GD); Below diagonal: average seed weight per plant (g).

Table 3. Values of correlation (r) between genetic distance (GD) and seed weight per plant, specific combining ability (SCA) and heterosis at two locations, Bajeng and Muneng, Indonesia.

GD estimated with morphological traits	Correlation value (r)		
	Bajeng	Muneng	Combining analysis
GD estimated with seed weight per plant	0.72	0.52	0.67
GD estimated with SCA	0.62	0.52	0.59
GD estimated with MPH	0.57	0.41	0.51
GD estimated with HPH	0.68	0.51	0.64

as new hybrid varieties with seed weight ranging from 176.05 g per plant to 181.24 g per plant. These six hybrids were: Bisma-140-2 × SP007-68, BM(S1)C0-172 × Mr4, SP007-68 × SP008-120, P5/GM26-22 × SP008-120, Bisma-140-2 × Mr14 and BM(S1)C0-172 × Mr14 (Table 3).

In this study, generally hybrids obtained from very closely or distantly related parents showed low seed weight per plant but those from parents of intermediate divergence tended to show higher seed weight per plant. This suggests that intermediate divergence of parental lines could give higher seed weight per plant. Morphological traits and SSR distances differentiate maize lines into heterotic groups from which superior hybrids can be derived. The most important objective of this study was to investigate the relationship of genetic distance with hybrid performance, particularly yield (seed weight per plant) and heterosis. It is clear from the data that the level of genetic diversity was high but the relationship between genetic distance and heterosis was of a medium level. This suggests the complexity of the genetic basis of hybrid performance and heterosis.

The results of this study indicate that both of the steps used in hybrid breeding programs, ie, testcrosses followed by diallel crosses, have a medium level of correlation between GD and morphological traits. This means that we can use only one of the two steps on the way to selecting parents of hybrids based on marker-based genetic distance. We we can thus cut one or two cycles to get to new candidate hybrids. However, we need more experience and knowledge to enhance our understanding of the relationship between genetic distance and hybrid performance, and develop a strategy for utilizing molecular markers in hybrid maize breeding programs.

Conclusions

- The values of correlation between GD and morphological traits, particularly seed weight per plant, of hybrids derived by the testcross as well as the diallel cross methods were of a medium level. This indicates that genetic distance values can be used to select candidate parents of hybrids with potentially high heterosis.
- On the basis of genotypic grouping, intergroup inbreds generally produced hybrids with greater yield potential than intragroup inbreds.
- Six single-cross hybrids showed potential to be developed as promising hybrids with seed weight ranging from 176.05 g per plant to 181.24 g per plant.

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Graphical Genotyping of Genomic Resources (QTL-NILs and RILs) and Transcriptome Profiling of Maize Genotypes in Response to Sorghum Downy Mildew (*Peronosclerospora sorghi*) in India

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Abstract. Sorghum downy mildew (Peronosclerospora sorghi) is among the major diseases of maize in tropical maize-growing countries worldwide, including India. In this study, a set of selected RILs and QTL-NILs, along with their parents (CM139 and NAI116) and two CIMMYT-ARMP downy mildew-resistant lines, were phenotyped for sorghum downy mildew (SDM) disease response under artificial inoculation at the University of Agricultural Science - Regional Research Station (UAS-RRS), Mandya during the kharif (rainy) season of 2007, and genotyped using microsatellite/SSR markers spanning the maize genome. Graphical genotypes were generated, and a subset of these genotypes with strong phenotypic contrast were further used for transcriptome profiling for identification of putative genes and pathways involved in SDM stress resistance in maize. Transcriptome profiling was undertaken on selected highly resistant, tolerant and highly susceptible maize lines using 'one-cycle eukaryotic target labeling assay' and hybridized with maize genome arrays (Affymetrix). The microarray data were analyzed using different statistical tools. The internal control plot, poly-A control and hybridization control plot and 3'/5' ratio of genes (internal and external controls in analysis) and the Scatter Plot indicated use of intact and high quality samples in microarray hybridization and highly significant and positive correlations (r = 0.98-0.99) among the replicates. The study revealed a significant number of differentially expressed genes in SDM-resistant genotypes vis-à-vis susceptible genotypes. Further annotation and validation of the putative genes is in progress.

Key words: Maize, downy mildew, QTL-NILs, RILs, transcriptome profiling

Introduction

Despite substantial advances in plant disease control strategies, global food supply is still threatened by a multitude of pathogens and pests. Plant diseases can dramatically reduce crop yield, and the impact of disease outbreaks is particularly acute in developing nations (McDowell and Woffenden 2003). Among the various biotic constraints affecting maize production and productivity, the downy mildews (DMs) are very important due to their geographical distribution and their potential ability to cause significant reduction in yield. Worldwide, 30% of the maize area in tropical lowland, subtropical, mid-altitude transition and highland zones suffers economic losses due to DM attack (Jeffers *et al*. 2000).

One of the important downy mildew pathogens of maize is *Peronosclerospora sorghi* (Weston and Uppal) shaw (Sorghum DM), which is an obligate fungus. The most efficient, effective, environmentally safe and economical means to control SDM of maize is the use of resistant varieties. Microarray analysis allows the expression profiling of thousands of genes simultaneously and provides an opportunity for identifying genes involved in resistance as well as changes in their activity in response to a specific biotic/abiotic stress or developmental context (Schena *et al*. 1995; 1996).

The present study was undertaken with the specific objectives of (i) identification of a set of resistant and susceptible genotypes; and (ii) further utilization of this set of lines for identification of a set of putative genes conferring resistance to SDM.

Materials and Method

Phenotyping

A set of QTL-NILs derived through molecular marker-assisted backcrossing, and another set of Recombinant Inbred Lines ($F_{7:8}$ RILs using CM139 and NAI116 as parents) obtained through the single seed descent method were developed at the Maize Genetics Unit of the Indian

Agricultural Research Institute (IARI), New Delhi. Phenotyping was done on this set of QTL-NILs and RILs in a replicated trial (two replications; two rows per replication). The RCBD trial was undertaken at the SDM hot spot at UAS-RRS, Mandya (Karnataka, India), along with the parents (CM139 and NAI116), under artificial inoculation during *kharif* (monsoon) season of 2007 using the standardized procedure (Craig *et al*. 1977; Nair *et al*. 2004; 2005). As a susceptible control, noninfected CM500 and as a resistant control NAIH 2049 seeds were planted after every 10th row of test materials. Severe infection (98-100% DM incidence) in the susceptible control rows across the experimental block indicated uniform and strong pathogen pressure, leaving no possibility for disease escape. The disease reaction was assessed 30 days after plant emergence by scoring for DM infection in individual plants. The percentage disease incidence in each test entry was determined. Inoculated plants that did not show systemic symptoms for SDM (emergence of characteristic chlorotic leaves) one month after artificial infection were considered resistant. Disease rating was undertaken using the following scale: 0-10% - Resistant (R); >10-30% - Moderately resistant (MR); >30-50% - Moderately susceptible (MS); >50-70% - Susceptible (S); >70-100% - Highly susceptible (HS).

Graphical genotyping

A selected set of phenotyping contrasting lines were selected and genotyped using microsatellite/SSR markers spanning the maize genome. The concept of graphical genotyping was applied to compare the genome structure of selected lines, and profile specific chromosomal regions influencing SDM resistance in maize (George *et al*. 2003; Nair *et al.* 2005). Graphical genotypes were generated using GGT v2.0 for foreground SSRs covering bins 3.04-3.05 (30 SSRs) and 6.05 (17 SSRs) as well as for background SSRs (109).

Transcriptome profiling

On the basis of graphical genotyping a subset of genotypes, *ie,* MAS267-2 (R), RIL66 (R/MR) and MAS291-1 (HS), were found with strong phenotypic contrast and further used for transcriptome profiling. For this study, the selected genotypes were raised under artificial SDM inoculation at UAS-RRS during *kharif* (monsoon) 2006. Leaf tissues (in replicates) were collected from selected resistant and susceptible maize genotypes. Total RNA was isolated using TRIZOL (Ambion) from tissues of control and stressed plants of the resistant as well as susceptible genotypes. Complementary RNA (cRNA) was synthesized using 'one cycle eukaryotic target labeling assay' for labeling and hybridized with maize genome arrays (Affymetrix).

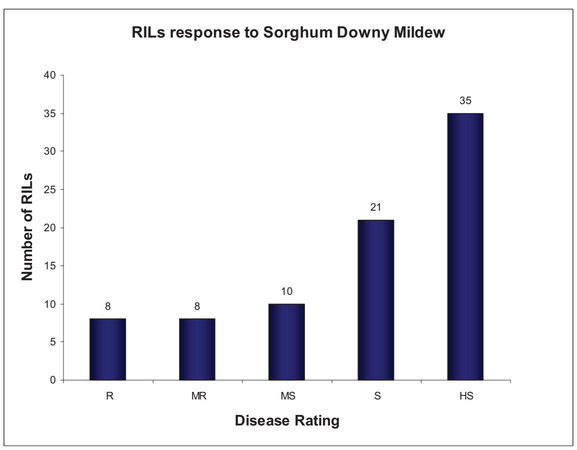
Results and Discussion

Phenotyping at UAS-RRS, Mandya revealed significant variation among the QTL-NILs and RILs for response to SDM. The study led to identification of lines showing highly contrasting phenotypic behavior against SDM (Figs. 1 and 2), while the other lines showed responses ranging from moderately resistant (MR) to highly susceptible (HS). Theses lines with highly contrasting behavior for SDM were selected for graphical genotyping. In the present study, the concept of graphical genotyping revealed a clear-cut visual differentiation among highly resistant, resistant and susceptible lines, and led to identification of a subset of three genotypes, ie, RIL66 (R), MAS267-2 (R) and MAS291-1 (S) showing strong phenotypic contrast and reduced possibility of genetic 'background noise' for undertaking transcriptome profiling.

Plants react to pathogen attack by expressing a set of specific genes. In order to identify these specific set of genes, we used microarray technology. Trancscriptome profiling was done using the maize genome array (Affymetrix) representing approximately 13 339 genes. Hybridization of samples with the maize array revealed a high degree of homology ranging from 30% to 57% with a mean of 50%. After hybridization, raw data was obtained and analyzed using different statistical tools.

Array diagnostic features like MA plot, volcano plot, etc. were used to check the quality of the data set. The analysis revealed the lack of any physical anomalies in the chips used as well as the excellent quality of data suitable for further statistical analyses. We found that the internal control plot, poly-A control and hybridization control plot and 3'/5' ratio of genes (internal and external controls in analysis) and the Scatter Plot indicated the use of intact and high quality samples in microarray hybridization and highly significant and positive correlations (r = 0.98-0.99) among the replicates. To remove the signal intensity bias, if any, and for adjusting the background, the GCRMA method of normalization was employed (Fig. 2b). For selecting differentially expressed genes and at the same time reducing False Discovery Rate (FDR), three stringent criteria were simultaneously used: P-values, Fold Change (FC), and average expression values (number of DE genes presented in Table 1).

Figure 1a. Selected lines under artificial inoculation at Mandya, Karnataka, India.



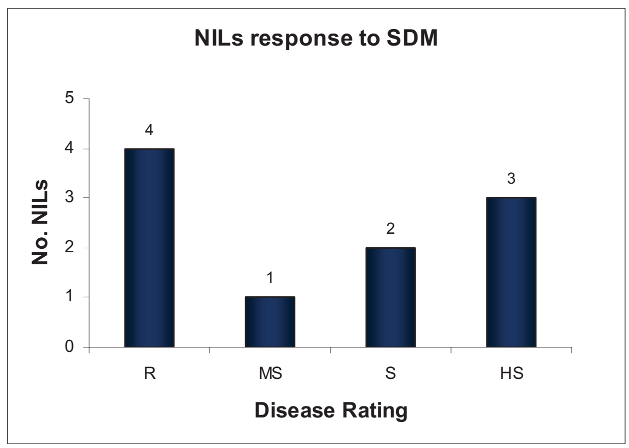


Figure1b. Responses of RILs and QTL-NILs for sorghum downy mildew (SDM).

Table 1. List of differentially expressed genes in all genotypes.

Genotype	Total no. of DE genes	No. of down-regulated genes	No. of total up-regulated genes	No. of significantly up-regulated genes
MAS267-2	1642	845	798	33
RIL66	1320	407	913	19
MAS291-1	1859	1028	831	33

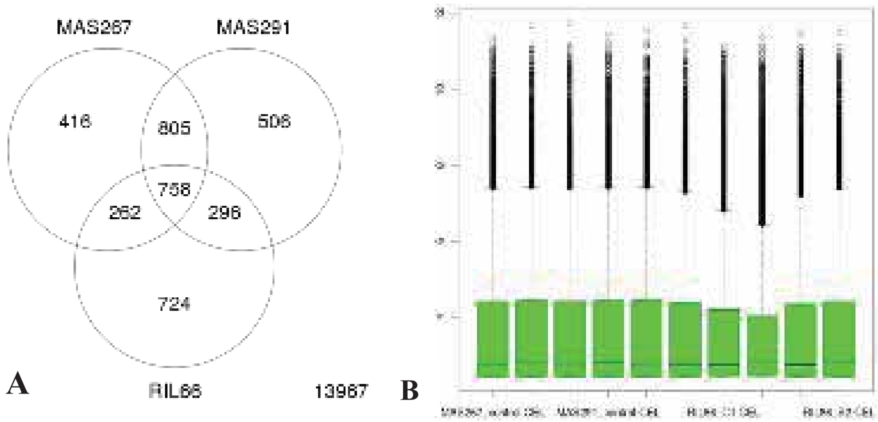


Figure 2. Venn diagram (A) revealing the number of commonly and differentially expressed genes among selected genotypes for SDM. (B) GC-RMA based normalization of microarray data.

The Venn diagram (Fig. 2a) indicates the differentially expressed genes among all genotypes. Annotation of the significantly up-regulated genes using different web-based existing databases and validation of the putative genes is under progress. This is the first study worldwide on transcriptome profiling for downy mildew resistance in maize.

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Allele Mining in the *Yellow1* (*Y1*) Gene Influencing Carotenoid Biosynthesis in Maize

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Abstract. Carotenoids are a class of fat-soluble antioxidant vitamin compounds that provide health benefits to animals and humans. Four carotenoid compounds are predominant in maize grain: â-carotene, â-cryptoxanthin, zeaxanthin and lutein. The present study pertains to an analysis of 24 maize inbred lines out of an association mapping panel for nucleotide diversity in the Yellow1 (Y1) gene which regulates an important step in carotenoid biosynthesis in maize kernels. The 5' untranslated region (UTR) of the Y1 gene was specifically targeted for study since an earlier analysis had revealed that most of the variation associated with this gene is likely due to cis-acting regulatory sequences. Three gene-specific primer pairs for PCR were designed using PRIMER3 software based on the gene sequence available in the public domain. The PCR products were purified and quantified prior to DNA sequencing. Sequence ambiguities, if any, were resolved by repeated sequencing (at least three replicates) from both ends. All the sequences were analyzed using different programs of Bioedit software, leading to identification of several SNPs and indels. A total of 15 sequence variations have been found in the present study with a frequency of 1 SNP per 87 bp. Haplotyping and intensive phenotyping of the selected inbred lines is in progress for identification of favorable allelic variants for carotenoid biosynthesis in maize.

Key words: Maize, Y1 gene, allele mining, β-carotene, Vitamin A

Introduction

Vitamin A is an important micronutrient and plays a vital role in human metabolism and health. Vitamin A deficiency affects over 250 million people, leading to susceptibility to infection, night blindness, rough and scaly skin, depressed immune system, diminished teeth and bone development (Underwood 2000). Adult males and females between 25 and 50 years of age require 1000 μg and 800 μg of vitamin A respectively in their daily diet (FAO/WHO 2000). This requirement cannot be fulfilled by synthetic vitamin A. Studies have indicated the ineffectiveness of high doses of synthetic â-carotene over natural carotenoids (van den Berg *et al*. 2000). Therefore, there is an utmost need to enhance vitamin A levels in the staple food crops in order to facilitate easy and efficient access to them in their natural form thereby meeting the daily metabolic micronutrient requirements of consumers.

Plant carotenoids are fat-soluble antioxidant vitamin compounds with two major classes: (i) carotene and (ii) xanthophylls. á-carotene and â-carotene come fall in the first class which contains carbon and hydrogen. Xanthophylls on the other hand contain oxygen. Lutein, β-cryptoxanthin and zeaxanthin are the major components of this class. β-carotene produces two molecules of vitamin A after digestion in the human body. The carotenoid biosynthesis pathway has been well characterized in the plant system involving many genes.

Several million people in developing countries in Asia, Africa and Latin America consume maize as an important staple food and derive their protein and calorific requirements from it (FAO 2004). White-kernel maize is devoid of â-carotene, and therefore, not desirable from the viewpoint of vitamin A content. The yellow/orange kernel type of maize genotypes vary considerably in terms of total carotenoid as well as â-carotene content, suggesting the possibility of selecting the most efficient *Y1* allele for breeding programs. Buckner *et al*. (1990) mapped and cloned the *yellow1/Y1* gene in maize in bin location 6.02 and demonstrated the relationship between the *Y1* gene and phytoene synthase enzymes (Buckner *et al*. 1996), which converts two molecules of geranylgeranyl pyrophosphate (GGPP) to phytoene in the first step of the carotenoid biosynthesis pathway, signifying the importance of the *Y1* gene in the pathway. The present study was undertaken to identify the sequence variation (SNP, indel) in the *Y1* gene among a set of maize genotypes varying in total carotenoid and β-carotene content with the ultimate aim of identifying favorable allele(s) for *Y1* gene with respect to â-carotene production in maize.

Materials and Method

An association mapping panel of 100 inbred lines has been formed at the Maize Genetics Unit of IARI in New Delhi for allele mining of the *Y1* gene. From this panel, a set of 24 lines were selected initially to optimize the procedure for analysis of allelic variation in the *Y1* gene. These 24 maize inbred lines included 13 lines from CIMMYT (HarvestPlus series, designated as HP lines) and 11 inbred lines developed at different maize breeding centers in India. Of the Indian lines, five were Quality Protein Maize (QPM) inbreds from the Directorate of Maize Research, New Delhi (designated as DMRQPM); two lines were from the Punjab Agricultural University (PAU), Ludhiana (designated as LM lines); two were from VPKAS, Almora (designated as V line), and one line each from Bajaura Centre, CSK-HPKV, Palampur (Bajim-2) and All India Coordinated Maize Improvement Programme (designated as CM lines). These lines were selected primarily on the basis of their kernel color, showing wide variation in the carotenoid level starting from deep yellow or orange kernel color to pale yellow and white kernel (Fig. 1). HP-10, a white-kernel inbred line, was taken as control.

Table 1. Primer pairs, their positions and amplicons size used in the 5' UTR region of the *Y1* gene.

S. No.	Primer pairs	Amplified region of 5' UTR (bp)	Amplicon size (bp)
1.	MGUY1a (F+R)	2 – 571	~570
2.	MGUY1b (F+R)	548 – 992	~445
3.	MGUY1c (F+R)	965 – 1336	~371

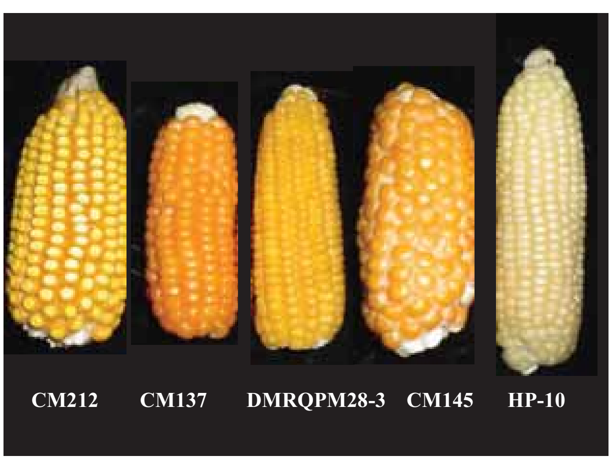

Figure 1. Variation in kernel color among the selected set of maize inbred lines.

The *Yellow1/Y1* sequence (Accession no. 32636) was used to design primer pairs for the 5' untranslated region (UTR) of this gene and PRIMER3 software was used to design a primer set, each resulting in an amplification product of 400-600 bp in the 5' UTR region of the *Yellow1* gene. Three primer pairs, MGUY1a, MGUY1b and MGUY1c, covering a ~1300 bp region of 5'UTR (Table 1), were used in the study. PCR optimization was done for each of the three primer pairs using different combinations of DNA concentration, annealing temperature, PCR program and other relevant components. Each genotype was amplified in 57.5 µL of PCR reaction volume in at least six replicates for purification. 1% agarose gel was used to check the size and concentration of amplicons before purification. A Qiagen gel extraction kit was used for purification of PCR amplicons and resolved again on 1% agarose gel before sequencing. Purified PCR amplicons of each genotype were sequenced in three replicates from both ends using forward and reverse primers to minimize any sequencing errors. Cap contig assembly program of Bioedit software was used to develop a consensus sequence from three replicates for each genotype. The consensus sequence of each genotype with MGUY1a, MGUY1b and MGUY1c was again subjected to the cap contig assembly program to get one final sequence which covers ~1.3 kb of 5' UTR of the *Y1* gene. These final ~1.3 kb sequences of all 24 inbred lines were analyzed using ClustalW program of Bioedit software to detect any sequence variation (base substitution/indel). The strategy followed in the present study is depicted in Figure 2.

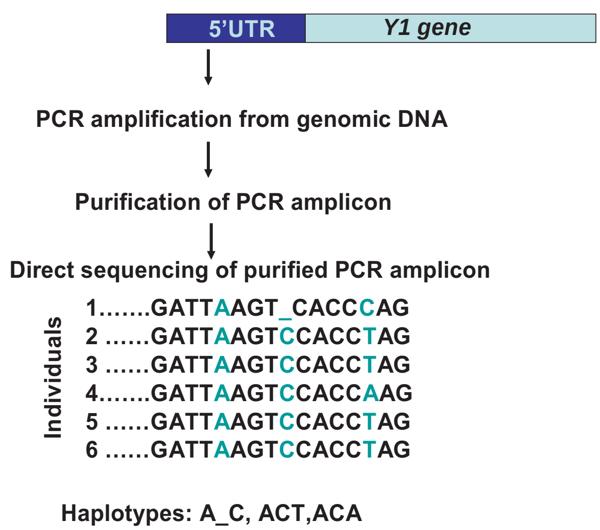

Figure 2. Strategy used for analysis of nucleotide polymorphism in the 5' UTR of the *Y1* gene.

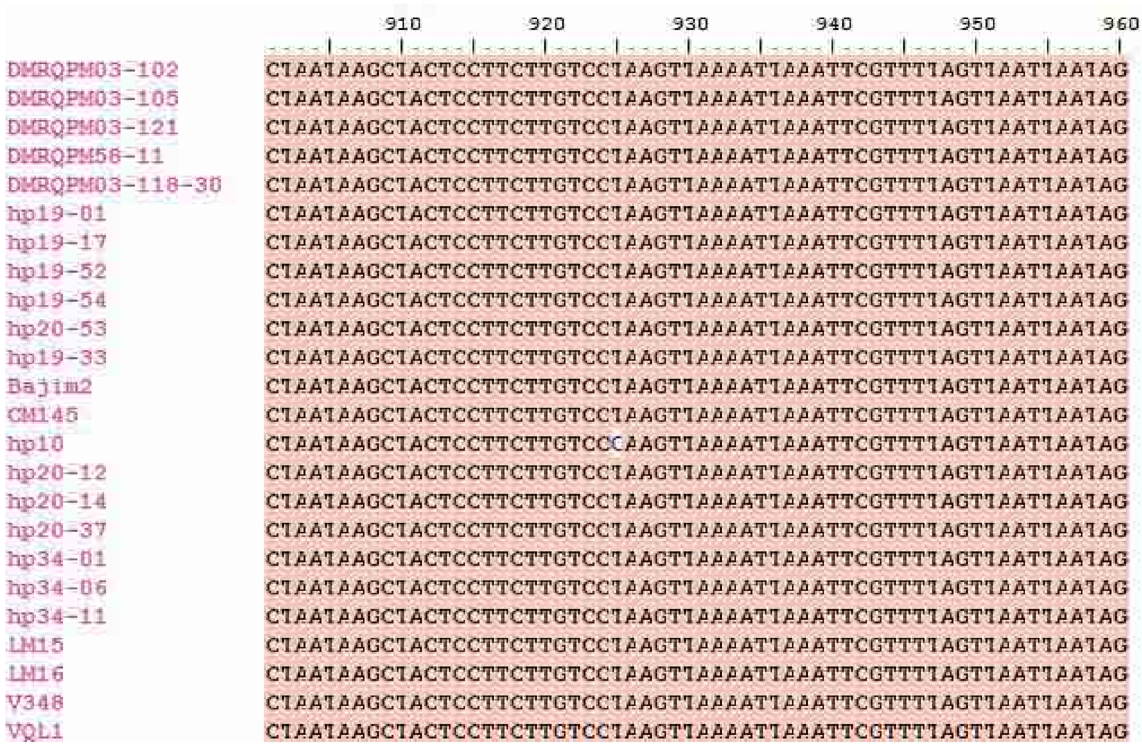

Figure 3. ClustalW analysis showing mononucleotide transition in one of the inbreds.

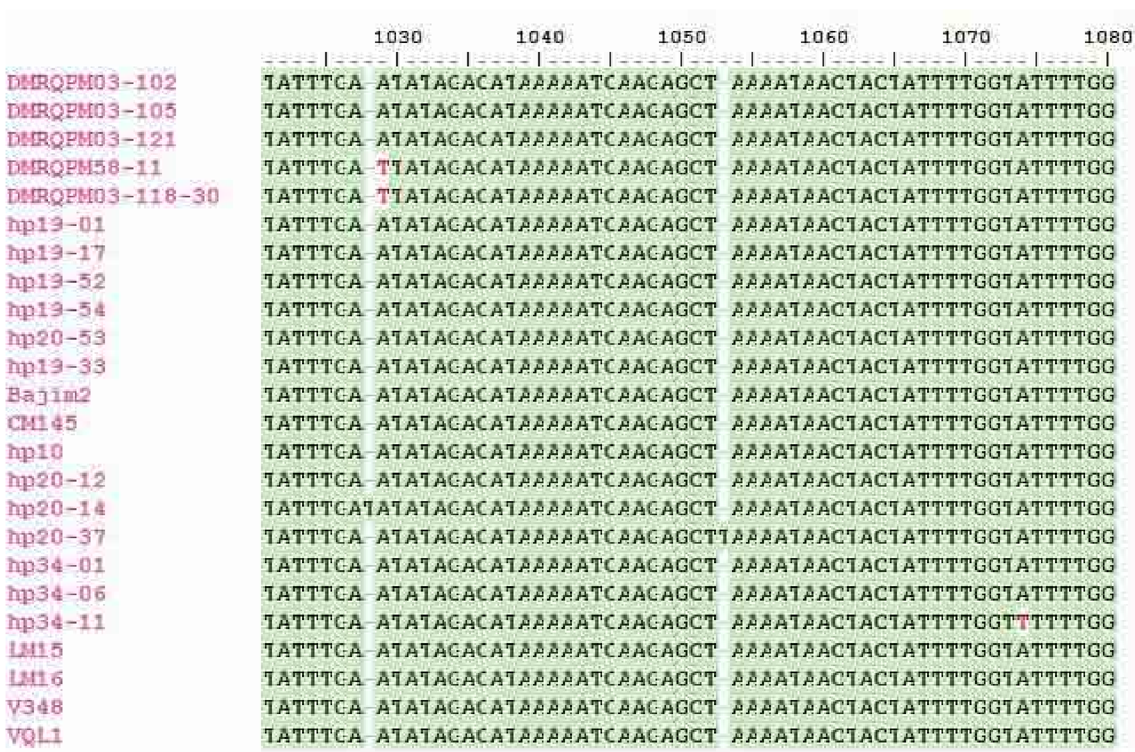

Figure 4. ClustalW analysis showing mononucleotide indel and transversion.

Results and Discussion

The study led to the detection of a total of 15 sequence variations, including 9 insertions/deletions (indels) of varying sizes, and 6 base substitutions (transitions/ transversions) with a frequency of 1 SNP per 87 bp (Fig. 3; Fig. 4). Out of the 9 indels, seven were single bp in length, one was of two bp and one of 14 bp. The MGUY1a primer set covered the 2-571 bp region, while MGUY1c covered the ~371 bp region from 965-1336 base pair of the 5' UTR of the *Y1* gene.

Primer pair MGUY1a revealed one indel of two bp length, while it did not show any base substitution in the given set of genotypes. Analyses with the MGUY1c primer pair led to identification of five indels of single base pair and one base pair transition at two different locations in the 5'UTR regions.

Primer pair MGUY1b, covering the 548-992 bp region of the 5' UTR of the *Y1* gene, amplified a ~445 bp sequence and showed one indel of 14 base pairs, two indels of a single base pair and four base substitutions with three transversions and one transition. Sequence analyses of this region revealed a 14-bp indel, detectable in 2% agarose gel (Fig. 5). This indel polymorphism effectively differentiates HP10, a white kernel line from the yellow/ orange colored lines. Palaisa *et al*. (2003) also reported the 14bp indel in a similar region of the *Y1* gene while analyzing a different set of maize genotypes. Validation of this indel is in progress with analysis of more number of white-kernel lines.

Of the three primer sets used, MGUY1b was found to be relatively more informative, revealing different kinds of sequence variations, namely, insertion-deletions, transitions and transversions. Thorup *et al*. (2000) also reported most of the nucleotide variations in the *cis*-acting regulatory sequences of the gene rather than in the coding regions. Palaisa *et al*. (2003) also reported a similar trend with the detection of 85 single nucleotide polymorphisms (SNPs) in the noncoding region and only 21 in the coding region of the *Y1* gene, supporting the hypothesis that noncoding regions are more sensitive for sequence variation than coding regions.

Presently, sequencing of the 5' UTR region of the *Y1* gene of the entire association mapping panel consisting of 100 maize inbred lines is under progress. Haplotyping and carotenoid profiling by the HPLC method is also under progress to detect favorable allelic variant(s) for the *Y1* gene.

Acknowledgement

The study was financially supported by the ICAR Network Project on Transgenics in Crops [Sub-project: Maize Functional Genomics] awarded to BMP.

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Genetic Variability for Kernel Iron and Zinc Densities in Selected HarvestPlus and Indian Maize Genotypes

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Abstract. This study was aimed at evaluation of kernel iron (Fe) and zinc (Zn) concentrations in a set of 72 maize genotypes from diverse agroecologies in India, besides CIMMYT, Mexico. A set of 22 selected genotypes were analyzed to understand the stability of kernel iron and zinc concentrations in three different environments, namely kharif (monsoon season) 2006 and 2007 at New Delhi, and rabi (winter season) at Hyderabad. The study revealed significant variation for the target traits among the genotypes. Kernel iron content among the 72 genotypes analyzed during kharif 2006 varied from 22.92 mg kg^{-1} to 49.85 mg kg^{-1}, while the range for kernel Zn was found to be from 14.48 mg kg^{-1} to 37.58 mg kg^{-1}. The genotype BAJIM-06-7 was found to have the highest mean of 55.56 mg kg^{-1} of kernel iron content across the three environments. V348 (47.96 mg kg^{-1}), IML273 (44.15 mg kg^{-1}), IML403 (43.79 mg kg^{-1}) and BAJIM-06-14 (43.08 mg kg^{-1}) were the other promising genotypes. For kernel zinc content, BAJIM-06-14 (41.37 mg kg^{-1}) was the best performing genotype in all the three environments. VQL1 (38.77 mg kg^{-1}), BAJIM-06-12 (35.07 mg kg^{-1}), IML119 (34.13 mg kg^{-1}) and BAJIM-06-10 (34.13 mg kg^{-1}) were the other promising genotypes for kernel zinc content. Considering both traits, BAJIM-06-6 and BAJIM-06-14 were the most promising. No strong correlation between the kernel iron and zinc contents was found, indicating the need for independent selection for enhancing the concentration for these traits. Stability analysis revealed the significant role of the environment and G × E interaction in determining both kernel iron and zinc content. V348 was found to be the most stable genotype for kernel Zn content with 30.15 mg kg^{-1}, 33.61 mg kg^{-1} and 30.77 mg kg^{-1} during kharif 2006, rabi 2006/07 and kharif 2007, respectively. IML273 and IML288 were the stable genotypes for kernel iron content. SSR polymorphisms grouped 24 and 22 selected inbred lines from diverse sources into three and four major clusters, respectively, indicating the genetic diversity of the inbred lines analyzed.

Key words: Maize, kernel iron, kernel zinc, genetic variability

Introduction

Malnutrition, often called "hidden hunger", has become an alarming problem in the developing world with an estimated three billion people afflicted with micronutrient-related malnutrition (WHO 1999). Further, two-thirds of all deaths among children are due to micronutrient deficiencies (Welch and Graham 2004). Billions of people worldwide are suffering from iron-related deficiencies such as anemia, goiter and eye problems along with profound effects on cognitive development, growth, reproductive performance and work productivity (Bouis 2002). Cichy *et al.* (2005) reported that 49% of the world's population is at risk due to low zinc intake, leading to anorexia, depression and psychosis, impaired growth and development, altered reproductive biology, gastrointestinal problems and impaired immunity (Solomons 2003).

Maize is the staple food for more than 1.2 billion people in the world and the most important cereal crop after wheat and rice. More than 50% of the world's cultivated land is devoted to wheat, rice and maize, and by 2020, almost 96% of the world's rice consumption, two-thirds of its wheat consumption and almost 60% of its maize population will be in the developing countries (Rosegrant *et al.* 1999). Medical supplements and fortification of food products have been tried in several countries for decades to ameliorate the devastating problem of 'hidden hunger' (Maberly *et. al.* 1994; Underwood 2000). However, these measures have their own limitations. Therefore, developing micronutrient-enriched staple plant foods either through plant breeding methods or via molecular biological techniques holds promise for sustainable food-based solutions (Banziger and Long 2000; Graham *et al.* 2001; Pfeiffer and McClafferty 2007). Therefore, the present study was undertaken to ascertain the genetic variability and stability of kernel iron and zinc densities in selected maize germplasm.

Materials and Method

A set of 72 maize genotypes, including 41 elite inbred lines developed at various breeding centers in India, 10 lines from the HarvestPlus (HP) Program, 3 CIMMYT Maize Lines (CMLs) and 18 Indian Maize Landraces (IMLs), were analyzed for kernel iron and zinc content at Delhi during the *kharif* (monsoon) season of 2006. A selected set of 22 genotypes were further analyzed for stability of kernel micronutrients across three environments during *kharif* 2006 at Delhi, *rabi* (winter) season of 2006/07 at Delhi and *kharif* 2006 at Delhi. The trials in Delhi were evaluated at IARI Experimental Farm during the *kharif* season, while the trial at Hyderabad was grown at the Maize Winter Nursery, Amberpet, Hyderabad, during the *rabi* season.

The maize genotypes were planted in an RCBD with three replications per entry and one row per replication with plant-to-plant spacing of 25 cm and row-to-row spacing of 75 cm. Standard agronomic practices were followed for raising and maintenance of the plants. After kernel maturation and plant drydown, ears with the husk were hand harvested and dried under the sun to lower the postharvest grain moisture content to 14%. The soil at the Delhi experimental location was a basically deep, well-drained, light alluvium which has been classified as Typic Haplustept belonging to the Mehrauli series, while the soil type at the Hyderabad location was a red sandy soil with low organic carbon, nitrogen and phosphorus. The ears were shelled carefully and the grain were bulked for each of the genotypes. Representative grain samples were collected by the ‘quartering method’ and individual dried grain samples were ground into a fine powder in an aluminum mill (1093 Cyclotec Sample mill, Hooganas, Sweden). Triplicate samples (1 g) were digested using standard protocol. The concentrations of trace minerals including iron and zinc in the samples were analyzed using an Atomic Absorption Spectrophotometer (AAS) (AAS-Perkin Elmer) at the Nuclear Research Laboratory, IARI, New Delhi. Kernel micronutrient data were analyzed for analyses of variance (ANOVA) using the SAS-6.12 program, while the Eberhart and Russel model of stability analyses was carried out using Windostat software.

Analysis of microsatellite/SSR polymorphisms was done in two sets of selected lines: (i) 24 lines including 10 BAJIM lines, 9 CM lines, 4 V lines and 1 H+ line using 50 SSR markers; and (ii) 22 lines including 14 H+ lines and 8 CM lines using 53 SSR markers, covering the entire maize genome. The SSR primers were synthesized from Research Genetics Inc., USA, based on primer sequence information available in the public domain. SSR analysis was carried out using the protocol standardized at the Maize Genetics Unit, IARI, New Delhi. The SSR data was analyzed using NYSYS-pc v2.11.

Results and Discussion

ANOVA revealed significant variation for both kernel Fe and Zn contents among the maize genotypes, indicating the presence of considerable genetic variability for the target traits. Kernel Fe content varied from 22.92 mg kg^{-1} to 49.85 mg kg^{-1} during *kharif* 2006, while the range for kernel Zn was 14.48 mg kg^{-1} to 37.58 mg kg^{-1}, which was a 2-4 fold increase over the poorest performing genotype. Banziger and Long (2000) also reported a kernel Fe range of 9.6 mg kg^{-1} to 63.2 mg kg^{-1}, and 12.9 mg kg^{-1} to 57.6 mg kg^{-1} for kernel Zn while undertaking 13 trials involving 1400 improved maize genotypes and 400 landraces at different locations in Zimbabwe and Mexico.

Among the 72 maize germplasm studied during *kharif* 2006, HP3 was found to be highly promising for kernel Fe content with 49.85 mg kg^{-1}. Other promising genotypes for kernel Fe content include V336 (49.67 mg kg^{-1}), BAJIM-06-1 (48.90 mg kg^{-1}), CML 354 (47.80 mg kg^{-1}) and CML356 (47.36 mg kg^{-1}). V340 (37.58 mg kg^{-1}), BAJIM-06-14 (35.41 mg kg^{-1}), V334 (35.19 mg kg^{-1}), IML119 (35.00 mg kg^{-1}) and IML303 (33.29 mg kg^{-1}) performed well for kernel Zn content. No correlation was found between kernel iron and zinc, indicating the need for independent selection for enhancing the concentration for these traits. Considering the common set of 22 genotypes evaluated for across environments, BAJIM-06-7 had the highest mean of 55.56 mg kg^{-1} kernel Fe content, with V348 (47.96 mg kg^{-1}), IML273 (44.15 mg kg^{-1}), IML403 (43.79 mg kg^{-1}) and BAJIM-06-14 (43.08 mg kg^{-1}) being the other promising genotypes (Table 1). For kernel Zn content, BAJIM-06-14 (41.37 mg kg^{-1}) was the promising in all the three environments. Among the other genotypes, VQL1 (38.77 mg kg^{-1}), BAJIM-06-12 (35.07 mg kg^{-1}), IML119 (34.13 mg kg^{-1}) and BAJIM-06-10 (34.13 mg kg^{-1}) were promising genotypes for kernel Zn content (Table 2). BAJIM-06-6 and BAJIM-06-14 were the most promising inbred lines for both kernel Fe and Zn contents.

Analysis by the Eberhart and Russel model revealed that the environment played a significant role in determining the kernel Fe and Zn contents (Table 3). Among the three environments, Hyderabad (*rabi* 2006/07) had the highest environmental index for both traits. The study also revealed significant G × E interaction for both kernel Fe and Zn contents. Banziger and Long (2000) and Oikeh *et*

Table 1. Kernel iron (Fe) content of the top 10 inbred lines/IMLs evaluated at Delhi (*kharif* 2006 and 2007) and Hyderabad (*rabi* 2006/07).

S.No.	Delhi *kharif* [1]2006		Hyderabad *rabi*[2] 2006/07		Delhi *kharif* 2007		Mean	
	Inbred/IML	Fe (mg kg^{-1})	Inbred/IML	Fe (mg kg^{-1})	Inbred/IML	Fe (mg kg^{-1})	Inbred/IML	Fe (mg kg^{-1})
1	V348	47.33	B[3]-06-7	79.40	V348	44.69	B-06-7	55.56
2	B-06-14	45.68	CM152	67.11	IML288	44.25	V348	47.96
3	IML273	44.35	IML403	66.80	B-06-14	36.97	IML273	44.15
4	IML288	44.25	CM145	64.08	IML273	36.87	IML403	43.79
5	B-06-6	42.25	B-06-10	63.51	IML119	36.67	B-06-14	43.08
6	IML119	39.34	B-06-6	60.13	B-06-7	36.23	IML288	41.41
7	IML403	37.97	B-06-5	54.58	V334	35.92	CM152	40.78
8	B-06-7	35.93	CM212	54.31	B-06-13	32.83	CM145	40.23
9	IML390	34.72	V348	51.87	VQL1	31.47	IML119	39.82
10	IML312	34.09	IML273	51.22	IML185	29.30	B-06-6	39.30
Mean[4]	-	34.96	-	51.91	-	27.83	-	-
CD (5%)[4]	-	2.65	-	6.20	-	4.32	-	-

[1.] Kharif = Monsoon crop.
[2.] Rabi = Winter crop.
[3.] B= BAJIM
[4.] Data taken from all the 22 genotypes.

Table 2. Kernel zinc (Zn) content of the top 10 inbred lines/IMLs evaluated at Delhi (*kharif* 2006 and 2007) and Hyderabad (*rabi* 2006/07).

S.No.	Delhi *kharif* [1]2006		Hyderabad *rabi*[2] 2006/07		Delhi *kharif* 2007		Mean	
	Inbred/IML	Zn (mg kg^{-1})	Inbred/IML	Zn (mg kg^{-1})	Inbred/IML	Zn (mg kg^{-1})	Inbred/IML	Zn (mg kg^{-1})
1	B[3]-06-14	35.41	B-06-14	53.37	VQL1	54.36	B-06-14	41.37
2	V334	35.19	IML312	53.10	B-06-10	49.87	VQL1	38.77
3	IML119	35.00	IML403	46.80	B-06-12	38.40	B-06-12	35.07
4	B-06-12	32.07	IML185	44.62	B-06-6	38.17	IML119	34.13
5	V348	30.15	VQL1	42.90	B-06-7	37.97	B-06-10	34.13
6	IML312	28.88	IML119	42.33	V334	37.23	V334	33.42
7	IML185	28.25	CM152	42.10	CM212	37.20	B-06-6	33.08
8	IML288	27.75	IML390	42.03	B-06-14	35.33	IML312	32.85
9	CM152	26.74	IML273	40.97	B-06-13	34.83	B-06-7	32.15
10	IML273	24.74	B-06-6	39.49	IML288	33.90	CM152	31.73
Mean[4]	-	25.40	-	38.15	-	30.24	-	-
CD (5%)[4]	-	2.92	-	2.38	-	3.69	-	-

[1.] Kharif = Monsoon crop.
[2.] Rabi = Winter crop.
[3.] B= BAJIM
[4.] Data taken from all the 22 genotypes.

al. (2003) also reported the significant role of the environment for both kernel Fe and Zn contents. Based on the mean performance, regression value and deviation from linearity, V348 was the most stable genotype for kernel Zn content with 30.15 mg kg^{-1}, 33.61 mg kg^{-1} and 30.77 mg kg^{-1} during *kharif* 2006, *rabi* 2006/07 and *kharif* 2007, respectively. IML273 and IML288 were the most stable genotypes for kernel Fe content. V348 and IML119 also performed well in relatively poor environments for kernel Fe content.

SSR polymorphisms using 50 polymorphic markers grouped a first set of 24 selected inbred lines from diverse sources into three major clusters with all the four VPKAS inbred lines (V lines) in cluster I and all the Bajaura inbred lines (BAJIM) except BAJIM-06-3 together in cluster II (Fig. 1). The majority of CM lines were grouped in Cluster III along with HP1. Analyses with a second set of 22 genotypes using 53 polymorphic primers revealed that the selected inbred lines were grouped into four distinct clusters, indicating the genetically diverse nature of inbred lines selected in the present study (Fig. 2). HP3, HP4 and

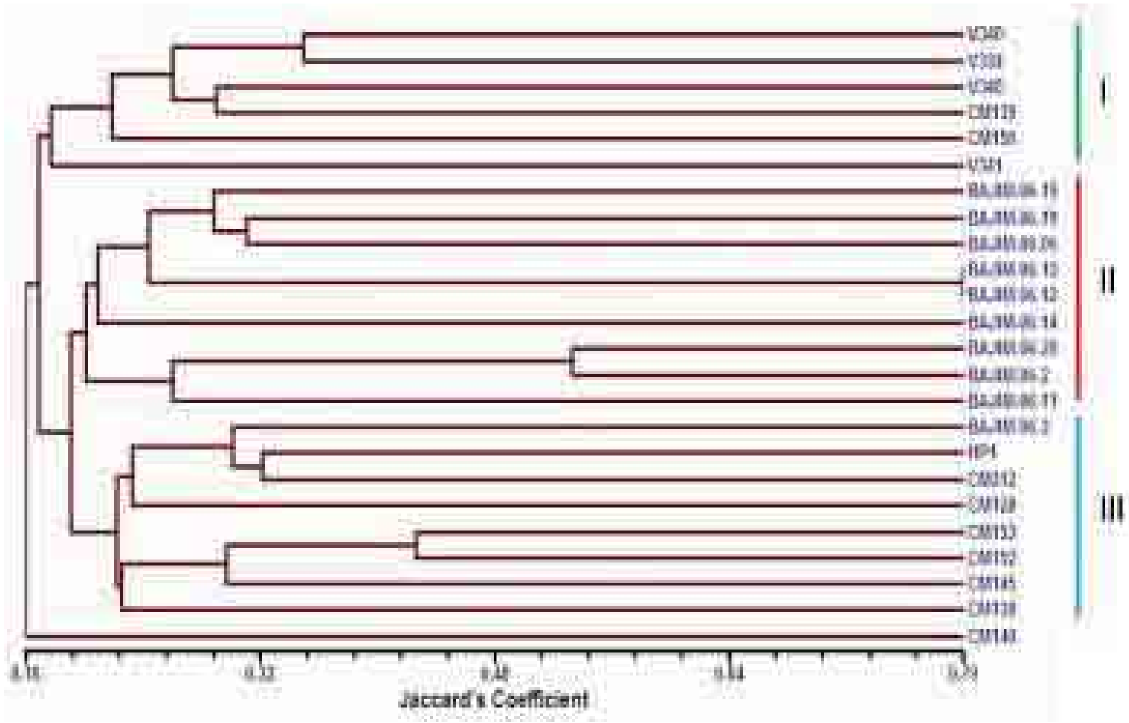

Figure 1. Dendrogram depicting the genetic relationship among 24 selected inbred lines using SSR data.

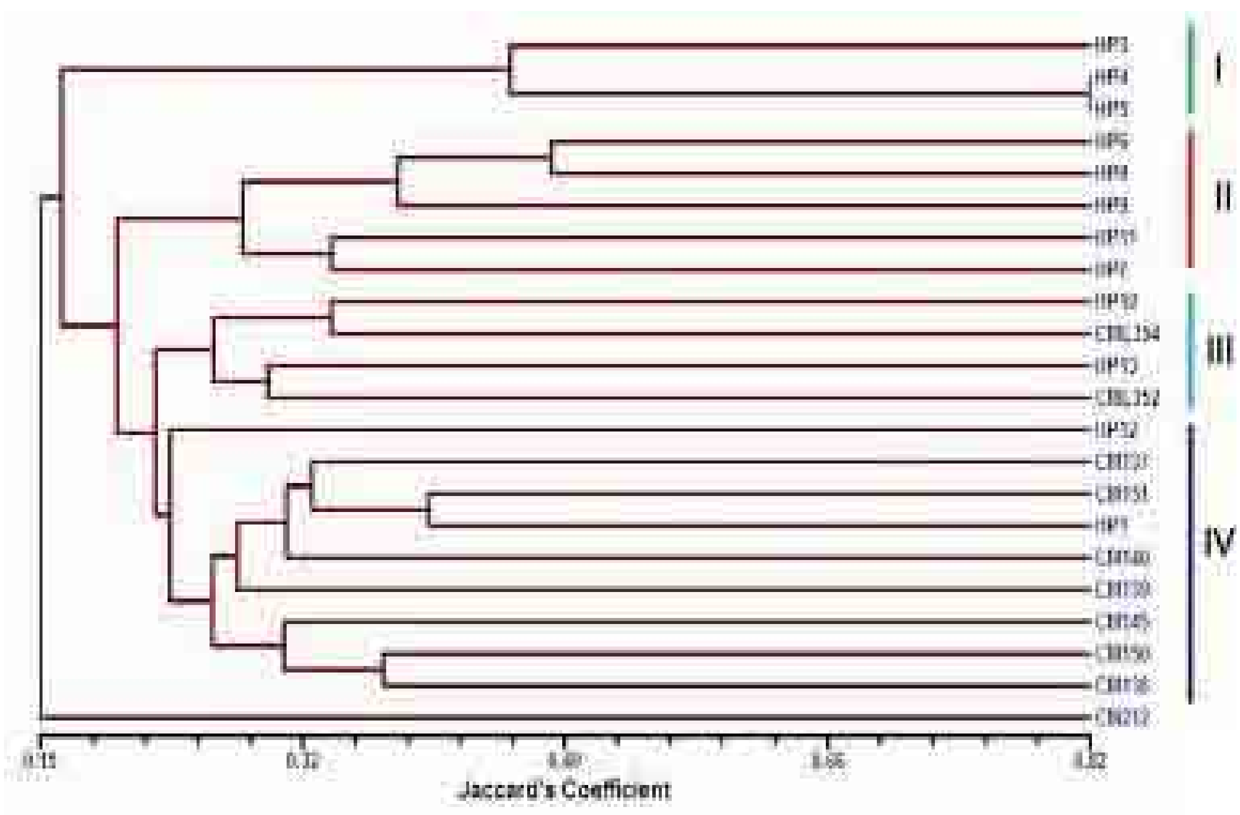

Figure 2. Dendrogram depicting the genetic relationship among 22 selected inbred lines using SSR data.

Table 3. ANOVA for stability analysis of target traits.

Source of variation	Df	MSS for kernel Zn content	MSS for kernel Fe content
Replication within env.	6	0.16	7.27*
Genotypes	21	51.30**	85.85**
Env. + (Gen. × Env.)	44	114.90**	229.03**
Environments	2	911.59**	3366.95**
Gen. × Env.	42	76.97**	79.61**
Environments (Lin.)	1	1823.17**	6733.90**
Gen. × Env. (Lin.)	21	43.0.43**	133.80**
Pooled Deviation	22	105.85**	24.27**
Pooled error	126	1.14	2.62
Total	65	94.36	182.77

HP5 were in cluster I and HP6, HP7, HP8, HP9 and HP11 in cluster II, showing the preponderance of HarvestPlus lines in clusters I and II. Most of the Indian maize inbred lines were in cluster IV. This phenotypic data, in combination with an understanding of the genetic relationships (based on SSR analyses), may be useful in generating suitable mapping populations for QTL analyses of kernel micronutrient content in maize.

Acknowledgement

This study was undertaken through the Department of Biotechnology (DBT) Network Project on Maize Biofortification. Our sincere thanks to HarvestPlus for sharing the germplasm.

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Conversion of Elite Maize Lines into QPM Versions Using Integrated Phenotypic and Molecular Marker-Assisted Selection

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Abstract. An integrated strategy of phenotypic selection for endosperm modifiers and molecular marker-assisted foreground and background selection has been adapted in this study for conversion of six elite maize inbreds in India, namely CM137, CM138, CM139, CM140, CM150 and CM151, into QPM versions. The donor lines used were CML161, DMRQPM-03-124 and DMRQPM-58. All the parental lines were first analyzed using 100 polymorphic microsatellite/SSR markers, which revealed high genetic divergence among the selected lines. Foreground selection was carried out on BC_1F_1, BC_2F_1 and BC_2F_2 progenies using opaque2 specific umc1066 and phi057 SSR markers. Nearly 30 polymorphic SSR markers covering the maize genome were employed in background selection for identification of BC_1F_1 and BC_2F_1 individuals with the highest recovery of recurrent parent genomic background. Phenotypic selection for endosperm modifiers was carried out (using the standard light box test) on the backcross progenies in each generation for recovery of segregants with good kernel texture. Biochemical analysis for total endosperm protein content and tryptophan content percentage was undertaken using standard procedures on the BC_2F_2 progenies for identification of promising o2/o2 genotypes with high nutritional value and proper kernel texture. During the winter season of 2007/08, the opaque2 homozygotes from the BC_2F_2 populations, identified using MAS, were further selfed to generate BC_2F_3 families. Further, these BC--$_2F_3$ was analyzed for endosperm protein and tryptophan content and selected genotypes were used to generate BC_2F_4. The study led to the conversion of elite maize lines that are parents of three popular single-cross hybrids (PEHM2, PEEHM5 and Parkash) into QPM versions.

Key words: Maize, QPM, MAS, endosperm modifiers, tryptophan, SSR markers

Introduction

With its high content of carbohydrates, fats, proteins and some of the important vitamins and minerals, maize has acquired a well-deserved reputation as a 'poor man's nutricereal' (Prasanna *et al.* 2001). The average protein content of common maize is about 9-10% and is inherently deficient in two essential amino acids, lysine and tryptophan (FAO 1985). The significant breakthrough came in 1964 when Mertz and his coworkers at Purdue University, USA, established the nutritional superiority of *opaque2* mutant with an enhanced concentration of lysine and tryptophan (Mertz *et al.* 1964). This finding provided an opportunity for breeding new cultivars with high lysine protein (Bjarnason and Vasal 1992; Prasanna *et al.* 2001). CIMMYT breeders successfully combined the high lysine potential of *opaque2* with genetic endosperm modifiers, releasing new maize genotypes which are collectively referred to as Quality Protein Maize (QPM) (Vasal *et al.* 1993; Prasanna *et al.* 2001). In view of the growing importance of QPM in India and to hasten the pace of progress of QPM cultivar development, it is important to develop more heterotic combinations with improved nutritional quality and kernel texture. In this direction, conversion of nonQPM hybrid combinations into QPM versions is an important viable option due to its quicker development, similar and tested heterotic potential, combining ability and adaptability of existing nonQPM hybrids.

Rapid advances in genome research and molecular technology have led to the use of molecular marker-assisted selection (MAS) in enhancing selection efficiency and expediting the development of new cultivars with higher yield potential (Ribaut and Hoisington 1998; Babu *et al.* 2004). The availability of *opaque2*-specific SSR markers offers an excellent opportunity for undertaking foreground selection, followed by marker-assisted background selection for rapid recovery of the recurrent parent genome in a backcross breeding program (Young and Tanksley 1989; Hospital *et al.* 1992; Babu *et al.* 2005). The present study, therefore, was undertaken to convert parental lines of three nonQPM single-cross hybrids into QPM versions using an integrated strategy of phenotypic selection for endosperm modifiers and grain quality attributes and molecular marker-assisted foreground and background selection.

Material and Methods

A set of six elite nonQPM maize lines, namely CM137 and CM138 (parental lines of PEHM-2), CM139 and CM140 (parental lines of Parkash), CM150 and CM151 (parental lines of PEEHM-5) were used as the recurrent parents, while DMRQPM-58, DMRQPM-03-124 and CML161 were used as the donor lines in the present study. In each of the two respective recurrent parents of a hybrid combination, two different donor combinations were used to facilitate better complementarity between the converted lines. The recurrent parents were crossed with their respective donors at the Maize Winter Nursery, Amberpet, Hyderabad during *the rabi* (winter season) of 2005/06, and each of the F_1 plants were backcrossed to their respective recurrent parents at the IARI Experimental Farm, New Delhi during *kharif* (monsoon) 2006. BC_1F_1 generations were further raised at the Hyderabad nursery during *rabi* 2006/07. The strategy used in the present study has been depicted in Figure 1.

DNA was extracted from all the nine parental lines for SSR polymorphisms using nearly 100 SSR polymorphic markers. Foreground selection in the backcross populations was carried out using *opaque2* gene-specific SSR markers. Thirty polymorphic SSR markers (on an average ~3 markers per chromosome) were used for background selection. In addition, chromosome 7 was specially targeted in background selection to minimize linkage drag, as the *opaque2* gene is present at bin 7.02. SSR analysis was undertaken as per the procedure standardized at the Maize Genetics Unit, IARI, New Delhi (Bantte and Prasanna 2003).

Endosperm protein content was estimated using the Micro-Kjeldahl Procedure (AOAC 1965), while the tryptophan content was estimated at the Maize Quality Laboratory, DMR, New Delhi using the colorimetric method (Hernandez and Bates 1969). Kernel modification of the QPM progenies was rated using a procedure reported by Bjarnason and Vasal (1992). For analysis of endosperm modification, the backlit kernels were rated on a scale of 1-5, with 1 indicating 100% opaque; 2 indicating 75% opaque; 3 indicating 50% opaque; 4 indicating 25% opaque; and 5 indicating 100% normal (vitreous). Endosperm modification scores were derived based on analysis of 100 randomly chosen kernels from the ears of QPM inbreds.

Results and Discussion

SSR polymorphism with nearly 100 polymorphic SSR markers revealed the genetic diversity in the recurrent and donor parents. For foreground selection, a total of 1164

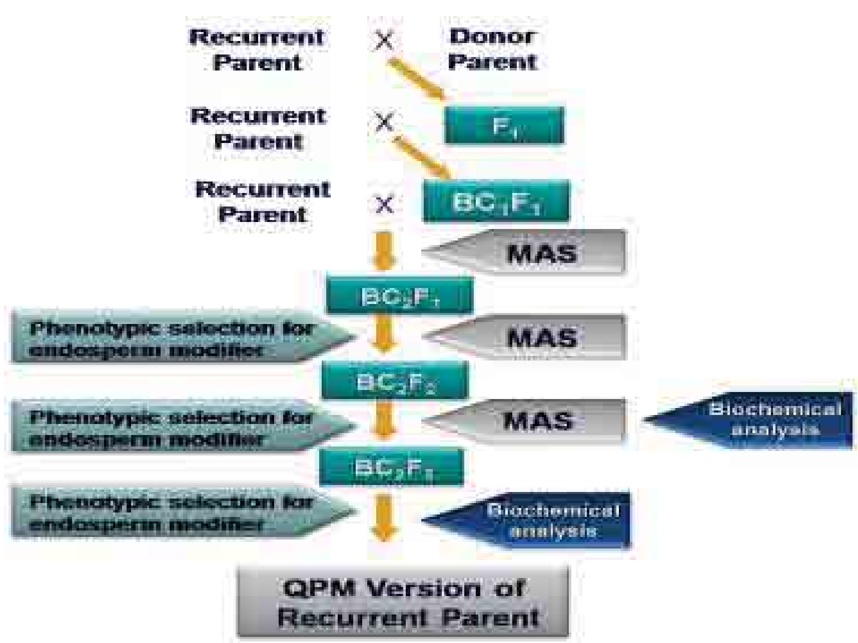

Figure 1. Strategy used for conversion of nonQPM lines into QPM versions.

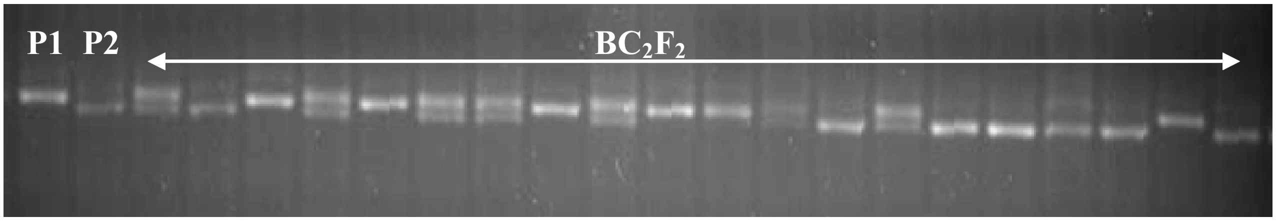

Figure 2. Foreground selection for o2/o2 homozygote in CM151-based BC_2F_2 population using *umc1066* marker.

Table 1. Number of individuals screened and heterozygotes found in each of the BC_1F_1 populations.

S.No.	BC_1F_1 population	No. of individuals screened	No. of heterozygotes selected
1.	CM137 × (CM137 × DMRQPM03-124)	176	41
2.	CM138 × (CM138 × CML161)	229	43
3.	CM139 × (CM139 × DMRQPM-58)	170	38
4.	CM140 × (CM140 × CML161)	188	14
5.	CM150 × (CM150 × CML161)	167	40
6.	CM151 × (CM151 × DMRQPM58)	234	81
	Total	994	219

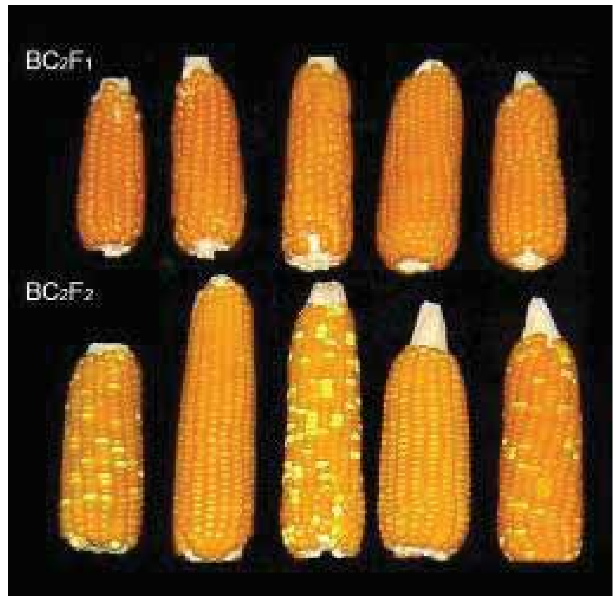

Figure 3. Comparison of kernel modification in BC_2F_1 and BC_2F_2 generations in CM137- based backcross-derived population.

individuals were screened in the BC_1F_1 population. The details of the number of individuals screened and heterozygotes identified in each population are presented in Table 1.

About 1655 individual plants were analyzed in BC_2F_1 populations for foreground selection and selected heterozygotes were further subjected to background selection. Families with a high recovery of the respective recurrent parent genome were selfed to get BC_2F_2 generations. Both *umc1066* and *phi057* were used to detect the heterozygosity status of the *opaque2* allele in both BC_1F_1, and BC_2F_1 generations, as well as the homozygotes in BC_2F_2 generations (Fig. 2).

Phenotypic selection on the basis of the ear phenotypes of each recurrent parent was carried out in order to further aid in the recovery of the recurrent parent phenotype. Kernel texture for each of the selected backcross ears was also estimated using the standard light box test. The backcross ears as expected showed varying degree of kernel modification (Fig. 3). Only ears with high resemblance to the respective recurrent parent and a high degree of kernel modification, in addition to the selection based on foreground and background selections, were finally selected for further advancement.

Grain quality analyses were carried out on all the six recurrent parents, with CM139 proving to be the best of them with 0.059% tryptophan in the endosperm, while CM150 had 0.036% of the amino acid in the endosperm (Table 2). While both CM137 and CM140 showed 0.039% endosperm tryptophan, CM151 and CM138 showed 0.037% and 0.043% endosperm tryptophan content respectively. Among the BC_2F_3 families, CM151-based progenies exhibited the highest mean percent endosperm tryptophan (0.095%), while both CM138- and CM140-based families showed 0.09% mean endosperm tryptophan. CM137, CM139 and CM150-based progenies exhibited 0.085%, 0.084% and 0.076% endosperm tryptophan content, respectively. These results indicate that the backcross-derived families are superior to the respective recurrent parents with a 2-2.5-fold increase in percent endosperm

Table 2. Percent endosperm tryptophan content in the recurrent parent and MAS-derived BC_2F_3 families.

S. No.	Recurrent parent	Percent tryptophan in endosperm of recurrent parent	Mean percentage of tryptophan in BC_2F_3 lines
1.	CM137	0.039	0.085
2.	CM138	0.043	0.090
3.	CM139	0.059	0.084
4.	CM140	0.039	0.090
5.	CM150	0.036	0.076
6.	CM151	0.037	0.095

tryptophan. Thus, an integrated approach of phenotypic and molecular marker-assisted selection aided in the conversion of six non-QPM inbred lines into QPM versions. Presently, these lines are in the BC_2F_4 stage and will be further tested for their heterosis and combining ability.

Acknowledgement

This study was undertaken as a part of the ICAR Network Project on Molecular Breeding [Sub-project: Marker-assisted selection for development of Quality Protein Maize]. We are grateful to the Directorate of Maize Research (New Delhi) and CIMMYT (Mexico) for providing the seed material for the donor parents used in this study.

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Optimization of Regeneration System of Some Maize (*Zea mays* L.) Varieties through Somatic Embryogenesis

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Abstract. Genetic variability of maize (Zea mays L.) can improved by in vitro culture. Transformation have big change to success by using somatic embryos because they come from a single cell. The aim of this experiment was to find a method to get embryogenic callus, somatic embryo, and plant regeneration of some maize varieties. The experiment was done at the Biology Cell and Tissue Laboratory of BB-Biogen Bogor, Indonesia in 2007. Four maize varieties (Antasena, Arjuna, Bisma and Sukmaraga) were used in this experiment. Young ears were harvested at 10, 12 and 14 days after anthesis. Explants were embryos from the young ear which were sterilized and isolated in laminar air flow. MS medium combined with 2,4-D (1 and 2 mg L^{-1}) + sucrose (3 and 12%) + manitol 1 % or prolin 4% were used as treatment for embryogenic callus induction. Combination of MS medium with BA (1, 2 and 3 mg L^{-1}) and IAA 0.5 mg L^{-1} were used as treatment for regeneration. The results of the experiment showed that of the four experimental varieties, Sukmaraga gave the best response to somatic embryogenesis using young ear explants which harvested at 10 days after anthesis (85.28%) with MS + 2,4-D 1 mg L^{-1} + sucrose 3% + manitol 1% as media for callus induction (81.67%) and MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1} as media for regeneration (6.07%), although Bisma gave the highest percentage of somatic shoot formation (7.98%).

Key words: Maize (*Zea mays* L.), somatic embryogenesis, regeneration, tissue culture

Introduction

In vitro culture is an alternative technology to improve varieties by improving genetic variability. Ahlowalia (1986) reported that *in vitro* culture can be employed for improving component variation that cannot be found in nature, thereby changing the characteristics of a cultivar. Che *et al*. (2005) found that regeneration of maize in tissue culture is important for production of transgenic corn and for crop improvement using genetic engineering approaches. Gene expression patterns were filed during somatic embryogenesis.

Plant improvement by *in vitro* culture or genetic engineering using somatic culture of embryos is preferred because the product comes from a single cell, has a higher probability of character change and avoids a chimera. Green and Phillips (1975) was the first researcher to regenerate maize in *in vitro* culture. Purnamaningsih (2002) reported that somatic embryogenesis has the capacity to produce a large number of propagules in a short time and speed up genetic engineering activities. For short- or long-term preservation, somatic embryos are ideal explants which can be regenerated into somatic shoots.

Some factors affect callus induction and plantlet development when young embryos are used as explants. Initiation, cell growth and success of regeneration in tissue culture depend on the genotype, part of the tissue, growth phase, culture medium and the environment of every phase (Phillips *et al.* 1988; Armstrong 1994).

The Murashige & Skoog (MS) medium is generally the basic medium used in tissue culture. In somatic embryogenesis, initiation often occurs in a medium containing auxins in high levels, especially 2,4-D (George and Sherrington 1984). Mariska (2003) reported the somatic embryogenesis of some soybean varieties with a high level of 2,4-D. Samaj *et al*. (2003) reported that a developmental switch from nonpolar preembryogenic units to polarized transition units in maize embryogenic calluses is caused by auxin deprivation in the culture medium. The switch is accompanied by cytoskeletal rearrangements in embryogenic cells.

The aim of our experiment was to optimize the method of producing embryogenic calluses, somatic embryos and plant regeneration of some maize varieties using young embryos as explants which can be used in *in vitro* selection or gene transformation in biotechnology.

Materials and Method

The experiment was conducted at the Biology Cell and Tissue Laboratory of BB-Biogen, Bogor in 2007. Four maize varieties (Antasena, Arjuna, Bisma and Sukmaraga) were used in this experiment, planted at the Cikeumeuh research facility in Bogor. Selfing was done for plants which had good vigor. Young ears were harvested at 10, 12 and 14 days after anthesis and then refrigerated until they were ready for use. The explants were embryos from young ears which were sterilized with 75% alcohol for 5 minutes, and isolated in laminar air flow. Ten embryos were planted in each bottle medium with 4 bottles per treatment. The treatments were media for embryogenic callus induction: MS + 2,4-D (1 mg L^{-1} and 2 mg L^{-1}) + sucrose (3% and 12%) + manitol 1% or prolin 4%. After embryogenic calluses were formed, they were subcultured in regeneration media: MS + BA (1, 2 and 3 mg L^{-1}) + IAA (0.5 mg L^{-1}). Observations were taken on the best time to harvest young ears as a source of explants to produce embryogenic calluses, the best variety to produce embryogenic calluses, the best media for callus induction, percentage of embryogenic callus formation, the best variety to regenerate calluses into somatic shoots, the best media for regeneration and percentage of shoot formation.

Results and Discussion

The results of the experiment showed that all the experimental varieties (Antasena, Arjuna, Bisma and Sukmaraga) could be used for producing embryogenic calluses at any harvesting time of young ears (10, 12 or 14 days after anthesis) (Fig. 1). Sukmaraga gave the best response for somatic embryogenesis with young-ear explants when harvested at 10 days after anthesis (85.28% embryogenic callus formation) (Table 1). Many researchers said have reported that young embryo are the best sources of explants to produce embryogenic calluses and for regeneration (Green and Phillips 1975; Freeling *et al.* 1976; Armstrong and Green 1985; Tomes and Smith 1985; Hodges *et al.* 1986; Tuberosa and Lucchese 1989). Green and Phillips (1975) and Finar (1998) also found that the age of the embryo, position in the medium and the composition of the medium are the main factors for the success of the somatic embryo method.

Embryogenic calluses visually are more friable than nonembryogenic calluses, with a globular structure on the surface (Fig. 2). Observations on the four combinations of media for embryogenic callus induction showed that MS + 2,4-D (1 mg L^{-1}) + sucrose 3% + manitol 1% was the best medium for embryogenic callus induction (81.67%) for the Sukmaraga variety (Table 2). This result was different from the findings reported by Almeida and Margarida (2000) that short-term tissue cultures were obtained on N6 medium

Table 1. Effect of variety and ear-harvesting time on embryogenic callus formation.

Variety	Days after anthesis	Embryogenic callus formation (%)
Antasena	10	52.08
	12	46.46
	14	46.67
Arjuna	10	38.19
	12	48.58
	14	63.95
Bisma	10	53.32
	12	52.29
	14	63.95
Sukmaraga	10	85.28
	12	82.87
	14	46.66

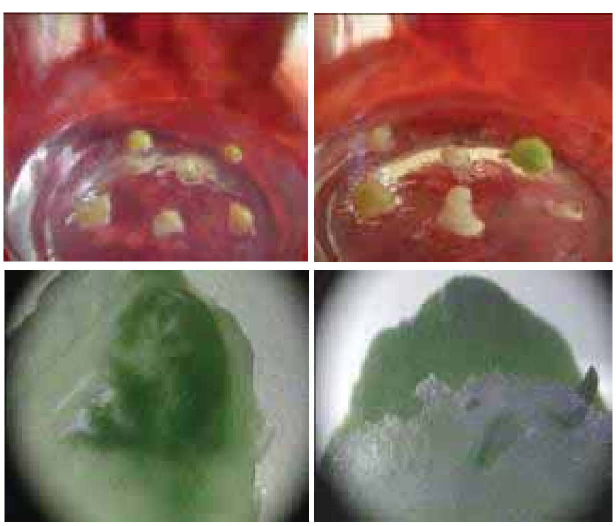

Figure 1. Embryogenic callus of maize and green somatic embryo.

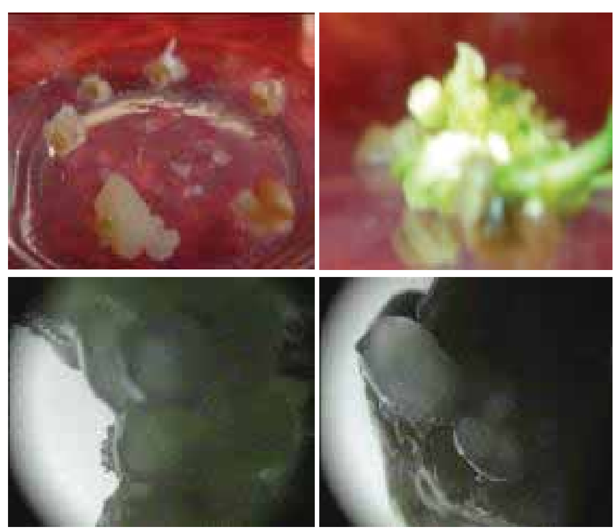

Figure 2. Globular structure of somatic embryo nodule candidate of somatic embryo.

Table 2. Effect of variety and medium on embryogenic callus formation.

Variety	Media MS+	Embryogenic callus formation (%)
Antasena	2,4-D 1 + sucrose 3% + manitol 1%	55.56
	2,4-D 2 + sucrose 3% + manitol 1%	53.61
	2,4-D 1 + sucrose 12% + prolin 4%	44.45
	2,4-D 2 + sucrose 12% + prolin 4%	40
Arjuna	2,4-D 1 + sucrose 3% + manitol 1%	56.95
	2,4-D 2 + sucrose 3% + manitol 1%	58.33
	2,4-D 1 + sucrose 12% + prolin 4%	37.56
	2,4-D 1 + sucrose 12% + prolin 4%	48.15
Bisma	2,4-D 1 + sucrose 3% + manitol 1%	58.32
	2,4-D 2 + sucrose 3% + manitol 1%	57.78
	2,4-D 1 + sucrose 12% + prolin 4%	50.56
	2,4-D 2 + sucrose 12% + prolin 4%	65.28
Sukmaraga	2,4-D 1 + sucrose 3% + manitol 1%	81.67
	2,4-D 2 + sucrose 3% + manitol 1%	70.18
	2,4-D 1 + sucrose 12% + prolin 4%	63.43
	2,4-D 2 + sucrose 12% + prolin 4%	73.15

supplemented with 1.5 mg L^{-1} 2,4-D and 12 m*M* L-proline. Sharp *et al.* (1980) reported that auxins induce embryogenetic determination in parts of cells in callus or suspension culture but at the same time also induce cell differentiation. Differentiation of the embryogenic mother cell only resumes in a medium with low levels of auxin. They concluded that active concentration physiologically and decreasing auxin may be the cause of metabolism or absorption.

When embryogenic calluses are subculture in a regeneration medium, not all of them regenerate. Some become white and brown. For Sukmaraga variety, the best regeneration medium to produce somatic shoots was MS + BA (1 mg L-1) + IAA (0.5 mg L^{-1}) (6.07 %) with the number of somatic embryo structrures per embryogenic callus being 3.06. Almeida and Margarida (2000) also found that the average number of fertile regenerated plants in 2-3-month-old cultures ranged from 2 to 8.15 per callus. The highest percentage of shoot formation was achieved by Bisma variety (7.98%) (Table 3) with MS + BA (1 mg L-1) + IAA (0.5 mg L^{-1}) as the regeneration medium (Fig. 3). Somatic shoots were produced in this experiment will be used for *in vitro* selection in the next experiment.

Conclusions

- Sukmaraga was the best variety to produce embryogenic calluses (85.28%)

Table 3. Number of somatic embryo structures produced per explant and percentage of somatic shoot formation.

Variety	Media MS+	Number of emb. som. structures per emb. callus	Shoot formation (%)
Antasena	BA 1 + IAA 0.5	2.77	7.12
	BA 2 + IAA 0.5	2.22	2.17
	BA 3 + IAA 0.5	2.67	1.08
Arjuna	BA 1 + IAA 0.5	2.72	0.76
	BA 2 + IAA 0.5	2.51	1.35
	BA 3 + IAA 0.5	2.92	5.95
Bisma	BA 1 + IAA 0.5	2.86	7.98
	BA 2 + IAA 0.5	2.40	1.67
	BA 3 + IAA 0.5	2.02	6.72
Sukmaraga	BA 1 + IAA 0.5	3.06	6.07
	BA 2 + IAA 0.5	3.79	4.17
	BA 3 + IAA 0.5	2.22	3.56

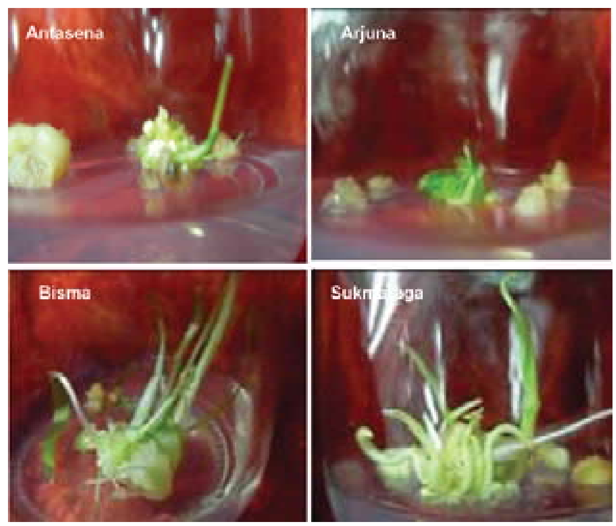

Figure 3. Regeneration of embryogenic calluses of four maize varieties (Antasena, Arjuna, Bisma and Sukmaraga) with media MS + BA (1 mg L^{-1}) + IAA (0.5 mg L^{-1}).

- Harvested kernel at 10 days after anthesis was the perfect time to produce embryogenic calluses for Antasena and Sukmaraga.
- The best media for callus induction was MS + 2,4-D 1 mg L^{-1} + sucrose 3% + manitol 1% and the best media for regeneration was MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}.
- The highest percentage of regeneration callus to become somatic shoots was 7.98% for Bisma variety.

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Genetic and Biochemical Analysis of Quality Protein Maize Lines in India

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Abstract. This study was aimed at evaluation of the agronomic performance and endosperm protein quality, besides responses to two major biotic stresses, of selected Quality Protein Maize (QPM) inbreds developed in India vis-à-vis some CIMMYT QPM lines. A set of 14 DMRQPM lines and three QPM inbreds from CIMMYT, Mexico, were utilized in generating a 14 × 3 line × tester (L × T) set and a 7 × 7 full diallel set. These experimental hybrids, besides parental lines, were evaluated at Delhi and Pantnagar, India, for yield-related and kernel quality characters. Analysis of the datasets revealed predominance of nonadditive gene action, significant effects of location on the test characters and excellent potential of DMRQPM-56, DMRQPM-401 and CML189 for QPM breeding programs at both locations for grain yield and related traits. The protein content among the QPM inbreds ranged from 7.30% to 11.40%, while tryptophan content in the endosperm protein varied between 0.72% and 0.98%. DMRQPM-403, DMRQPM-401 and CML189 were the best inbreds in terms of both quality traits. CIMMYT QPM inbreds showed better grain modification that the DMRQPM inbreds. Significant variation among the QPM genotypes was observed for their response to banded leaf and sheath blight (BLSB; Rhizoctonia solani f.sp. sasakii) and grain weevil (Sitophilus oryzae). Most of the QPM genotypes showed severe susceptibility to BLSB isolates at the three test locations (Delhi, Pantnagar and Udaipur) in India. Two QPM hybrids, Shaktiman-1 and Shaktiman-2, and one nonQPM hybrid (Parkash), along with their parental lines, also showed high vulnerability to BLSB. In contrast, two QPM genotypes (DMRQPM-60 and CML167) were found to be highly promising for resistance to stored grain weevil, with minimum kernel weight loss (3.70% and 5.01%, respectively) and insect multiplication (5.66 and 8.66, respectively), while the majority of QPM lines were susceptible. The study highlighted the need for genetic enhancement of maize germplasm in general, and QPM in particular, for resistance to BLSB and grain weevil infestation.

Key words: QPM, combining ability, tryptophan, kernel texture, BLSB, grain weevil

Introduction

Maize is a vital source of protein and calories to billions of people worldwide, particularly in Africa, South America and Asia. The average protein content in the maize kernel is about 9-10%; however, endosperm protein is deficient in two essential amino acids, lysine and tryptophan (FAO 1985). The discovery of the nutritional value of the *opaque2* mutation in maize was a significant breakthrough as it was found to alter the amino acid composition of the endosperm protein, resulting in enhanced concentration of lysine and tryptophan (Mertz *et al.* 1964). CIMMYT scientists successfully combined the high lysine potential of *opaque2* with genetic endosperm modifiers, and released new maize genotypes which are collectively referred to as Quality Protein Maize (QPM) lines (Prasanna *et al.* 2001; Bjarnason and Vasal 1992). Several countries in Asia, Africa and Latin America, including India, now have active QPM breeding programs which mainly concentrate on developing new arrays of QPM inbred lines, testing different cross combinations and converting elite nonQPM inbred lines. In view of the growing importance of QPM in India and to hasten the pace of progress of QPM cultivar development, it is important to develop broad-based QPM germplasm and to identify QPM genotypes, including inbred lines, with high *per se* productivity, combining ability, nutritional quality and considerable resistance to major biotic stresses.

Materials and Method

The genetic materials for the present study consisted of (i) a set of 13 QPM inbred lines developed from high-lysine *opaque-2* composites, including Shakti-1, under the All-India Coordinated Maize Improvement Project (AICMIP) [designated as 'DMRQPM' lines]; (ii) one QPM inbred line of Caribbean origin; and (iii) a set of 10 QPM inbred lines developed at CIMMYT, Mexico, designated as CML series.

Two sets of mating design, (i) 7 × 7 diallel set (including reciprocals) involving DMRQPM-56, DMRQPM-60, DMRQPM-401, DMRQPM-28-3, DMRQPM-403, DMRQPM-17-4 and DMRQPM-45 as parents and (ii) 14 × 3 line × tester set comprising DMRQPM genotypes as lines and CMLs as testers, were generated during *rabi* (postrainy season) 2002/03 at the Winter Maize Nursery in Hyderabad. For the line × tester analysis, DMRQPM17-1, DMRQPM17-4, DMRQPM28-3, DMRQPM28-5, DMRQPM45, DMRQPM56, DMRQPM58, DMRQPM60, DMRQPM65, DMRQPM401, DMRQPM402, DMRQPM403, DMRQPM404 and Tuxpeno Caribbean were used as lines, while CML166, CML167 and CML189 were utilized as testers. All the experimental crosses, along with the parents, were evaluated in a randomized complete block design with three replications per entry at two locations: (i) IARI Experimental Farm, New Delhi, and (ii) Crop Research Centre, GBPUAT, Pantnagar, during *kharif* (rainy season) 2003. Yield and yield contributing traits were recorded as per the standard method. All the experimental QPM hybrids were analyzed for heterosis against two QPM (Shaktiman-1 and Shaktiman-2) and three nonQPM controls (PEHM-1, PEHM-2 and PEHM-3). Combining ability analysis was done using SPAR-1 software, developed by the Indian Agricultural Statistical Research Institute (IASRI), New Delhi while ANOVA and LSD were estimated using SAS Version 6.12.

Endosperm quality traits, namely endosperm protein and tryptophan content, were analyzed using data from a separate trial planted in a complete randomized block design with two replications during *kharif* (rainy season) 2003 at the IARI Experimental Farm, New Delhi. The trial was control-pollinated by bulk sibbing to avoid the xenia effect due to open-pollination. The endosperm protein content was estimated using the Micro-Kjeldahl Procedure (AOAC 1965), while the tryptophan content was estimated by colorimetric method as suggested by Hernandez and Bates (1969) at Maize Quality Laboratory, Directorate of Maize Research, New Delhi.

Kernel modification attributes like endosperm modification, crown modification and ear appearance of the QPM genotypes were rated using a procedure reported by Bjarnason and Vasal (1992). For analysis of endosperm modification, backlit kernels were rated on a scale of 1-5, with 1 indicating 100% normal (vitreous), 2 indicating 25% opaque, 3 indicating 50% opaque, 4 indicating 75% opaque and 5 indicating 100% opaque. Endosperm modification scores were derived based on an analysis of 100 randomly chosen kernels from the ears of QPM inbreds and experimental crosses. To judge the effect of two pollination systems (open- and control-pollinated) on grain modification, QPM inbred lines and their experimental crosses were scored separately for the open- and control-pollinated sets planted during *kharif* 2003. Cumulative indices for the genotypes were computed using the LSD ranks for individual attributes as per the procedure suggested by Arunachalam and Bandopadhyay (1984).

A set of 24 QPM genotypes including three controls [Shaktiman-1 and Shaktiman-2 and Parkash] were evaluated for their responses to banded leaf and sheath blight (BLSB) pathogen *Rhizoctonia solani* f.sp. *sasakii* at three locations in India: (a) IARI Experimental Farm, New Delhi (*kharif* 2004); (ii) GBPUAT, Pantnagar (*kharif* 2003 and 2004); and (iii) MPUAT, Udaipur (*kharif* 2003 and 2004) under artificial inoculation conditions using the procedure devised by Ahuja and Payak (1978). Observations were recorded 35-40 days after inoculation on a standard 1-5 disease rating scale (a score of 1.0 was rated as resistant, 1.1 to 2.5 as moderately resistant, 2.6 to 3.5 as susceptible and 3.6 to 5.0 as highly susceptible).

Twenty-four QPM inbred lines along with two QPM hybrids [Shaktiman-1 and Shaktiman-2], four nonQPM hybrids [Parkash, PEHM-1, PEHM-2 and PEHM-3], four nonQPM inbred lines [CM139, CM140, LM5 and LM6], ‘Surya’ (composite) as resistant control and Basi Local as susceptible control were used for evaluating the responses of the QPM germplasm to stored grain weevil (*Sitophilus oryzae*) using standard methodology. Data on kernel weight loss due to insect infestation and number of insect progeny emerged were recorded.

Results and Discussion

For most of the yield-related traits, DMRQPM-56 and DMRQPM-401 were identified as promising lines. At Pantnagar, DMRQPM-56 recorded the highest GCA effects for grain yield, followed by DMRQPM-17-4 and DMRQPM-60. Pooled analyses also revealed the promise of DMRQPM-56 as the best general combiner for grain yield and the majority of yield-related traits. Analyses in the L×T set identified DMRQPM-65 and DMRQPM-28-5 as the best general combiners at Delhi and Pantnagar, respectively, considering grain yield and other related characters. Among the testers, CML166 and CML189 were the best general combiners at Pantnagar, while CML189 performed well at Delhi for the majority of yield-related characters. Among the crosses, DMRQPM-404 × CML189 showed high SCA effects for the majority of yield and yield-contributing traits at both locations, indicating its adaptability. The study also revealed that in most of the cases, at least one parent involved in experimental crosses had desirable GCA effects, indicating the importance of selection of parents on the basis of GCA effects (Vasal *et al.* 1993a & b).

The significant effects of locations on the test characters were very apparent. Variance due to dominance effects was also found to be much higher than variance due to additive effects, signifying the utility of heterosis breeding in the QPM genotypes. Tuxpeno Caribbean × CML189 at Pantnagar and DMRQPM-401 × DMRQPM-56 at Delhi were the best cross combinations showing 14.28% and 11.11% standard heterosis, respectively, over the nonQPM control PEHM-1. The high correspondence between SCA and heterosis (Bhatnagar *et al.* 2004) was observed in many combinations, indicating nonadditive gene action as an important contributor of heterosis. However, in general, a low extent of heterosis was displayed by the QPM experimental hybrids over the controls (Kassahun 2001), indicating the need for further genetic enhancement of the QPM germplasm by synthesizing broad-based QPM heterotic pools and isolation of QPM inbred lines with desirable attributes and combining ability. The study also revealed the importance of cytoplasmic effects in terms of both heterosis and combining ability, indicating the importance of choice of proper female parent in the hybrid breeding program (Khalil *et al.* 2005).

Protein content among the QPM inbreds ranged from 7.30% to 11.40%, while tryptophan content in endosperm protein varied from 0.72% to 0.98%. Vasal *et al.* (1993a); and Kassahun and Prasanna (2004) also reported a similar range of protein content in the QPM genotypes. CML167 (10.85%), CML166 (10.65%) DMRQPM-403 (10.55%), CML189 (10.45%) and DMRQPM-45 (10.00%) were the best QPM inbred line in terms of endosperm protein content. Among the cross combinations, DMRQPM-17-4 × CML167 (11.85%), DMRQPM-402 × CML167 (11.62%), DMRQPM-403 × CML167 (11.45%), DMRQPM-60 × DMRQPM-403 (11.35%), DMRQPM-17-4 × MRQPM-56 (11.35%), DMRQPM-403 × DMRQPM-45 (10.85%), DMRQPM-45 × DMRQPM-17-4 (10.80%) and DMRQPM-17-4 × DMRQPM-45 (10.65%) were excellent in terms of endosperm protein quality.

All the QPM genotypes recorded an almost 2-2.5-fold higher tryptophan content in endosperm flour as compared to the nonQPM control (Surya), confirming the nutritional superiority of QPM. DMRQPM-28-5 (0.99%), DMRQPM-401 (0.98%), DMRQPM-56 (0.89%) and DMRQPM-17-4 (0.82%) among the inbred lines, and DMRQPM-56 × CML189 (1.03%), DMRQPM-56 × DMRQPM-28-3 (1.02%), DMRQPM-60 × DMRQPM-17-4 (1.02%), DMRQPM-60 × DMRQPM-401 (1.01%), DMRQPM-60 × DMRQPM-45 (1.01%) and DMRQPM-401 × DMRQPM-60 (1.01%), among the experimental hybrids, were found to be superior for tryptophan content in endosperm protein. Among the testers, CML189 was the best donor for tryptophan content as compared to CML166 and CML167, indicating the presence of favorable alleles in CML189 for this specific trait. Taking into account both endosperm quality attributes, DMRQPM-65 × CML189 and DMRQPM-60 × DMRQPM-403 among the crosses, and DMRQPM-403, DMRQPM-401 and CML189 among the inbred lines were the best genotypes identified in our study.

The study revealed significant differences among the QPM genotypes for the three kernel attributes, ie, endosperm modification, crown modification and ear appearance, indicating the presence of enough variation for these kernel attributes among the experimental QPM genotypes. ANOVA also showed significant interaction of the pollination mode with genotype, suggesting the significance of the pollen source and its genetic constitution in affecting kernel modification in QPM genotypes. However, the effect of pollination mode (open vs controlled) on kernel modification was found to be nonsignificant for all the three kernel traits. A comparison of the mean values for various attributes revealed that the kernel texture of the majority of QPM inbred lines and their experimental crosses was better than that of the QPM control Shakti-1**.** However, the study emphasized further need for accumulation of endosperm modifiers genes in the Indian QPM lines. Cumulative indices (CI) for kernel modification computed for the genotypes, based on LSD ranks of genotypes for the individual attributes, showed CML166 (2.71), CML167 (2.81), DMRQPM-403 (2.65) and DMRQPM-56 (2.49) among the inbred lines as the most promising genotypes under controlled pollination. Our study also suggests that controlled pollination should always be followed to select QPM genotypes in terms of kernel texture as the data based on open pollination could be misleading which in turn may lead to faulty selection of genotypes.

Our study indicated severe susceptibility of the majority of QPM genotypes to BLSB isolates at the three experimental locations (Lal *et al.* 1980). DMRQPM-401 (Pantnagar-2004); DMRQPM-17-1 and Tuxpeno Caribbean (Udaipur 2004) revealed moderate tolerance; however, the expression was not consistent in other locations and even in different years at the same location. This differential behavior could be due to various reasons including (i) differences in the virulence of the BLSB isolates at the three locations; (ii) environment × year × genotype interaction; and/or (iii) inadequate disease pressure in one of the years. Therefore, these lines may still be categorized as susceptible to BLSB. It was also found that Shaktiman-1, Shaktiman-2 and Parkash, which are popular maize hybrids in India, are highly vulnerable to BLSB. All the parental lines (CML142, CML150, CML176 and CML186) of the two QPM hybrids (Shaktiman-1 and Shaktiman-2) were also highly susceptible to BLSB.

Our study revealed significant variation among QPM lines for kernel weight loss and for the number of insect progeny, indicating the presence of different sets of genes for tolerance/susceptibility to stored grain weevil in the genotypes under study. Sharma (2000) also screened several nonQPM lines and reported significant variation for this trait. DMRQPM-60 was found to be the best genotype in terms of resistance to the stored grain weevil, with minimum kernel weight loss (3.70%) and insect multiplication (5.66), followed closely by CML167. A moderate degree of tolerance was exhibited by DMRQPM-28-3, CML189 and CML176, which could be due to the accumulation of a few favorable genes for this trait. Santos *et al.* (1996) also identified some QPM maize hybrids with resistance to *S. zeamais*. The study also revealed the high susceptibility of DMRQPM-56, DMRQPM-401, DMRQPM-402, DMRQPM-403, DMRQPM-58, DMRQPM-17-1, CML171, CML150 and CML186 to stored grain weevil infestation, while the responses of the rest of the QPM lines ranged from moderate susceptibility to susceptibility. The nonQPM hybrids (PEHM-1, PEHM-2 and PEHM-3) as well as the nonQPM inbred lines (CM140, CM139, LM5 and LM6) were also found to be vulnerable to stored grain weevil infestation, indicating that genetic enhancement for resistance to stored grain weevil is not only important for the QPM germplasm but also for the nonQPM genotypes in India.

Acknowledgement

We are grateful to Dr. N.N. Singh (former Project Director, DMR, New Delhi), Dr. Ganesan Srinivasan and Dr. Ashish Srivastava (former scientists with CIMMYT) for providing the seed materials of DMRQPM and CIMMYT QPM lines, respectively, for this study. We also thank Dr. M.Z.K. Warsi (GBPUAT, Pantnagar, Uttarakhand), Dr. S.B. Singh (DMR, New Delhi), Dr. Rajpal Singh (DMR, New Delhi); Dr. O.P. Sharma (DMR, New Delhi), Dr. P. Kumar (DMR, New Delhi), Dr. R.K. Sharma (Division of Entomology, IARI, New Delhi), Dr. R.C. Sharma (Division of Plant Pathology, IARI, New Delhi), Dr. R.S. Rathore (MPUAT, Udaipur), and Dr. Rajesh Kumar (Division of Genetics, IARI, New Delhi) for their kind support and suggestions.

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In vitro Selection for Increasing Maize Tolerance in Acid Soil

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Abstract. Expansion of maize cultivation in Java is difficult. It is only possible by using marginal lands with acid soils. Aluminium (Al) toxicity is a problem in such soils due to low pH. Liming and increasing fertilizer dosage are expensive options and not feasible for farmers. Using maize varieties with tolerance to Al in acid soils is an alternative technology that is both cheaper and environmentally safe. In vitro culture can be used to produce maize varieties with Al tolerance. This technique allows any variability to be screened in the cell phase. In vitro selection has been done by culturing populations of embryogenic cells in media enriched with high concentration of $AlCl_3$ and low pH, and modified MS basic media by decreasing the concentration of ions P^+, Ca^{++} and K^+. In this experiment, we used the maize varieties Sukmaraga, Bisma and Arjuna. Induction of embryogenic cells was done by using young embryos as explants cultured in JG1 media. Embryogenic cells were selected in media with $AlCl_3$ (0; 250; 500; 750 mg L^{-1}) as component selection. The results of the experiment showed that the variety Arjuna gave the highest response of embryogenic cells. Adding $AlCl_3$ in selection media with low pH decreased the regeneration ability of all varieties. Sukmaraga, which is relatively tolerant to acid soil, gave the highest regeneration compared with the other varieties, and moreover showed the same tendency in acclimatization. Bisma and Arjuna, which are not tolerant to acid soils, gave good results with production of survived shoots in the media and acclimatized. All somatic seeds which survived in acclimatization were moved to an experimental farm and produced ears with plenty of seeds.

Key words: Maize (Zea mays L.), in vitro selection, aluminium tolerance

Introduction

High demand and low productivity of maize (*Zea mays* L.) in Indonesia necessitate import of the commodity every year. In 2007 maize imports were 600 000 t (Anonymous 2007) at a cost of Rp. 1.68 trillion. National production was only 577 513 t from 113 373 ha (BPS Propinsi Jawa Barat 2008). Maize prices and demand have been increasing in recent years. However, raising productivity and expanding the maize area remains difficult because agricultural lands are increasingly being put to nonagricultural use, especially in Java. So expansion of maize area is only possible by using marginal lands. Marginal soils, especially acid soils, account for about 50.94 million ha in Indonesia, spread over Sumatra, Kalimantan, Papua, Maluku and Sulawesi islands (Makarim 2005).

Acid soils have low fertility due to the high content of aluminium (Al), iron (Fe) and manganese (Mn) which are toxic to plants, while minerals needed by them, such as calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) are very low (Samac and Tesvayi 2003). Aluminium poisoning causes slow root development, with yellowish red leaves which become pale between the veins and tip. The margins of leaves become dry and growth is stunted (Morimura *et al.* 1978; Wagatsuma 1988). Growth of maize is disturbed when aluminium content is more than 60% (Sanchez 1976). Maize yield is relatively high even at 40% aluminium (Kamprath 1970). Ismunadji (1976) reported that in acid soils with high aluminium content maize growth is short with a weak stem and yellowish leaves turning brown and drying up.

Problems in acid soils have been conventionally resolved by liming with $CaCO_3$ (Delhaije and Ryanm 1995), using organic matter, alley cropping or fertilizer application (Adiningsih and Sudjadi 1993). But these methods are difficult to adopt for small farmer because they are quite expensive and not very efficient (must be repeated many times instead of leaching by rain) (Nirmala 1999; Mulyani *et al.* 2003). The most effective, efficient and cheap way of solving the problem is using new cultivars that are tolerant to acid soil.

In vitro culture is an alternative method of improving maize varieties. In this method, a component of variation that is not found in nature can be added to improve cultivars (Ahlowalia 1986). By culturing cells or embryogenic structures in a medium consisting of the metabolite pathogen (filtrate) or toxic compound (Al, PEG), we can produce new varieties that are tolerant to biotic and abiotic stresses (Starvarek and Rains 1984; Ahlowalia 1986). *In vitro* selection is an alternative method that can produce a new variety that has disease tolerance inheritable by to the progeny. Mutation or modified characters can be produce in the free cell phase or in the callus in *in vitro* culture or in an explant with mutant cell in the tissue. Maize has been somaclonally modified to produce tassels earlier (Brook Houzen *et al.* 1984).

In vitro selection for tolerance to aluminium and low pH has been done on potato and tomato (Stavarek and Rains 1984), soghum (Smith *et al.* 1983) and soybean (Mariska 2003). Tolerance induced in cell-stage embryonic mutants in soybean has been reported to be stable until the sixth generation in acid soils (Mariska 2003). Improved varieties obtained through *in vitro* culture using somatic embryo structures are preferred because they come from a single cell with high assurance. Variation in the cell population is important in the *in vitro* selection process. It can be improved by using plant growth regulators from the auxin group which promote cell differentiation continuously (Bordallo e*t al*. 2004; Hitomi *et al*. 2006).

A maize cell population with high variability followed by *in vitro* selection using $AlCl_3.6H_2O$ as the selection component can hopefully produce maize new lines with tolerance to Al in acid soils. The aim of this experiment was to improve the tolerance of Indonesian maize varieties (Bisma, Arjuna and Sukmaraga) to aluminum.

Materials and Method

The experiment was conducted at the Biology Cell and Tissue Laboratory and the Cikeumeuh experimental farm of BB-Biogen Bogor in 2007. Three maize varieties, Bisma, Arjuna (both susceptible to acid soil) and Sukmaraga (moderately tolerant) were used in the experiment. Embryos of young ears harvested 10 days after anthesis were used as explants to produce embryogenic calluses. The MS medium modified by increasing NH_4NO_3, decreasing $CaCl_2.2H_2O$ and KH_2PO_4, and adding Fe_2SO_4 was used as the selection medium to increase aluminium toxicity in the *in vitro* culture. The young zygotic embryos (1.5 mm) isolated from the ears were sterilized with alcohol 96%, 70% and bleached, and then planted in the medium containing MS + 2,4-D 1 mg L^{-1} + sucrose 3% + manitol 1%. The calluses thus induced were selected in a selection medium comprising MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1} to which the selection component $AlCl_3.6H_2O$ was added (0; 250; 500; 750 mg L^{-1}). The calluses or embryo somatic structures rproduced were subcultured in a medium containing MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1} without a selection component. Somatic shoots formed with $AlCl_3.6H_2O$ as the selection component were subcultured in an MS medium without plant regulators to promote root development. Acclimatization was done for planlets with complete leaf and root in room temperature of about 25°C and lighting for 16 hours per day. The fresh seedlings were moved to the greenhouse and then transferred to the field (experimental farm) until harvesting time.

Results and Discussion

The results of the experiment showed that media with aluminium decreased the average number of somatic embryo structures per explant whether of the moderate variety (Sukmaraga) or the susceptible varieties (Bisma and Arjuna). Data presented in Table 1 show that the control medium (without $AlCl_3.6H_2O$) gave a higher number of somatic embryo structures per explant than media with different concentrations of $AlCl_3.6H_2O$. The lowest number of somatic embryo structures per explant was given by a medium with 500 ppm of $AlCl_3.6H_2O$, not the treatment with 750 ppm of $AlCl_3.6H_2O$. This phenomena was noted for all the three experimental varieties. It may be caused by induction of somaclonal variation that may have modified tolerance among the cell population using $AlCl_3.6H_2O$.

The growth of somatic embryo structures to become shoots was better in the control medium than in the selection medium although the number of shoots in the selection medium were higher than in the control. Shoots in the selection medium tended to be weaker and light green in color. Shoots of the variety Sukmaraga grown in a selection medium with 750 ppm $AlCl_3.6H_2O$ were stronger than in the control medium. This may be due to the fact that Sukmaraga is a more tolerant variety than Bisma and Arjuna. Increasing tolerance of Bisma and Arjuna was indicated by shoot formation in media with a high level of $AlCl_3.6H_2O$ (750 ppm) (Table 2).

Table 1. Average number of somatic embryo structures/explant in selection medium with aluminium and low pH

Variety	Aluminium treatment (ppm)			
	0 (control)	250	500	750
Sukmaraga	12	9.97	7.66	10.13
Bisma	10	7.66	5.93	9.33
Arjuna	10	9.6	9.44	9.77

Table 2. Average number of shoots/embryonic calli in selection medium with aluminium and low pH.

Variety	Aluminium treatment (ppm)			
	0 (control)	250	500	750
Sukmaraga	0.4	0.93	0.51	1
Bisma	2.6	0	0.06	0.62
Arjuna	0.66	0.88	0.22	0.22

Figure 1. Responses of maize calli in selection medium with aluminium and low pH and root induction of selected somaclones by Al and low pH.

Figure 2. Variation of selected somaclones from vegetative to reproductive phase.

The success of acclimatization needs good root development. Even though roots or primary roots are already formed in the somatic shoot/seedling in control or in selected medium, the seedling must be subcultured in a root induction medium (MS) without any plant growth regulator. Data in Table 3 show that roots were formed in both control and selected media, but the roots in the selection medium were better than that in the control medium. Except in Bisma variety, somatic seedlings in medium with $AlCl_3.6H_2O$ 500 ppm primary root failed to grow.

Seedlings or planlets which successfully acclimatized ranged from 17.39% to 29.41% for Sukmaraga variety (Table 4). For Arjuna variety the range of success rate was 25-100%, even though only 1 seedling survived until planted in the field. The results of acclimatization showed that survived planlets/seedlings were 19 for Sukmaraga variety and 2 for Arjuna. All seedlings of Bisma failed to acclimatize.

Table 3. Average number of roots/shoot in selection medium with aluminium and low pH.

Variety	Aluminium treatment (ppm)			
	0 (control)	250	500	750
Sukmaraga	3.66	1.08	15.55	21.53
Bisma	4	0.66	0	4.956
Arjuna	8.66	8.88	14.44	10.88

Table 4. Percentage of selected somatic seedlings, 3 weeks after acclimatization.

Variety	Aluminium treatment (ppm)			
	0 (control)	250	500	750
Sukmaraga	0	17.39	29.41	17.24
Bisma	0	-	0	-
Arjuna	0	100	-	25.0

Table 5. Number of selected somaclones which planted and selfed in the field.

Variety with $AlCl_3.6H_2O$ (mg L^{-1})	Number of planted somaclones	Number selfed	Notes
Sukmaraga 250	4	1	-
Sukmaraga 500	1	3	One plant with 2 ears
Sukmaraga 750	0	1	-
Arjuna 250	5	0	-
Arjuna 750	11	1	3 branches, 2 of them selfed
Total	21	6	

Acclimatization is a critical process in *in vitro* culture. In maize the process is quite difficult because even roots were formed; death level is very high. In fact no seedling from the control medium survived.

Observations in the field showed no difference in the number of leaves (7-13 leaves per plant). But in terms of plant height, there was 1 plant of Sukmaraga variety from the 250 ppm $AlCl_3.6H_2O$ treatment that was shorter than the other plants and did not produce tassels completely. From the 21 somaclones which were acclimatized, only 6 were selfed to produce quite a number of seeds for the next experiment (Table 5). The seeds which were thus produce will be selected in an acid soil in the next experiment.

Conclusions

- Tolerance in Arjuna (susceptible) and Sukmaraga (moderate) varieties was relatively not different. Calluses of Sukmaraga were easier to regenerate than Arjuna variety.
- Nineteen Sukmaraga and 2 Arjuna which were tolerant to aluminium were achieved through *in vitro* selection, and they produced seed by selfing.

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'Sikkim Primitives' Reveal Significant Phenotypic and Molecular Distinctness from Other Maize Landraces in India

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Abstract. Mmaize landraces, particularly from the northeastern Himalayan (NEH) region in India, are important from an evolutionary viewpoint due to the presence of some unique landraces such as 'Sikkim Primitives' which have a high prolificacy (6-9 ears on a single plant). This study pertains to intensive phenotypic and molecular analyses of a set of 48 selected landrace accessions in India with specific focus on the 'Sikkim Primitives'. Multilocation evaluation of these accessions revealed significant genetic variability for grain yield and its components. The landraces/locals were also genotyped using 42 fluorescent-labelled SSR markers, which revealed a high mean PIC value of 0.60. Estimation of F-statistics and Analysis of Molecular Variance (AMOVA) indicated high levels of genetic differentiation within and among accessions. Cluster analysis using Rogers' (1972) genetic distance revealed the distinct genetic identity of the 'Sikkim Primitives' from the rest of the accessions from India, including Sikkim. This germplasm harbors a rich diversity that could provide a range of useful genes that could be efficiently utilized in future maize breeding programs and for population improvement.

Key words: Maize, landraces, simple sequence repeats, phenotypic diversity, genetic diversity, Sikkim Primitives

Introduction

Maize is one of the most important cereal crops in the world with its enormous role in food, feed and nutritional security. Originating in Mexico, maize has spread to all the important agroecologies in tropical, subtropical and temperate regions of the world. The unparalleled genetic richness of maize is evident from the existence of well-described landraces from diverse countries. Landraces, which are a natural treasure for useful genes and alleles, are the germplasm maintained by farmers over decades or centuries. They were evolved and selected to thrive under particular environmental conditions and to meet local food preferences.

India is a rich hub of diversity of maize landraces. Extensive variability in plant, ear and tassel characteristics is observed in the northeastern and northwestern highlands of India. Relatively less varietal diversity is available in the plains (Prasanna and Sharma 2005). Particularly impressed by the diversity of maize in the northeastern Himalayan (NEH) region, Anderson (1945) considered maize to have an ancient Asiatic origin. Dhawan (1964) rechristened a prolific popcorn variety of Sikkim as 'Sikkim Primitive' (SP1 and SP2). The most important physiological attributes of Sikkim Primitive maize are a complete lack of apical dominance, prolificacy (5-9 ears) with uniformity in ear size; erect leaves for developing maize varieties for high population density, top bearing habit and drooping tassel to ensure effective fertilization (Sachan and Sarkar 1982). This landrace, locally known as "Murli makai" stays green after maturity, and thus serves farmers well for fodder purposes. Thirteen different strains of the primitive maize, also designated as SP strains, are distributed throughout the NEH region other than Sikkim (Sachan and Sarkar 1986).

Most of maize diversity remains undescribed, poorly understood or underutilized in modern plant improvement largely because of the difficulty in identifying useful genetic variants hidden in the background of local varieties (Tanksley and McCouch 1997). A bottleneck in utilizing and managing these important genetic resources could be the lack of knowledge about the agronomic performance and genetic architecture of these landraces. The first detailed molecular characterization and population genetic analysis of selected Indian maize landraces was initiated by Prasanna *et al*. (2005), wherein 17 selected landraces from diverse agroecologies were analyzed using 27 polymorphic microsatellite or simple sequence repeat (SSR) markers. This study revealed greater genetic divergence among the NEH landraces than landraces from other regions.

The present study pertains to an intensive phenotypic and molecular characterization of Indian maize landraces with particular focus on the Sikkim Primitives.

Materials and Method

A set of 48 maize landraces/locals representing the diverse agroecologies of India were selected for the present study. Of these, 8 accessions represented Sikkim Primitives (collected from villages in different districts of Sikkim in Nov 2005), 21 NEH landraces (other than Sikkim Primitives), 4 from non-NEH tribal hill regions, and 15 belonging to the plains of India.

Evaluation of the agronomic performance of the selected accessions was undertaken during 2006-2007 at three locations in India: (i) Bajaura, Himachal Pradesh; (ii) New Delhi; and (iii) Hyderabad, Andhra Pradesh. Apart from data on days to anthesis and silking, various yield parameters, including grain yield, 100-kernel weight, ear length, ear diameter, number of kernel rows per ear, number of kernels per ear were recorded. The datasets were analyzed for ANOVA (analysis of variance) and least significant differences (LSD).

The genomic DNA was isolated from each of the selected landraces using a 'population bulk strategy' with each bulk prepared by pooling an equal amount of leaf material from 15 individuals (Rebourg *et al*. 2001). DNA extraction was performed after the pooling step, using a modified CTAB procedure according to CIMMYT Applied Biotechnology Center's Manual of Laboratory Procedures (http://www.cimmyt.org/ABC/Protocols/ manual ABC.html). Forty-two fluorescence-labelled polymorphic SSR loci, distributed on the 10 chromosomes, were selected for DNA profiling of the selected populations. The amplicons were resolved using MegaBACE automated DNA Sequencer. An automated DNA sequencer increases the accuracy of allele sizing by the use of an internal size standard in each lane and the availability of automated allele-calling algorithms. Data generated in the form of peaks was processed into allele sizes using the software 'Fragment Profiler v1.2' (GE Biosciences). This was followed by estimation of SSR allele frequency using FreqsR software (Dubreuil *et al*. 2006) and subsequent conversion of frequencies to lengths using FtoL software. The datasets were further subjected to statistical analysis using PowerMarker v3.25 (Liu and Muse 2005). Cluster analysis was done based on Roger's (1972) genetic distance and an unrooted tree was obtained through UPGMA clustering algorithm (Fig. 2). To statistically assess genetic variation "within" and "among" populations, we performed an Analysis of Molecular Variance (AMOVA) with the software package Arlequin, version 2.0 (Schneider *et al*. 2000).

Results and Discussion

Agronomic performance of the accessions

Multilocation evaluation of the agronomic performance of the selected accessions revealed significant variation for various yield parameters, including days to anthesis and silking, plant height, ear height, ear length, ear diameter, number of kernel rows per ear, number of kernels per ear, 100-kernel weight and grain yield. The performance of some accessions with respect to grain yield and its components was on par or significantly higher than the high-yielding controls used in the study. Several promising accessions with adaptation to specific agroecological zones as well as across zones were identified for grain yield and its components vis-à-vis controls (Table 1). Sikkim Primitives showed good agronomic performance, including prolificacy at Bajaura (Himachal Pradesh, India) as they are particularly adapted to high-altitude regions (Fig. 1).

Genotypic diversity

The population bulk strategy, used in the current study, made it possible to estimate accurately the within-population diversity. This approach could thus serve as a powerful tool to analyze numerous populations from gene banks and help in the maintenance and utilization of maize

Table 1. Promising maize accessions identified on the basis of performance of accessions at three locations in India [Bajaura (B); Delhi (D); Hyderabad (H)].

Accession	Collection from	Promising for the traits[1]
IML112	Himachal Pradesh	GY(H); HKW (H)
IML181	Himachal Pradesh	GY (D,H); NKR (B); KPR (H); KPE (H)
IML215	Arunachal Pradesh	GY (B); HKW (B)
IML282	Arunachal Pradesh	GY (B)
IML293	Bihar	GY (D)
IML298	Meghalaya	GY (B, D); HKW (B)
IML423	Bihar	GY (H)
IML452	Madhya Pradesh	GY (B,D); HKW (B); KPR (D); KPE (D)
IML454	Madhya Pradesh	GY (D); HKW (B,D); NKR (H); KPR (D); KPE (D)
IML615	Sikkim	GY (B,D); NKR (H); KPE (H)

[1.] GY: Grain yield; HKW: 100-kernel weight; NKR: Number of kernel rows per ear; KPR: Kernels per ear row; KPE: Kernels per ear.

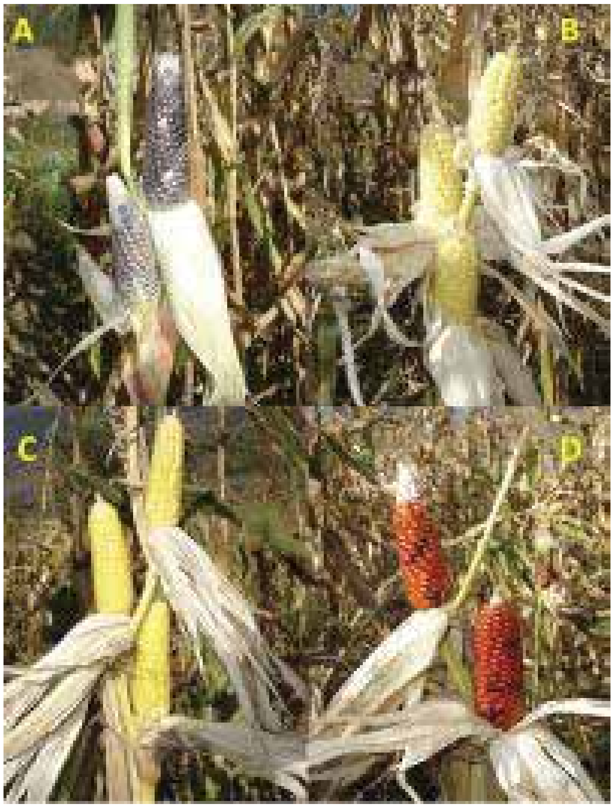

Figure 1. 'Sikkim Primitives' (SP) showing prolificacy at Bajaura, Himachal Pradesh, India: (A) IML203 (Nagaland SP); (B) IML592 (SP); (C) IML602 (SP); (D) IML637 (Mizoram SP).

diversity. The total number of alleles detected across 42 SSR loci was 547 alleles, with a high mean of 13 alleles per locus. All SSR loci selected (except *phi075* and *phi112*) had tri-nucleotide or higher repeat motifs in order to limit the stuttering often associated with di-nucleotide SSR loci. Since tri-nucleotide or higher repeat SSR loci are less polymorphic as compared to the di-nucleotide repeat SSRs (Vigouroux *et al*. 2002), the high allelic richness obtained explains the high levels of diversity present among the maize accessions under study. The PIC values of the 42 SSR loci ranged from 0.18 (*phi062*) to 0.85 (*phi083*), with a high mean PIC of 0.60, thus reinforcing the importance and utility of SSR markers in the study of population genetics. There was a positive correlation between the PIC values and the number of alleles detected at many of the SSR loci. But it is not possible to predict which SSRs will be the most discriminatory on the basis of PIC alone (Warburton *et al*. 2002). Thus, both allelic richness and their respective PIC values should be considered to objectively evaluate the polymorphism of different loci and genetic diversity of accessions. Rare alleles with a frequency less than 0.005 were identified at several loci.

A large number of 'private or unique alleles' (163 or 29.8%) were detected, leading to an effective differentiation of 44 out of the 48 accessions. Also, the unique alleles observed indicate that there is considerable variation in these accessions to exploit for maize improvement programs. *phi014* and *phi062* showed one highly frequent SSR allele each, with frequency as high as 75% and 85% respectively. *phi014* tags the *rip1* (*ribosomal inactivating protein1)* gene which plays a role in pathogen resistance, while *phi062* tags the *mgs1 (male gametophyte specific1)* gene that controls pollen fertility. Favorable alleles at loci controlling agronomic traits or environmental adaptation often get selected in landraces/locals either by natural selection or by selection enforced by farmers in their fields.

Estimation of Wright's *F*-statistics revealed F_{IS} values ranging from -0.11 (IML602) to 0.13 (IML592), indicating low levels of inbreeding and negligible nonrandom mating within each population. Significant variation in the F_{ST} values was found between accessions. The highest F_{ST} value (0.80) was found between IML181 and IML210 while the lowest value (0.11) was between IML608 and IML610, with a mean F_{ST} value of 0.38. According to the interpretation of F_{ST} given by Wright (1978), values of F_{ST} above 0.25 indicate a very great genetic differentiation among the accessions under study. AMOVA, implemented through Arlequin, showed that 60% of the genetic variation in these accessions was within the individuals, 37% variation was among populations within a group, while 3.19% and 0.24% of variation was among groups and among individuals within a population, respectively. Diversity in farmers' fields is a dynamic process maintained by an influx of new genes, together with farmer selection. In addition to this, landraces themselves are constantly evolving, thus maintaining a high level of variation.

Since most SSR loci exhibit a complex model of evolution, the distance between two accessions was estimated by the Rogers' (1972) Distance, which is free of any assumptions about mutation model at marker loci. An unrooted UPGMA tree, generated using PowerMarker revealed the distinct genetic identity of the Sikkim Primitives from the rest of the accessions in India (Fig. 2). This distinct clustering can be attributed to SSR alleles associated with the unique features of this landrace, most important being prolificacy and popcorn type. Accessions from other regions in India were found to form different clusters, based on their geographical region, while none of the Sikkim Primitive accessions featured in either of these clusters. This enhances their distinctness as a separate group of maize landraces and supports the fact that they are grown in very remote and isolated pockets of states in the NEH region.

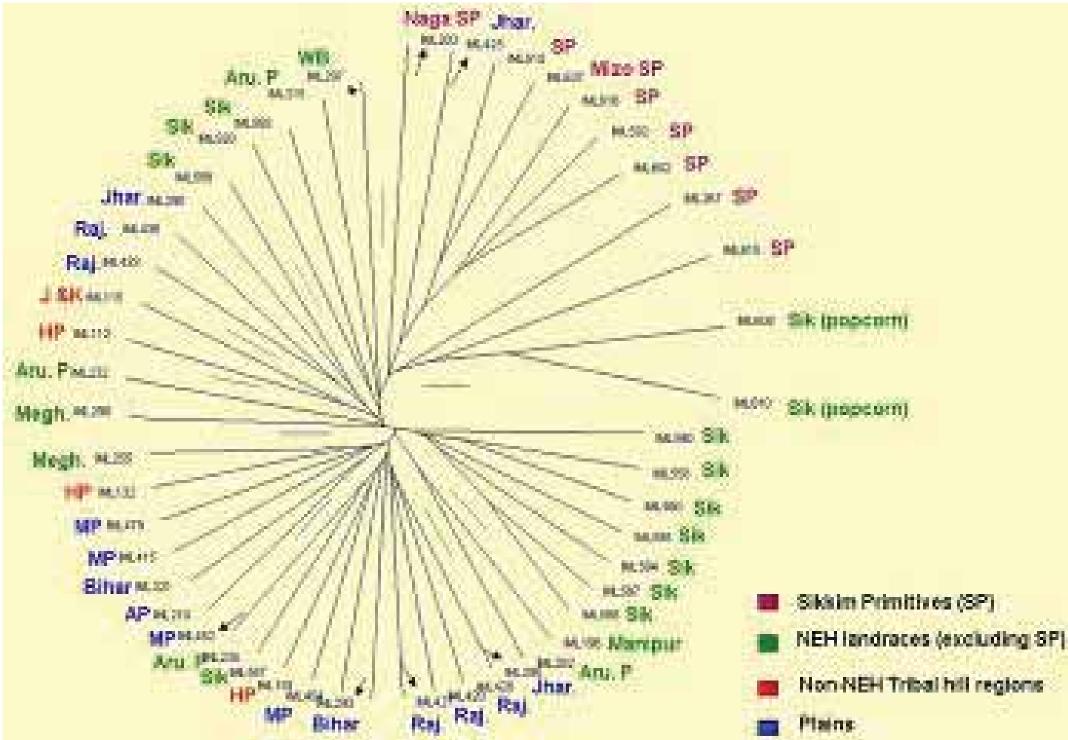

Figure 2. Tree depicting the genetic relationships among the selected maize landrace accessions in India through analysis of SSR data. Note the genetic distinctness of the 'Sikkim Primitives' from other maize landrace accessions.

Conclusion

Germplasm collections as a source of genetic diversity must be well characterized for efficient management and effective exploitation. The present study is an initial effort to characterize maize landraces in India at intensive phenotypic and molecular levels. Multilocation analysis of selected accessions led to identification of some promising landrace accessions that could be potentially utilized in breeding programs. SSR markers analyzed using DNA Sequencer technology led to an effective differentiation of accessions. Sikkim Primitives have been demonstrated to be distinct both at the phenotypic and molecular levels as compared to other landrace accessions in India.

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A Precise Means to Confirm Purity of Commercial Maize Varieties on the Basis of SSR Analysis

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Abstract. Until recently, estimation of seed purity exclusively depended on the grow-out test, which is based on the assessment of morphological and floral characteristics in plants grown to maturity. This requires intensive inspections at different stages and is sometimes imprecise when the stage cannot be exactly defined due to plant damage caused by weather and pests or diseases. Hence, a complementary tool to identify maize cultivars and to rapidly check the identity of seed lots is highly desired. As locus-specific codominant PCR-based markers, microsatellites (SSR) are good candidates for this purpose. In this study, 7 hybrid and 3 open-pollinated varieties were genotyped with 100 SSR markers that evenly covered the whole maize genome. Two hybrids shared the male parent; one was a three-way hybrid and one a double hybrid. The utilization of genetic analyzer ABI3130 enabled us to observe accurately the size of each allele. In total, 432 SSR fragments were identified with a mean of 4.4 alleles per locus. The obtained DNA fingerprinting serves as genetic markers for those cultivars. In general, four random SSR loci were sufficient to discriminate two cultivars. To reduce the cost, we focused only on markers that were able to resolve on 2% agarose gel for the genetic purity test. In combination with our simple DNA isolation method that normally employs 100 dried maize kernels per seed lot, a precise purity percentage of different seed lots can be provided in just two working days.

Key words: DNA extraction, DNA fingerprinting, purity assessment, single seed, SSRs, maize varieties

Introduction

Assessment of seed purity is one of the most important quality control components in hybrid seed production. For years, the method used to check hybrid seed purity has been the grow-out test. This consists of growing a representative sample of the F_1 seed and later classifying it as true hybrid seed or off types on the basis of descriptors. Normally a high number of traits have to be observed during field inspections (Taixing *et al.* 1998). Inspections are needed at different stages of the crop. The increasing number of cultivars makes this process more and more complex. Sometimes the resulting information is ambiguous due to unfavorable weather or pests or disease attacks. Hence, a complementary tool to identify maize cultivars and to rapidly check the identity of seed lots is highly desired.

DNA fingerprinting approaches based on polymerase chain reaction have become methods of choice for germplasm characterization, gene tagging, diversity studies and also seed purity assays. A variety of DNA markers are now available for fingerprinting cultivars and for marker-assisted selection. Of these, microsatellites (SSRs) are the preferred type of markers due to their ease of assay, high level of polymorphism, codominant inheritance, and their abundant distribution throughout the genome (Sharapova *et. al.* 2002; McCouch *et al.* 2002).

Fluorescence-based SSR detection and allele sizing on an automated DNA fragment analyzer have been recognized as the fastest and most accurate methods for SSR genotyping (Ziegle *et al.* 1992; Mansfield *et al.* 1994). Another advantage of this system is that several SSRs can be simultaneously separated in a single capillary or gel lane providing the SSR fragments have nonoverlapping sizes. In instances where SSR allele sizes are overlapping, coseparation can be achieved by labeling the SSR products with fluorescent dyes that have different emission wavelengths (Hayden *et al.* 2007).

Considering these beneficial reasons, we genotyped 10 varieties with 100 SSR markers that evenly covered the whole maize genome. Visualization of their alleles with genetic analyzer ABI3130 enabled us to observe precisely the size of each allele. This high accuracy and good scoring system is very useful to establish the DNA fingerprints of the cultivars tested. However, a simple and rapid DNA extraction method as well as high throughput MAS system is a necessity when utilizing molecular markers in practical breeding. Here the selection procedure is described of appropriate SSR alleles, combined with our single seed extraction as an alternative feasible strategy for cultivar identification and purity test of maize cultivars.

Materials and Methods

Plant materials

Three open-pollinated varieties (OP-1, OP-2 and OP-3) and 7 hybrid varieties (F1_A-F1_G) were employed as materials for genotyping. F1_D and F1_E possessed the same male parent; F1_F was created through three-way crossing; and F1_G was produced by double crossing. The remaining 3 F_1 varieties were derived from single crosses.

DNA extraction

Two DNA extraction methods were utilized in this experiment. For the DNA fingerprinting work, we isolated genomic DNA from young leaf material according to the CTAB method of Saghai-Maroof *et al.* (1984). The genomic DNA of each line/variety was assembled by mixing equal amounts of DNA from 10 individual plants.

For the genetic purity test work, DNA was isolated from a single maize kernel following the conditions described by Hee *et al.* (1998) with a modification of the incubation time and the grinding method. Briefly, the methods were as follows. All seeds were incubated in 400 µL of extraction buffer containing proteinase-K (50 µg) at 55°C for 2 hours prior to grinding either manually or by our 2 semiautomatic machines (one has 4 grinders and other 24) that were specially designed to crush the seed. Principally, the instruments worked as a drilling machine. Seed was drilled by its grinder at a constant spinning rate for 5-10 seconds or till the seed became destructive. Then, 400 µL of CTAB solution (2%) was added to the seed extract, followed by gentle extraction using chloroform:isoamyl alcohol (24:1). After centrifugation at 12 000 rpm, 4°C for 10 min, the supernatant was transferred to new tubes. The supernatant was further purified for a second time in chloroform:isoamyl alcohol (24:1). After this second purification, only 200-400 µL of the supernatant was taken and added to 0.1 mg concentrate RNase and incubated for about 1 hour at room temperature. About one volume of cold isopropanol was added to precipitate the DNA. After 10 min of incubation at room temperature, the solution was centrifuged at 12 000 rpm and 4°C. The DNA pellet was then washed twice with 200 µL 70 % ethanol, the second time overnight. The washing solution was removed and the DNA pellet was dried thoroughly and dissolved in TE buffer. The DNA was then diluted to a concentration of 10 ng μL^{-1} before being used in the SSR genotyping experiment.

PCR analysis

One hundred publicly available SSR markers that provide an even coverage of the maize genome (Heckenberger *et al.* 2002) were utilized to establish DNA fingerprinting of our cultivars. The sequences of those primers were kindly provided by Dr. Heckenberger, but also can be obtained from www.maizegdb.org. All forward SSR primers were labeled with 6-FAM. The PCR reaction was set up in a total volume of 20 µL reactions containing 1X PCR Buffer (-$MgCl_2$), 0.2 mM dNTPmix, 0.25 µM of each primer, 3.5 mM $MgCl_2$, 1 U *Taq* Polymerase (New England Biolabs Inc.) and 20 ng of DNA template. PCR cycles were carried out according to the following temperature profile (Gene Amp PCR System 9700 ABI): 35 cycles of 94°C for 10 s, 60°C for 10 s and 72°C for 30 s with final extension of 10 min at 72°C. The PCR products were resolved on 2% (w/v) agarose gel (Roche Diagnostic, USA) stained with ethidium bromide and visualized with GelLogic200 (Kodak MI) for genetic purity test and ABI 3130 for DNA fingerprinting.

Generation of SSR data

Two µL of an appropriate dilution of the PCR product was added to 6 µL of deionized formamide containing 0.2 µL of GeneScan 500 LIZ size standard (Applied Biosystems). The mixture was denatured at 95°C for 5 min and then cooled on ice. The samples were then run on an ABI 3130 Genetic Analyzer using 36 cm capillary arrays and POP-7 polymer and the default settings of the manufacturer-supplied run module. Semiautomated SSR allele sizing was performed using GeneMapper v4.0 software (Applied Biosystems). SSR primer pairs that produced double bands for F_1 combining each from its parental lines were further selected for seed purity works. The purity of each seed lot was represented by the total number of true-to-type seeds in 100 randomly taken maize kernels from that lot.

Results and Discussion

Hybrid varieties offer a number of potential benefits including uniformity, heterotic vigor and by virtue of the inbred lines a proprietary shield for the developer. To achieve the full potential of hybrid varieties, it is essential that the hybrid seed lots be genetically pure. Contamination with seeds can originate from sib or self-pollination and result in off-types appearing in the field, which may reduce yield and uniformity (Arús *et al.* 1982). Therefore, assurance of seed purity is one of the most important considerations that should be meticulously maintained by a seed company.

Genotyping of 10 Maize varieties

In order to obtain efficient markers, we first genotyped our varieties with 100 SSR primer pairs that were evenly distributed over the maize genome. This enabled us to obtain specific markers for the genetic purity test of each hybrid variety and at the same time to establish DNA fingerprinting of each inbred (parental lines of hybrids) and open-pollinated variety. The utilization of fluorescence-based SSR facilitated scoring all SSR alleles with high confidence. With the assistance of internal size standard (GeneScan500 LIZ), 1 bp distance among different alleles as shown in Figure 1 can be accurately and conveniently observed. This ensures that all obtained alleles are highly reliable for further use in identifying different cultivars and also for purity test.

Out of 100 SSR primer pairs tested, two primer pairs, bnlg2047 and bnlg2086 failed to produce any amplicon in all samples. The remaining primer pairs generated 432 DNA fragments scattered over 10 maize varieties. Hence, on an average we obtained 4.4 alleles per locus. Ninety-six of them successfully amplified polymorphic alleles at least for one variety, while the other two primer pairs, phi050 and phi017, produced the same alleles for all varieties. An example of DNA fingerprinting based on 10 loci data can be seen in Table 1. Aside from screening potential markers for the purity test, establishment of DNA fingerprinting is

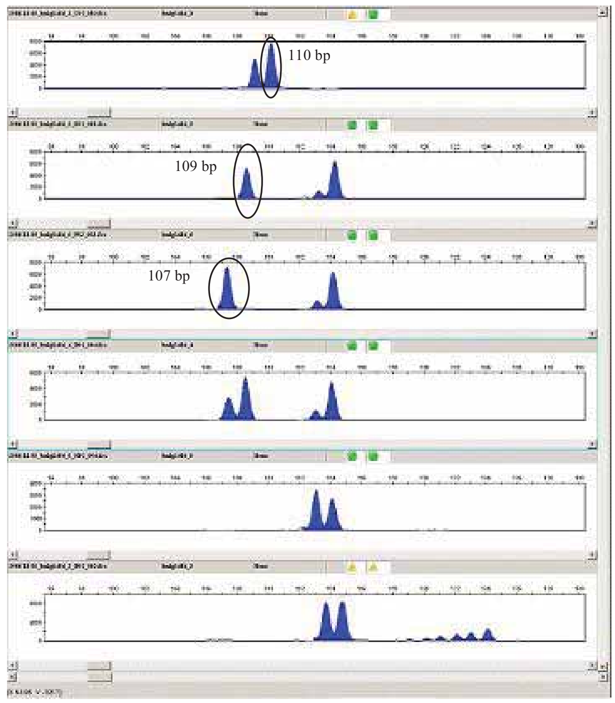

Figure 1. DNA fingerprinting of six commercial maize varieties after amplification with SSR primer pairs bnlg1484.

Table 1. DNA fingerprinting of 10 maize varieties based on 8 SSR primer pairs.

Variety	SSR locus-allele size (bp)							
	bnlg1017	bnlg1237	bnlg1429	bnlg1526	bnlg1662	bnlg1720	bnlg1940	phi037
OP_1	165-169, 177-178	153-156	185-188, 190-200	106, 109-113	120-124, 147-158	230-246	217-219	154-155
OP_2	163-165, 177-180	153-156, 176-180	175-177, 187-194	109-113	136-143, 146-151	230-233	216-230, 234-240	126-129
OP_3	185-190, 196-202	153-156, 174-182, 188-195	180-186	107-113	149-156	231-233	206-213, 217-228	126-135, 154-155
F1_A	166-169	153-158	188-195	107-109, 113	136-141, 150-156	232-236	215-219, 223-230	127-129, 154-155
F1_B	167-171, 194-200	153-158, 180-190	175-188	107-113	136-143, 151-156	158-160, 164-169	216-230	126-130
F1_C	180-184, 192-196	200-212	105-106, 108-120	120-124, 153-158	230-236	208-213, 219-223	130-133, 160-161	
F1_D	166-169, 177-180	153-158, 176-180, 188-195	188-195	109-113	139-145, 156-163	223, 236, 245-251	213-219	127-129, 154-155
F1_E	166-169, 177-180	153-156, 188-195	188-195	109-113	145	223, 226, 237-239	213-219	127-129
F1_F	165-169	170-180	185-189, 189-201	106, 109-121	120-126, 148-158	231-233, 240-245	206-213, 221-228	154-155

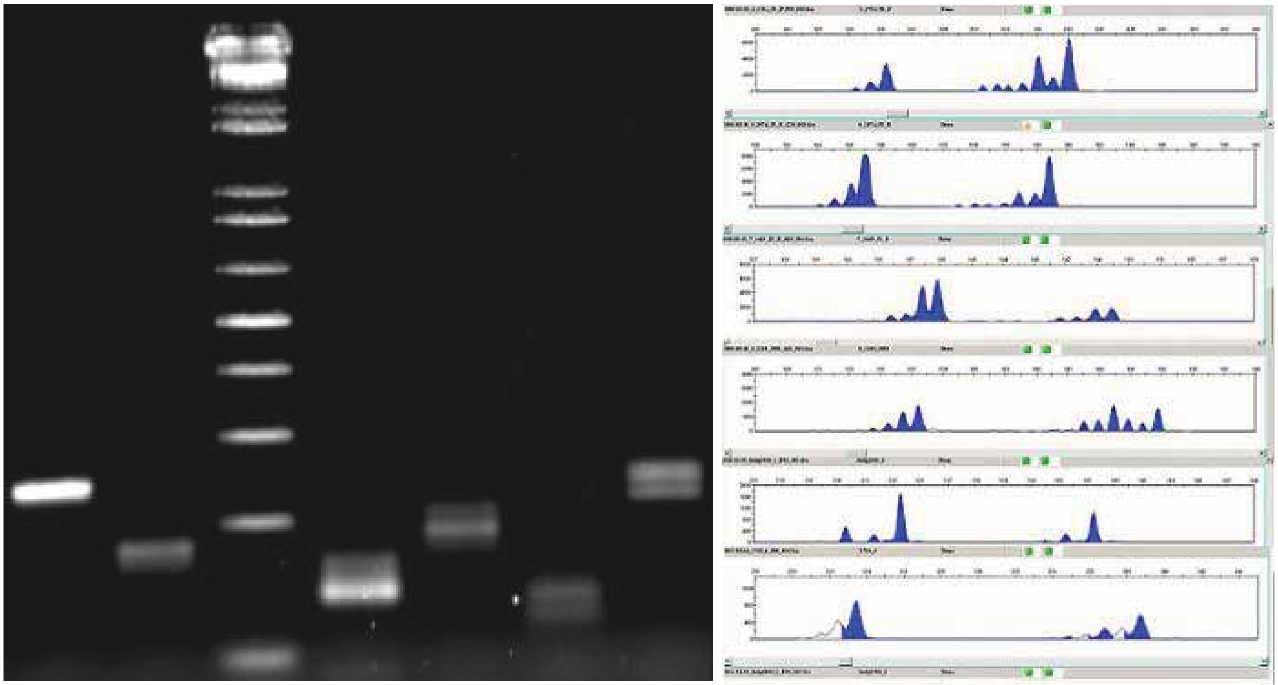

Figure 2. Comparison between agarose gel and electropherogram in visualizing 5-10 bp distance between two alleles. A. bnlg1784 - F1_F, B. bnlg1074 - F1_E, C. bnlg1449- F1_D, D. bnlg2238 - OP3, E. bnlg1808- F1_D, F.bnlg1720- F1_E, M= 1 kb Plus DNA Ladder.

very useful for the purpose of protection of our varieties against piracy, counterfeiting and illegal cultivation.

The different genetic background of varieties enabled us to examine differences in term of polymorphism between two varieties. On an average, 81 of the 98 primer pairs (data not shown) were polymorphic between two random cultivars, both for hybrids or open-pollinated varieties. However, genetic similarity between two cultivars that shared one parent (F1_D and F1_E) was higher. Only 32 of the 98 SSR primer pairs were able to generate polymorphic alleles. This information can be so interpreted that we need only 2 SSR primer pairs for distinguishing two maize cultivars having no relationship, while we require at least 3-4 primers to differentiate cultivars that share one parental line. From this information, we can deduce that 4 randomly selected SSR primer pairs should be sufficient to discriminate any two cultivars.

Selection of SSR alleles appropriate for purity test assessment

Allele uniqueness and codominance are two absolute criteria that should be fulfilled for selecting molecular markers to be further employed for purity tests. The unique allele size facilitates rapidly differentiation of the desired genotyped F_1 from other cultivars, while codominance plays a significant role as a reliable marker to confirm the allelic position of parental lines. These characteristics are not always easy to obtain, especially with a limited amount of markers. As a solution, multiplexing of two promising dominant markers can be performed (Fig. 3). SSR primer pair bnlg1189 produced an identical pattern for F_1 and its female, while bnlg1194 generated the same allele for F_1 and its male. Thus, multiplexing between bnlg1189 and bnlg1194 is very effective to produce a heterozygous pattern for F1_C that possesses 4 alleles, combining each 3 alleles from its parental lines.

Though conveniently utilized, the operation of Genetic Analyzer requires many expensive consumables. This certainly contributes to the high cost of data point per sample, a fact that limits its utilization for routine application of genetic purity tests. For this reason we intentionally selected markers that could be clearly separated on 2% agarose gel. Different primer pairs that were able to generate 2 alleles possessing size differences between 5 and 10 base pairs were tested (Fig. 2). The result revealed that 6 bp distance is the critical point where two alleles begin to resolve on 2% agarose gel after running for 3 hours. Hence, for the purpose of certainty, we preferred to utilize markers that were able to produce 2 alleles having a distance between 7 and 10 bp. Based on all the conditions mentioned above, we finally obtained 11 potential SSR primer pairs as markers for the seeds purity test of our 10 varieties (Table 2).

DNA isolation from 100 dried maize kernels

An ideal DNA isolation technique should be simple, result in high DNA quantity and quality, and only require a short period to perform. To produce high quantity and quality of DNA, we modified several existing DNA isolation methods. We incubated seeds for 2 hours to let them became easier destructed. Such a long incubation in extraction buffer containing proteinase K was very useful to eliminate high protein and other cell debris from the seed. Further purification of other contaminants that normally disturb the PCR process was performed by two-time extraction with chloroform:isoamyl alcohol (24:1). We observed that two-time purification with this solution produced better DNA quality than one time (data not shown). Following this modification, at the end we obtained about 500 ng of good quality DNA that was suitable for PCR analysis.

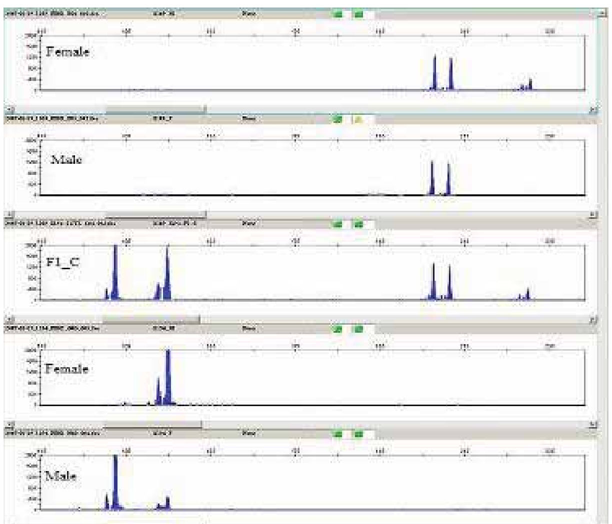

Figure 3. Multiplexing between bnlg1189 and bnlg1194 that can be employed as a marker for the purity test of F1_C. A, B. bnlg1189; C. bnlg1189-1194; D, E. bnlg1194.

At the beginning a manual grinder (like screwdriver) was employed to crush the seeds. But this process required different time depending on the person performing the task. Therefore, two semiautomatic seed grinding machines were designed, combining an adjustable lever and the power of machine; these could easily crush the seeds that were immersed in a syringe tube containing the extraction buffer in only a few seconds. The first instrument with 4 axes enabled us to pound 100 seeds in only 2 hours. To further shorten the grinding time, we designed another instrument possessing 24 axes. This significantly accelerated the grinding process to 4 times faster compared with manual grinding. Overall, we can draw a conclusion that our system is simpler, faster and more convenient compared to other seed-based DNA extraction methods.

Seed purity test

As described above, the conventional Field Purity Test is time-consuming and complicated. Furthermore, the results can be ambiguous occasionally due to bad weather (too much rain or too high temperature) or heavy infestation by pests and diseases. Moreover, sometimes marketing teams require quick information about the seed status before selling it to the public. In these situations, genetic purity tests will play a role as an alternative supporting tool for field purity tests.

Figure 4 illustrates a typical genetic purity test that normally compares samples with true-to-type control and

Table 2. Unique SSR markers that can be used to identify cultivars and also for purity tests.

No.	Variety	SSR markers	Allele size (bp)		
				Male	Female
1	OP_1	bnlg0615	194-198, 207-217	-	-
2	OP_2	bnlg1074	167, 178-183	-	-
3	OP_3	bnlg0619	225-230, 242-246	-	-
4	F1_A	nc009	112, 127-139	112	127-139
5	F1_B	bnlg1237	153-158, 180-190	180-190	153-158
6	F1_C	bnlg1189-bnlg1194*	208-212, 226-230 and 133-135,142-145	133-135, 142-145, 208-212	133-135, 208-212, 226-230
7	F1_D	phi031	185-186, 222	185-186	222
8	F1_E	bnlg1360	107-109, 124-130	107-109	124-130
9	F1_F	dup028	76-78, 91-96,103-112	103-112	76-78,91-96
10	F1_G	bnlg2122	210-212, 215-220, 227, 229-233	227,229-233	210-212, 215-220

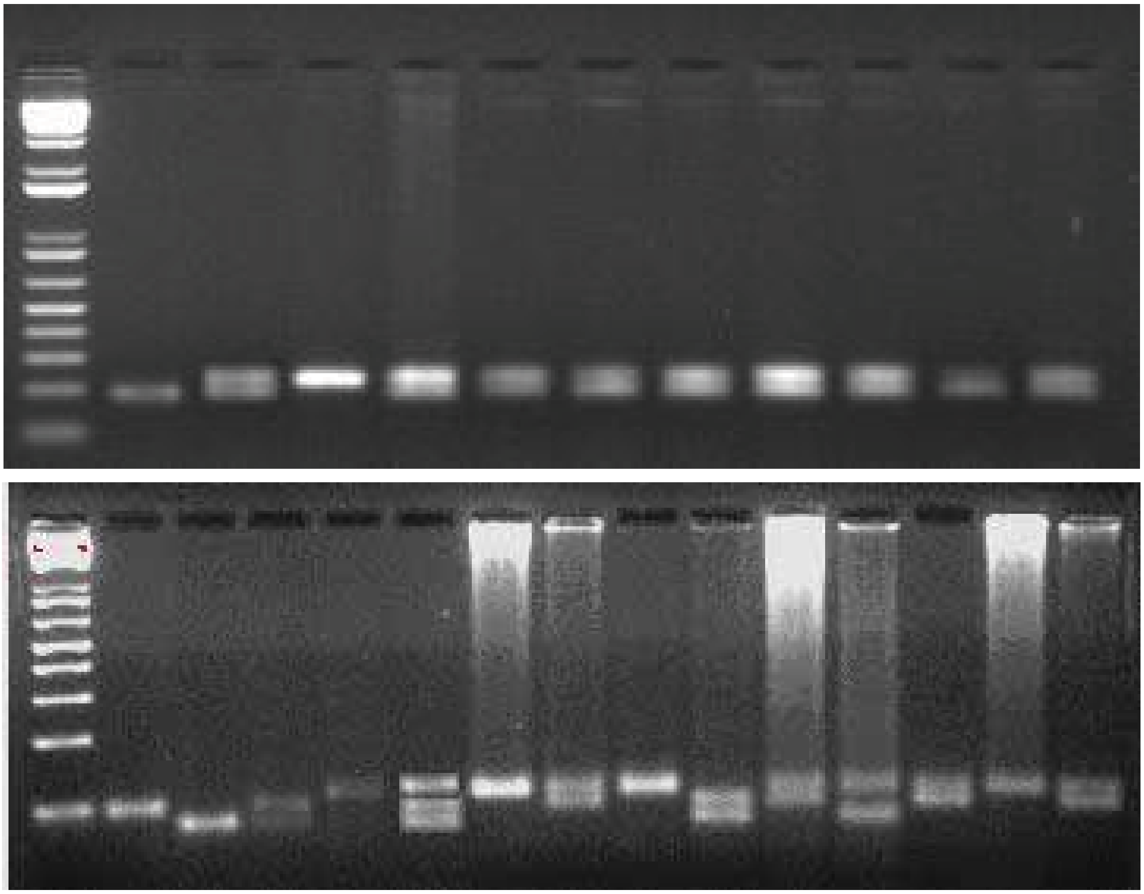

Figure 4. Representative genetic purity test of true-to-type samples of F1_D (a) and off-type samples of F1_F (b) after separation on 2% agarose gel for 180 minutes. M=1kb Plus DNA Ladder.

its parental lines. The good result is shown by Figure 4a, wherein all tested F1_D samples possessed an identical pattern to the control. Hence, the purity of those samples reached 100%. In contrast, none of samples evaluated in Figure 4b have the same pattern such as revealed by the control of F1_F. This means the purity of these samples was zero. Four different off-type patterns were observed in samples after amplification with dup028 primer pairs: samples possessed the same allele with the male (X1); allele of samples was identical with the female allele (X2); the third and fourth off-type pattern represented what happened if 2 inbred lines as female components were mixed up in a female group. By knowing how many off-type samples were found in 100 seeds tested, precise information of purity can be quickly calculated. The DNA isolation work takes only one day, while it takes also one day to accomplish the genetic purity test. In general we only require 2 working days to provide purity data of 3-4 different seed lots.

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Analysis of mutation types for *waxy* gene of waxy maize landraces from South-Western China

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Abstract. Germplasms of waxy maize in Southwestern China are extremely abundant. To further understand the mutant mechanism and gene evolution of *waxy* gene, six waxy maize landraces and two flint maize landraces from Southwestern China and key inbred line Huangzaosi, which was representative flint maize germplasm from huanghuaihai area of China, were used to clone the integrated coding region of *waxy* gene. Through aligning the sequence of *waxy* gene, the molecular mechanism of waxy phenotype of waxy maize landraces was analyzed and predicted. Except several synonymous substitutions in extrons and insertions/deletions in introns, we have found that a 16-bp insertion in the ninth exon in three waxy maize landraces from Southwestern China, which might cause frame-shift mutation of translation and terminating codon occurred early. At the same time, five of six Chinese waxy maize landraces contain deletion of 15bp in the tenth exon, which is the starting site of the conserved domain of gycosyl transferases group 1, and it could induce inactivation of the granule bound starch synthase. Therefore, mutations described above, which are different from the reported waxy mutant arose by transposable elements and spontaneous mutation, are presumably responsible for the mutant phenotype in part of Southwestern China waxy maize. We could even presume that the mutations were new mutant types of *waxy* gene.

Keywords: waxy maize landrace; *waxy* gene; DNA mutation; mutation type

Introduction

The *waxy*(*wx*) locus of maize encodes a starch biosynthetic enzyme responsible for the synthesis of amylose in the endosperm, pollen and embryo sac(Tsai 1974; Kloesgen *et al*. 1986). The glutinous endosperm of the waxy maize is controlled by recessive mutation of *waxy* and it is almost composed of all the amylopectin. So waxy maize with many excellent characters, is mainly used in food production, textile, adhesive and paper industries.

Waxy maize is a special type of cultivated maize and has grown in China for long history. A wide range of genetic diversity has been observed in Chinese waxy maize germplasms(Huang and Rong 1998). There are about 901 waxy maize landraces in China, approximately 80% of them distribute in Southwestern China, which is the original area of Chinese waxy maize. We have carried out a lot of research for partial genetic resources and observed that there are abundant genetic diversity on agronomic traits, quality characters, molecular markers and other aspects in waxy maize landraces from Southwestern China. Genetic diversity among 22 inbred lines of waxy maize has been analyzed using random amplified polymorphic DNA (RAPD) markers (Huang and Rong 1998). Liu *et al*.(2005) carried out comparative analysis of genetic diversity in landraces of waxy maize fromYunnan and Guizhou using simple sequence repeats (SSR) markers. Recently, Tian *et al*.(2008b) studied the systematic position of waxy maize in genus *Zea*, and investigated the way of origin and dynamics of population evolution for Chinese waxy maize. They concluded that Chinese waxy maize originated from *waxy* gene mutation from Chinese flint maize. However, the formation mechanism and biological effects of mutations still remain obscure.

During the last century, many mutant alleles have been isolated and carried out genetic research because of the viable and easily scored phenotypic expression of *Wx* mutants. The molecular mechanisms of *waxy* mutants for American waxy maize have been analyzed and the results have shown that there are unsatble and stable *wx* mutations. The molecular basis of unstable *wx* alleles are that the transposable elements *Activator*(*Ac*), *Dissociation*(*Ds*), *Suppressor-mutator*(*Spm*), *Enhancer*(*En*) and *retrotransposon* insert into flanking region0intron and exon of *waxy* gene(Fedoroff *et al*. 1983; Pereira *et al*. 1986). In addition, analysis of stable *wx* mutants demonstrated that spontaneous mutations usually involved the insertion or deletion of DNA(Wessler and Varagona 1985). Nowadays, the research on molecular and genetic mechanism of phenotypic expression of *Wx* mutants for Chinese waxy maize has been reported fewly. Comparison of the sequences of exon 9-14 from waxy maize landraces from Southwestern China with the published *wx* sequences identified a 15 bp deletion in exon 10 of partial landraces (Tian *et al*. 2008a). Interestingly, the 15 bp deletion in exon 10 and a 30 bp deletion removing the last 4 bp of exon 7 were observed in partial Chinese waxy maize respectively by Fan (Fan *et al*. 2008).

The above results show that there are differences of wx mutations between Chinese waxy maize and American waxy maize. To further understand the mutant mechanism for the rise of glutinous phenotype and gene evolution of waxy gene in waxy maize landraces from Southwestern China, the integrated coding region of waxy gene should be sequenced. In this study, genomic sequences of exon 2-14 of waxy maize landraces from Southwestern China are sequenced. Compared these sequences with *wx* sequences of nonglutinous maize, we have found some mutation types that are different from the reported *wx* mutant and are recognized as new mutant type of *waxy* gene.

Materials and methods

Plant materials

The strains including six waxy maize landraces, two flint maize landraces from Southwestern China, key inbred line Huangzaosi, which was representative flint maize germplasm from huanghuaihai area of China are used in this study (Table 1). All of them are supplied by Maize Research Institute of Sichuan Agricultural University and are studied on genetic diversity widely. The sequences of *waxy* gene for American nonglutinous maize are downloaded from GenBank and used as comparisons in this study. The sequence accession numbers of American nonglutinous maize are X03935, AF544096 and AF544098, respectively.

Genomic DNA extraction

All strains were grown from seeds and DNA was extracted from 20d leaves using the protocol described by Sambrook and Russell with minor modifications (Sambrook and Russell 1989). The quality and concentration of genomic DNA were detected by agarose electrophoresis and ultraviolet spectrophotometer.

PCR and DNA sequencing

Primers for three overlapping regions for exon 2-14 were designed based on conserved regions of full length sequence of waxy gene that had been published in GenBank(Fig.1). The sequences of primers were as follows:

1F: 5'-CAGGTTCTGTTCCGTTCCGA-3',

1R: 5'-GGACTGGTAGTTGCTCTTGAG-3'ÿ

2F: 5'-AGATCAAGATGGGAGACGGGTA-3',

2R: 5'-AGGCGCATGATGTTGTCGAG-3'ÿ

3F: 5'-TGCGAGCTCGACAACATCATGCG-3',

3R: 5'-AGGGCGCGGCCACGTTCTCC-3'.

The amplified region of the three pairs of specific primers covered the integrated coding sequence of *waxy* gene. Primers were designed using DNAMAN program. PCR products were purified by Agarose Gel DNA Purification Kit (TaKaRa Ver.2.0), and were cloned into PMD18-T vector (TaKaRa) and sequenced using the forward and reverse primers. At least three independent clones were sequenced on ABI 3730 Automated DNA

Table 1 Experimental plant materials used in this study

Classification	Cultivar	Sources	Accession numbers
Waxy maize from Southwestern China landraces	Liuchengnuo	Guangxi, Liucheng	EU747866
	Zaobainuo	Yunnan, Mengzi	EU747869
	Piantounuo	Yunnan, Zhenkang	EU747867
	Silunuo	Yunnan,Xishuangbanna	EU747868
	Huanuo	Guizhou, Liping	EU747865
	Changchongbainuo	Guizhou, An'long	EU747864
Flint maize from Southwestern China landraces	Xiaobainian	Guizhou, Jian'e	EU747870
	Xiaohuang	Guizhou, Ceheng	EU747871
Inbred line (nonglutinous maize)	Huangzaosi	Huanghuaihai area	EU747872
Ameician maize (nonglutinous maize)	M24258		X03935
	Pa91		AF544096
	Tx601		AF544098

Sequencer. Sequence data have been deposited in GenBank under accession numbers EU747864- EU747872.

Data Analysis

Sequenced fragments of waxy gene for each strain were spliced by Contig-Express program, then full length sequence of *waxy* gene for 12 materials were aligned using ClustalX program.

Results

The results of sequence alignment for *waxy* gene show apparent sequence difference between the glutinous and nonglutinous strains, which may be responsible for the mutant phenotype. The mutations contain several synonymous substitutions and insertion /deletion in introns and exons. Particularly, short direct repeat diversity is observed in intron 1, insertion mutation is in exon 9 and deletion mutation is in exon 10.

Mutations in the first intron

In the first intron, we identified the mutation of short tandem repeats at two positions through comparison of waxy sequences of glutinous with nonglutinous maize. At the first position, the insertion of short direct repeat was only found in nonglutinous maize. Two copies of 13bp (GTTCCGTTCCGAT) repeat was found in Xiaobainian, the flint maize from Southwestern China, and three copies of 5bp(CCGAT) tandem repeat were found in other nonglutinous strains (Fig 2A). However all the glutinous maize from Southwestern China did not contain direct repeat sequence at the position. At the second mutation position of the intron, the sequences of all the glutinous maize and xiaobainian are identical, they are more four copies of 2-bp (TC) repeat unit with the boundary sequences of "TG" than other strains in this study(Fig 2B). All the insertions of repeat unit in the first intron are located in the interior of intron, so they are not presumably responsible for the mutant phenotype.

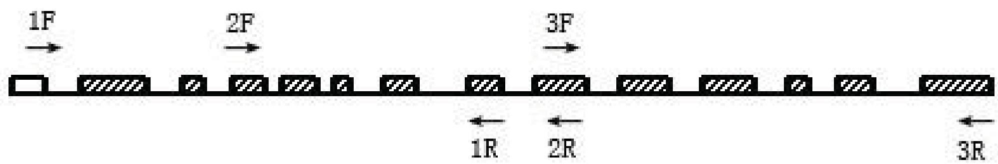

Fig.1. Schematic diagram of *waxy* gene in maize. Boxes represent exons and only shadows are translated; lines between exons represent introns. Arrows indicate locations and directions of the PCR primers used in this study.

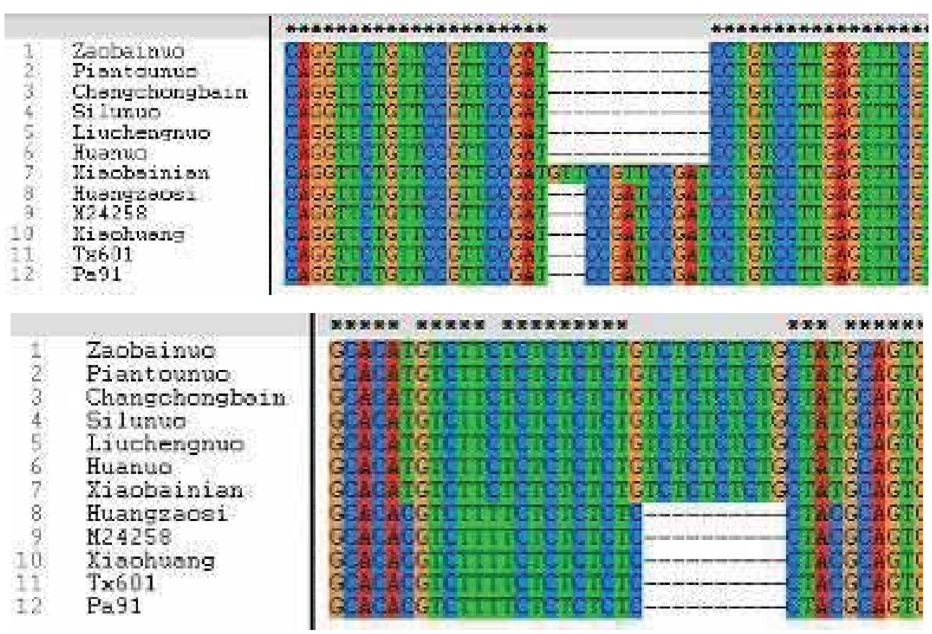

Fig. 2. The mutations in intron 1.

A insertion mutation in the ninth exon

In the ninth exon, a 16-bp(ACAACATCATGCGCCT) direct repeat are identified in Zaobainuo,Piantounuo and Changchongbainuo, but the same insertion is not found in nonglutinous maize and other glutinous maize from Southwestern China (Fig.3). The 16-bp direct repeat in the ninth exon results in frameshift mutation and terminating codon occurred early and generated incorrect production of translation. So the insertion is presumably responsible for the mutant phenotype.

A deletion mutation in the tenth exon

By sequences aligning, a 15-bp(AACAAGGA GGCGCTG) are absent of the exon 10 in Liuchengnuo, Silunuo, Changchongbainuo, Zaobainuo and Piantounuo (Fig 4). For the six glutinous maize landraces from Southwestern China, the deletion is not found in Huanuo only. The deletion of *Wx* do not lead to frameshift mutation for translation, but removing five amino acids0(NKEAL) of integrity protein. Further analysis of function domain for granule-bound starch synthase indicates that amino acid sequences, NKEAL, coded by the 15-bp deletion locate in the starting site of the Glycosyl transferases group 1 domain of GBSS1 (Fig 5). So the deletion is presumably made the enzyme inactivation.

Discussion

In cereals, the glutinous phenotype has been shown to be resulted from a dramatic reduction in synthesis of amylose due to mutations or insertion/deletions in the *waxy* gene. The *waxy* phenotype is a easily scorable, nonlethal mutant phenotype, so a large number of wx alleles mutants have been isolated and realized molecular characterization. For example, a single-nucleotide mutation in an intron 1 splice donor site of the *Waxy* gene was responsible for the change in endosperm starch leading to the glutinous phenotype of rice, and the splice donor mutation had a single evolutionary origin and that it probably arose in Southeast Asia(Olsen and Purugganan 2002; Olsen *et al.* 2006). In maize, many *Waxy* mutations are caused by insertions of transposable element. For example, the *wx* mutants of *wx-m9* and *wx-844* contained the *Activator*(*Ac*) and *Suppressor-mutator*(*Spm*) transposable element respectively, and the studies of three wx alleles—*wx-m9* , *wx-B4,* and *wx-m1*— demonstrated that the transposable *Dissociation*(*Ds*) element could function as an

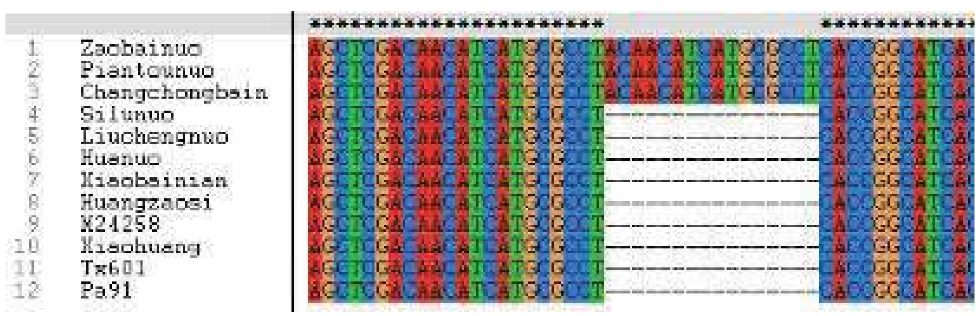

Fig. 3. A 16-bp insertion in exon 9.

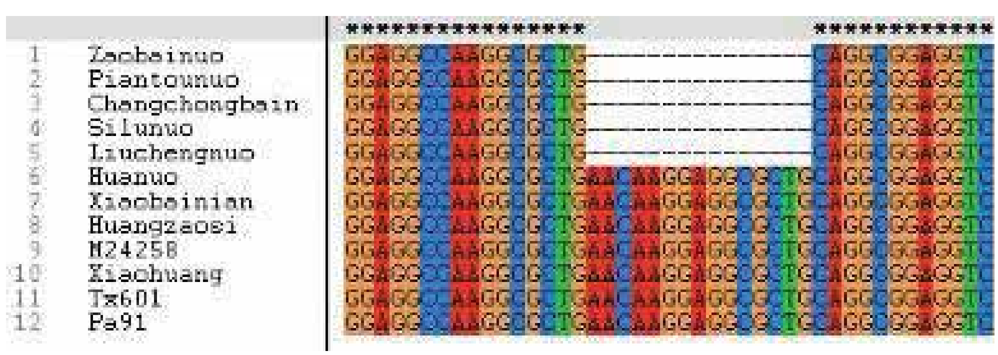

Fig. 4. A 15-bp deletion in exon10.

Fig. 5. The prediction result of conserved domain for GBSS1. Glycos_transf_1: the domain of glycosyl transferases group 1; GlgA: the domain of glycogen synthase

intron(Fedoroff *et al.* 1983; Pereira *et al.*1985; Wessler 1989, 1991). Retrotransposons are important agents of spontaneous mutation in maize. The wx alleles of *wx-Stonor*, *wx-B5,* and *wx-G* were found to contain retrotransposon insertions, but the insertion sites and relative orientations were different. *wx-Stonor* was inserted at the intron 5-exon 6 junction, and both the *wx-B5* and *wx-G* elements were inserted into introns, *wx-B5* and *wx-G* in intron 8(Wessler and Varagona 1985; Varagona *et al.* 1992). However, we have not found transposable element in the sequenced region of *waxy* gene for the six waxy maize landraces from Southwestern China in this study, and the glutinous phenotype of them presumably are caused by other mutations.

The insertion or deletion of DNA is one of the main *wx* spontaneous mutation in maize. The size and position of insertion or deletion are the important influential factors for the expression of waxy gene. Wessler et al.(1990) reported that the molecular basis of the mutant phenotypes for *wx-B1*, *wx-B6*, *wx-B*, and *wx-C4* was evident from their respective deletions. *wx-B1* is missing 655 bp upstream of the transcription start, all of exon 1, and part of introns. *wx-B* is also missing sequences upstream of the transcription start site in addition to exon 1, intron 1, and the AUG start in exon 2. *wx-B*, *wx-B6*, and *wx-C4* are missing the amino terminus which encodes the transit peptide. However the deletion in a nonmutant allele *wx-W23* is restricted to intron 6, and the RNA splicing is normal(Wessler *et al.*1989). In this study, we find a 16-bp insertion in exon 9 of three glutinous maize landraces from Southwestern China (Fig 3), and the sequences of the insertion are identical with the vicinity sequence. So there are two copies of the 16-bp tandem repeat in Changchongbainuo, Piantounuo and Zaobainuo. The mutation of insertion may result in frameshift mutation and is responsible for the mutant phenotype. The mutation type of 16-bp insertion for waxy gene is firstly reported in this study, but not other cereals strains (e.g., rice, wheat and barley). We identified an identical mutant mode in intron 1 of Xiaobainian the flint maize from Southwestern China, and it contained two copies of a 13-bp repeat when insertion mutation happened. However, at the position, there are three copies of the 5-bp(CCGAT) tandem repeat in other nonglutinos maize, and there is not 5-bp repeat in all the waxy maize landrances from Southwestern China. The mutant mode of repeat units added or deleted were also found in wx alleles of *Wx-LineC*, *wx1240* and *wx-c*(Okagaki *et al.* 1991). The repeat of the intron sequences TCCA, which is located in intron 7 and found two times in *Wx-LineC*, and three times in *wx1240* and *wx-c*. Okagaki et al. (1991) reported that the above deletion formation initiated when short direct repeats aligned out of register during DNA replication, the process was called slipped mispairing (Glickman and Ripley 1984). So in this study, we have infered that the mutation of repeat units added or deleted containing in two positions of intron 1 may be caused by slipped mispairing too. However, the insertion mutation in extron 9 is different from the above deletion polymorphism in intron 1, so the mechanism of mutant formation is not identical. The further research is needed in order to know the mechanism of insertion formation in exon 9.

We identified a 15-bp deletion in exon 10 of five waxy maize landraces besides above insertion or deletion mutations. Interestingly, the 15-bp deletion was also observed in partial Chinese waxy maize by Fan and waxy maize landraces from Southwestern China by Tian recently (Fan *et al*. 2008; Tian *et al*. 2008a). Because of the occurrence of the same deletion in multiple independent alleles, we presume that this may be a favored site for deletion formation and the mutation may be one kind of major mutation type of Chinese waxy maize,especialy waxy maize landraces from Southwestern China. But the mutation type have not been observed in *waxy* gene of other cereals so far. The 15bp deletion results in removing five amino acids (NKEAL) of granule-bound starch synthase protein. The deletion of peptide segment locates in the initiation region of an important function domain (Fig 5). So we infer that the deletion is responsible for the mutant phenotype too. But we know nothing about the mechanism of deletion formation.

The insertion in exon 9 and the deletion in exon 10, either of them may be result in the mutant phenotype. In this study, we have found that three waxy maize landraces contain both 16-bp insertion in exon 9 and 15-bp deletion in exon 10. Do the mutations of the two positions generate simultaneously or independently. If the mutations generate independently and represent independent mutational events, which mutation is the main cause for the mutant phenotype, which mutation do generate early, or are there correlation between the two mutations. All the above problems need to be investigated further.

Acknowledgements

We thank academician Tingzhao Rong for direction. This work was supported by State Key Development Program for Basic Research of China (grant no. 2006CB101700) and Key Project of Department of Education, Sichuan Province (grant no.2006A018).

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Formation of maize haploid plant through Bulbosum method

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Abstract. Hybrid variety can improve maize productivity relatively higher than that of open pollinated variety. One alternative technology which used to speed up producing pure line was anther culture, but success of maize anther culture to produce haploid plant was still low. To anticipate the problem, Bulbosum method will used in this research. The method was used to be done in Australia, Germany and New Zeland. One parent of crossing in Bulbosum method was known as haploid inducer line which has RI-nj gene and expressed with anthocyanine color in seed from crossing. Haploid seeds were produced from crossing identified mark with purple color. Sexual cross between non commercial line with purple seed color (No. 3059 and No. 3490) and Bisma, Sukmaraga and Arjuna varieties were done in Cikeumeuh experimental farm of ICABIOGRAD. Chromosome analysis was done at tip roots of germination purple and yellow seeds. Result of sexual crossing showed that there was no purple seeds produced by F1, but it was produced by BC1 (F1 x No. 3490). The highest percentage of purple seed formation was 31.58 % which came from BC1 [F1 (Bisma x No. 3490) x No. 3490] with percentage of purple seed per ear was 1.5 % and average of purple seed per ear was 3.82. Result of chromosome analysis showed that chromosome number of germinated purple seed was a half (10 $\pm$ 1) of that of germinated yellow seed. Back cross was done to get haploid inducer line especially for tropical maize which produced significantly increasing purple seeds per ear.

Key words : Maize (*Zea mays* L.), haploid plant, bulbosum method

Introduction

Maize (*Zea mays* L.) is the second important food crops after rice in Indonesia. New variety with high yielding is needed to increase national productivity. Highly demand of maize in Indonesia as food and poultry cause highly imports. One alternative method to increase productivity is developing hybrid maize. According to minister of Agriculture, increasing productivity of maize was be connected to seed (Apriantono, 2008). Right now use of hybrid maize only 40 % of total using seed. Replace composite with hybrid seed was expected to increase national productivity to supply of demand or even export.

Thamrin (1988) proved that using hybrid maize was effective method to increase productivity. Dihaploid homozygous plant (pure line) is needed in breeding to produce hybrid variety. Bulbosum method can be used to speed up producing haploid plant in barley (Devaux, 2003; Campbell *et al.*, 2003). Bulbosum method was crossing between 2 wild relatives species or crossing between the same species which one of them have ability to induce haploid plant.

Some maize cultivar naturally produces haploid plant even in low percentage or less than 0.001 % (Phoelman and Borthakur, 1969). Base on the ability of developing bulbosum method in maize, crossing was done between two maize cultivars which one of them has ability to induce haploid plant. Chromosome of the haploid plant can be multiply to be double haploid homozygous. By this method dihaploid homozygous plant can be achieved in 2 generations only, while conventionally dihaploid homozygous need 7 or more generations (Araus, 2008). In last decade, the bulbosum method was reported in Australia and China even in Europe (Eder and Chalyk, 2002). Crossing was done between the same lines which call inducer haploid line. Result of crossing was haploid maternal, so the characteristic if inducer line was not affected to haploid line which produced (Eder and Chalyk, 2002).

The aim of experiment was to get haploid plant of maize and maize line which can induce haploid plant.

Materials and methods

The experiment was conducted at Biology Cell and Tissue Laboratory and Cikeumeuh experimental farm of ICABIOGRAD Bogor, Indonesia in 2008. Three maize varieties (Bisma, Sukmaraga and Arjuna) and non commercial line with purple seed color (No. 3059 and No. 3490) were used in this experiment. Crossing was done at Cikeumeuh experimental farm with non commercial line (No. 3059 and No. 3490) as male parents and Bisma, Sukmaraga and Arjuna varieties as female parents. Haploid seeds of

F1 with purple seed in endosperm were selected and isolated. Back cross were done between F1 as female parent and the same non commercial line as male parent. BC1 chromosome was analyzed at tip roots of germination and compare between purple seed and yellow seed. BC1 with purple seed were back cross again with the same male parent. The haploid seed which purple color in endosperm were isolated and selected.

Result and Discussion

Result of the experiment showed that the first crossing between Bisma, Sukmaraga and Arjuna varieties as female parents with non commercial line (No. 3059 and No. 3490) as male parents was success 100 %. Nothing seed with purple color was found in endosperm from all ear of F1.

To get purple seed, F1 seeds were use as female parent of crossing (back cross) with non commercial line (No. 3059 and No. 3490) as male parents. This crossing was also success 100 %. Seeds of BC1 were produced from back crossing between F1 (Bisma x 3490), F1 (Sukmaraga x 3490), F1 (Arjuna x 3490) as female parents with No. 3490 as male parent.

The highest percentage of ear with purple seeds produced by BC1 from back cross between F1 (Bisma x 3490) with No. 3490 (31.58 %). Even though the percentage of ear with purple seed was quite high, percentage number of purple seed per ear was very low (1.50 %) and the average of purple seed was 3.82 per ear (Table 1). This result was lower than that of Eder (2002) which percentage of haploid seed was 2.7 – 8.0 %.

Percentage of ear with purple seeds of Sukmaraga variety was 14.29 % with percentage of purple seed per ear was 1.41 % and average of purple seed was 3.67 per ear. For Arjuna variety percentage of ear with purple seeds was same with Sukmaraga. Percentage of purple seed per ear was lower (0.57 %) and average of purple seed was 1.62 per ear (Table 1).

From back cross 1 showed that only line No. 3490 had ability to induced ear with purple seed. Line No. 3059 had no ability to induced ear with purple seed (Fig. 1). The purple seed was expected had only a half set of chromosome than the parents, so chromosome analysis must be done.

Result of chromosome analysis showed that chromosome number of germination from purple seed was 10 ± 2. Chromosome number of tip root germination from yellow seed was 20 ± 1 (Fig. 2). Description of Bisma variety said that chromosome number of Bisma was 20. So if chromosome number was only 10 means that purple seed was only carried a half set of the chromosome.

Table 1. Formation of purple seed from backcross 1

Backcross (BC1)	% of ear with purple seeds	% of purple seed/ar	Average of purple seed/ear
F1 (Bisma x 3490) x No. 3490	31.58	1.50	3.28
F1 (Sukmaraga x 3490) x No. 3490	14.29	1.41	3.67
F1 (Arjuna x 3490) x No. 3490	14.29	0.57	1.62

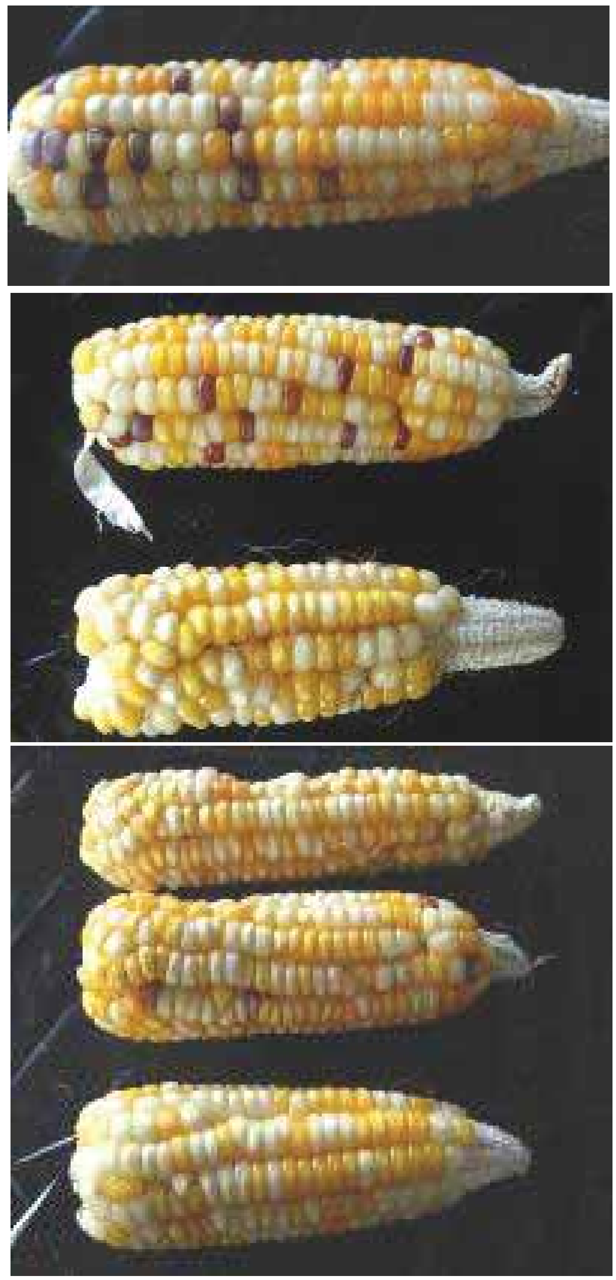

Figure 1. Ear of maize from Back Cross 1

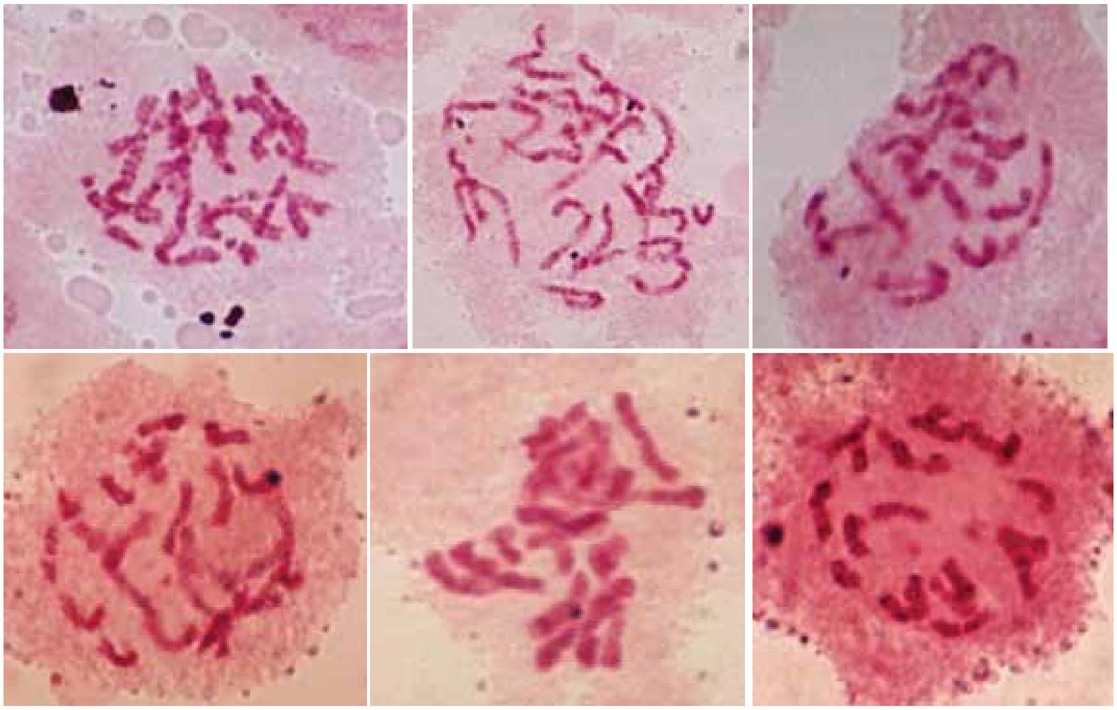

Figure 2. Chromosome analysis of BC1
Upper= Chromosom of BC1 with yellow seed: 20±2
Lower = Chromosom of BC1 with purple seed: 10±2

Figure 3. Ear of maize from Back Cross 2
Upper Bisma x 3490) X 3490 (upper)
Lower BC1 (Sukmaraga x 3490) X 3490 (lower)

Table 2. Formation of purple seed from backcross 2

Backcross (BC2)	% of ear with purple seeds	Average of purple seed/ear	Average of purple seed/total ear
BC1 (Bisma x 3490) (white seed)	16/56 (0.29)	325/16 (20.31)	325/2591 (0.11)
BC1 (Bisma x 3490) (yellow seed)	5/43 (0.12)	102/5 (20.4)	102/18 (0.12)
BC1 (Sukmaraga x 3490) (white seed)	2/26 (0.08)	26/2 (13.0)	26/390 (0.07)
BC1 (Sukmaraga x 3490) (yellow seed)	0	0	0
BC1 (Arjuna x 3490) (white seed)	0	0	0
BC1 (Arjuna x 3490) (yellow seed)	0	0	0

Backcross 2 was done to multiplying the purple seed with numbers of BC1 as female parent which had ability to induced purple seed formation.

Result of BC2 showed that the highest percentage of ear with purple seeds produced by BC1 with white seed as female parent (29 %) (Table 2). Although the percentage was not more than that of BC1, average of purple seed per ear at BC2 was higher (20.4 and 30.31) than that of BC1 (3.82). Back crossing of BC1 (Arjuna x 3490) as female parent was failed to produced purple seed or white seed. Purple seeds of Sukmaraga were produced from BC1 with white seed, but not with yellow seed.

Producing purple seed in BC2 was better than that of producing haploid seed by Eder (2002), but chromosome of the purple seed did not analysed yet. Analysing or calculating number of chromosome and technique of polyploidisation will be done in the next experiment.

Conclusions

- Purple seeds were produced after back cross 1 which F1 was used as female parent and non commercial line as male parents. Non commercial line which induce purple seed was No. 3490 and the best variety was induced to produce purple seed was Bisma.
- The highest percentage of ear with purple seeds was 31.58 %, produced by BC1 back cross between F1 (Bisma x 3490) with No. 3490 with average of purple seed per ear was 3.82. Percentage of ear with purple seeds formation in BC2 were lower than that of BC1, but average of purple seed per ear were higher than that of BC1 (20.31 and 20.4).
- Number of chromosome at root tip of germination from purple seed (BC1) was a half of that of yellow seed of Bisma.

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Study on selection of waxy-QPM maize lines with SSR markers

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Abstract. Both waxy corn and Quality Protein Maize (QPM) are high quality feed and food. Most waxy corn is consumed as food in Asia for its nutritional value and edible value, however, the development has been limited for the narrow genetic background. This study was aimed to create a new type of corn with advantages of waxy corn and QPM and seek for a new way to expand the genetic background of QPM and waxy corn germplasm in order to meet the demand of maize production and breeding. Eighteen stable waxy-QPM lines were developed from 6 crosses combination of 2 QPM and 6 waxy lines with SSR marker assisted selection. The total amino acid and lysine content of most lines was improved and most of the lines were preferable in term of disease resistances, endosperm hardness and agronomic traits.

Key words: Selection Waxy-QPM Line SSR Marker Amino acid Lysine

Introduction

Both waxy corn and QPM are popular in Asia, especially in China because they are superior in terms of nutritional value and edible value compared to the normal maize. They are not only good for feed but also high quality food. Waxy corn possesses higher content of water-soluble, salt-soluble protein and low alcohol-protein content than the normal maize. It also has high content of lysine and tryptophan which are the essential amino acids the human body cannot synthesize. Its lysine content is about 16% -74% more than normal maize. It is the favorite food for its special flavor, taste and tender kernel. QPM's prominent characteristic is its high lysine and tryptophan content. It's edible and raises value are superior than the common maize similar to the waxy corn. The biology experiment shown that, the digestibility and biological value of normal maize protein are 71.45% and 19.45, but the opaque-$_2$ maize is 95.00% and 78.25 respectively. Compared to the opaque-$_2$ maize, QPM and waxy corn has better resistance to disease. However, the development of waxy corn and QPM has been limited for their narrow genetic background.

There was evidence that lysine and tryptophan content of waxy-opaque-$_2$ maize were obviously higher than waxy corn in the same heredity background, its lysine content is higher than opaque-$_2$ maize as well. However, it has never been used in the production for its seed weight and density, resistance to diseases was worse than normal maize. It seems to be the floury original opaque-$_2$ parent cause the soft endosperm of waxy-opaque$_2$ maize and the wx gene can modify the endosperm trait of opaque-$_2$ maize for its seed weight and density higher than opaque-$_2$ maize.

The endosperm trait and resistance to ear diseases should be improved by utilizing QPM (hard endosperm) to developed waxy-QPM maize. Meanwhile, the o_2o_2 genotype was identified easiely after the SSR marker phi057, which was codominance and closely linkage with o_2 gene, and the technology of using this marker to identify the genotype were developed. Therefor, it is possible to develop waxy-QPM variety for the production. In theory, waxy-QPM maize can be used to develop not only waxy-QPM but also QPM and waxy corn variety (OPVs and hybrid). This study was aimed to create a new type of corn with advantages of waxy corn and QPM and find out a new way to expand the genetic background of QPM and waxy corn germplasm in order to meet the demand of maize production and breeding.

Materials and Methods

Materials for waxy-QPM inbred development

The materials for waxy-QPM inbred development were six crosses developed from two QPM lines combined to four waxy inbred lines. The two QPM lines were the parents of one certified QPM hybrid, and two waxy lines were the parents of two certified hybrids, the other two waxy lines were derived from two certified hybrids (Table1).

Method of waxy-QPM inbred developdevelopment

The 6 combinations were selfed to get S_1, then the waxy seeds in the S_1 ears were taken and planted in the following season. The leaves were taken for DNA extraction for S_1 plant during 4-6 leaves stage and identification of the O2O2, O2o2, o2o2 genotypes was done with SSR primer phi057 developed by Pioneer Company and Missouri University in the laboratory. The S_1 of the o_2o_2 genotypes were selfed for 5 generations continuously forming their pedigrees. lysine content selection was not done, but agronomic traits and resistance to diseases were considered during the selfing generations.

Table1. Materials and their parents for waxy-QPM inbred developing

Code	Materials	Female	Male
1	CML161×M606	Female of YMD102(QPM)	Male of YMD606(wx)
2	CML165×M604	Male of YMD102(QPM)	Male of YMD604(wx)
3	CML161×M604	Female of YMD102(QPM)	Male of YMD604(wx)
4	Ln5-1×CML161	From Ln 5(wx)	Female of YMD102(QPM)
5	Ln1-3×CML161	From Ln 3(wx)	Female of YMD102(QPM)
6	Ln1-3×CML165	From Ln 3(wx)	Male of YMD102(QPM)

Method of amino acid analysis

The kernels of S_6 waxy-QPM lines and their original parents were analyzed with Acid Hydrolysis Solution, but the tryptophan could not be analyzed because the tryptophan was destroyed with this method.

Results

Eighteen stable waxy-QPM inbred lines were developed in the study. Four of them were from CML161×M606, three from CML165×M604, one from CML161×M604, three from Ln5-1×CML161, five from Ln1-3×CML161 and two from Ln1-3×CML165 (table 2).

The total amino acid content of the waxy-QPM inbred

The total ***amino acid*** content of the waxy-QPM lines were ranged from 8.71% to 13.64%, eleven of them are higher than the average of their original parents, and the differents were ranged from 0.91% to 33.37%. The two parents' average of CML161×M606 content was low in the six crosses, however, the content of three lines from this cross were higher than the average and only one line was lower than 10%. The contents of 3 lines from CML165×M604 were ranged from 11.54% to 12.53%, higher than the average of their original parents. The 3 lines from Ln5-1×CML161 were ranged from 9.00% to 10.31%, two of them were lower and one was higher than the average of

Table 2. The total amino acid content of the waxy-QPM lines & their original parents (%)

Line code	Pedigree	Waxy-QPM	Original parents			Compare to the original parents		
			QPM	waxy	average	average	QPM	Waxy
1	(CML161×M606)-1	10.23	8.87	10.81	9.84	3.96	15.33	-5.37
2	(CML161×M606)-2	10.59	8.87	10.81	9.84	7.62	19.39	-2.04
3	(CML161×M606)-3	11.58	8.87	10.81	9.84	17.68	30.55	7.12
4	(CML161×M606)-4	9.93	8.87	10.81	9.84	0.91	11.95	-8.14
5	(CML165×M604)-1	11.24	10.11	10.37	10.24	9.77	11.18	8.39
6	(CML165×M604)-2	12.75	10.11	10.37	10.24	24.51	26.11	22.95
7	(CML165×M604)-3	12.53	10.11	10.37	10.24	22.36	23.94	20.83
8	CML161×M604	12.83	8.87	10.37	9.62	33.37	44.64	23.72
9	(Ln5-1×CML161)-1	10.31	8.87	11.22	10.05	2.64	16.23	-8.11
10	(Ln5-1×CML161)-2	9.00	8.87	11.22	10.05	-10.40	1.47	-19.79
11	(Ln5-1×CML161)-3	10.04	8.87	11.22	10.05	-0.05	13.19	-10.52
12	(Ln1-3×CML161)-1	13.64	8.87	13.62	11.25	21.30	53.78	0.15
13	(Ln1-3×CML161)-2	11.55	8.87	13.62	11.25	2.71	30.21	-15.20
14	(Ln1-3×CML161)-3	11.92	8.87	13.62	11.25	6.00	34.39	-12.48
15	(Ln1-3×CML161)-4	10.53	8.87	13.62	11.25	-6.36	18.71	-22.69
16	(Ln1-3×CML161)-5	8.71	8.87	13.62	11.25	-22.54	-1.80	-36.05
17	(Ln1-3×CML165)-1	11.85	10.11	13.62	11.87	-0.13	17.21	-13.00
18	(Ln1-3×CML165)-2	9.35	10.11	13.62	11.87	-21.20	-7.52	-31.35

original parents, but the difference between the parents average and the lines was not high. Although the average content of CML161×M604 parents was 9.62%, it was the lowest among the six crosses, the content of the only line derived from this cross reached 12.83%. The average content of Ln1-3×CML161 parents was 11.25%, the 5 lines from this cross were ranged from 8.71% to 13.64%, the differences between their original parents average and their offsprings were very high, it was ranged from -22.54% to 21.30% and both lines with the lowest and highest total amino acid content in the 18 waxy-QPM lines were derived from this cross. The average content of Ln1-3×CML165 parents is highest in the six crosses, but the content of the 2 lines from this cross were not very high and lower than their original parents average, the difference were -0.13% and -21.20% (table 2). The result indicated that the waxy-QPM line with high total amino acid content couldn be developed from the cross of both high or low average total amino acid content maize.

The total amino acid content of all waxy parents were higher than that of QPM parent in the six crosses. The total amino acid content of most of waxy-QPM lines were 10% higher than their original QPM parent . In the opposite, there were 12 waxy-QPM lines which have lower total amino acid content than their original waxy parent (table 2). It seems to be that the improvement of total amino acid content of waxy-QPM line was contributed by its original waxy parent.

The analysis showed that correlation coefficient between waxy-QPM lines and their original waxy, QPM parent and the average of the 2 parents in term of total amino acid content were 0.233, 0.006 and 0.084,ÿthey were not significant at 0.05 probaility level. It was indicated that the total amino acid content of waxy-QPM line was not correlated significantly with that of its original parent.

The lysine content of the waxy-QPM line

The lysine content of the 18 waxy-QPM lines was quite high, ranged from 0.36% to 0.54%. Most of them were higher than the average of their original parents. Three of them were over 0.50% and the highest one reached 0.54%, eleven of them were ranged from 0.4% to 0.49%, only 5 lines were lower than that of their original parents and the differences were only ranged from 1.27% to 10.34%. On the other hand, there were thirteen lines whit lysine content was 1.15% to 27.06% higher than that of their original parents average. Ten of them were 10% and three of them were 20% higher than the average respectively (table 3).

The content of the 4 lines from CML161×M606 was ranged from 0.47%-0.51%, they were 17.50%^ÿ27.50% higher than the average of their original parents which was 0.415%. The content of CML165×M604 parents average was 0.385%, the lowest among six crosses. The contents of the 3 lines from this cross were lower comparably, only 0.36%00.39%00.43% respectively, and the lowest among 18 lines was derived from this cross. It is only 0.395% of CML161×M604 and Ln5-1×CML161 parent average, however, the only line from CML161×M604 and 2 of three lines from Ln5-1×CML161 with lysine content

Table 3. The lysine content of the waxy-QPM lines and their original parents (%)

Line code	Pedigree	Waxy-QPM	Original parents			Compare to the original parents		
			QPM	waxy	average	average	QPM	Waxy
1	(CML161×M606)-1	0.47	0.40	0.43	0.415	13.25	17.50	9.30
2	(CML161×M606)-2	0.51	0.40	0.43	0.415	22.89	27.50	18.60
3	(CML161×M606)-3	0.50	0.40	0.43	0.415	20.48	25.00	16.28
4	(CML161×M606)-4	0.47	0.40	0.43	0.415	13.25	17.50	9.30
5	(CML165×M604)-1	0.39	0.38	0.39	0.385	1.30	2.63	0.00
6	(CML165×M604)-2	0.43	0.38	0.39	0.385	11.69	13.16	10.26
7	(CML165×M604)-3	0.36	0.38	0.39	0.385	-6.49	-5.26	-7.69
8	CML161×M604	0.44	0.4	0.39	0.395	11.39	10.00	12.82
9	(Ln5-1×CML161)-1	0.45	0.40	0.39	0.395	13.92	12.50	15.38
10	(Ln5-1×CML161)-2	0.39	0.40	0.39	0.395	-1.27	-2.50	0.00
11	(Ln5-1×CML161)-3	0.46	0.40	0.39	0.395	16.46	15.00	17.95
12	(Ln1-3×CML161)-1	0.40	0.40	0.47	0.435	-8.05	0.00	-14.89
13	(Ln1-3×CML161)-2	0.44	0.40	0.47	0.435	1.15	10.00	-6.38
14	(Ln1-3×CML161)-3	0.49	0.40	0.47	0.435	12.64	22.50	4.26
15	(Ln1-3×CML161)-4	0.47	0.40	0.47	0.435	8.05	17.50	0.00
16	(Ln1-3×CML161)-5	0.39	0.40	0.47	0.435	-10.34	-2.50	-17.02
17	(Ln1-3×CML165)-1	0.54	0.38	0.47	0.425	27.06	42.11	14.89
18	(Ln1-3×CML165)-2	0.41	0.38	0.47	0.425	-3.53	7.89	-12.77

reached 0.44%, 0.45% and 0.46% respectively, only one line from Ln5-1×CML161 with 0.39% lysine content. The lysine content of Ln1-3×CML161 parents average was 0.435%, the highest among six crosses, one line from this cross was 0.39%, the other 4 lines were ranged from 0.40% to 0.49%. The average lysine content of Ln1-3×CML165 parents was 0.425%, the content of two lines from this combination was 0.41% and 0.54% respectively, and the highset lysine content among 18 waxy-QPM lines was from this cross (table 3). The above results indicated that the lysine content of waxy-QPM line was not related to that of the average of their original parents, the high lysine content line could be derived from the cross of low lysine content average of parents and low content line derived from the cross of high lysine content average parents . However, there was a big chance to produce higher lysine content line derived from the cross of parents with 0.4%lysin content average. .

There are 15 waxy-QPM lines, three lines ranged from 0.0% to 7.89%, eight lines ranged from 10% to 17.5% and four lines ranged from 22.5% to 42.11%, which lysine content higher than that of their original QPM parent, the lysine content of three other lines were lower than that of their original QPM parent, but the differences ranged only -2.5% to-5.26%. Similarly, lysine content of most of the waxy-QPM lines were higher than or equal to that of their original waxy parent, only 5 lines were lower than that (table 3).

The analysis showed that correlation coefficient between waxy-QPM lines and their original waxy, QPM parent and the average of the two parents in term of lysine are 0.249, 0.334 and 0.293ÿthey are not significant at 0.05 probability level . It was indicated that the lysine content of waxy-QPM line was not significantly correlated with that of its original parent. The promotion of the lysine content of waxy-QPM line was possibly caused by the interaction of wx and o_2 gene.

The traits and the resistance to diseases

Most of the waxy-QPM lines were preferable in term of disease resistance (not artificial inoculated), agronomic traits and endosperm hardness. They could be utilized for developing hybrid. The endosperm hardness improvement was probably contributed by the endosperm modified-gene of QPM parents and waxy gene. On the other hand, the preferable disease resistance and agronomic traits were genetically derived from their parents.

Discussions and Conclusions

1. It is quite effective method to create the waxy-QPM inbreds. It should be a way to develop waxy-QPM OPVs theoretically.
2. In order to create a waxy-QPM germplasm (inbred and OPVs), the agronomic traits, endosperm hardness, disease resistance and the lysine content of the original parents must be considered.
3. It will be necessary to study the combining abilities of the waxy-QPM lines and they are crossed to waxy line and QPM line.

Acknowledgements

The study was supported by Guangxi Science Foundation for Young Scholars project (GKQ0728076)

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Molecular marker-assisted pyramiding of genes conferring resistance to Turcicum Leaf Blight and Polysora Rust in maize inbred lines in India

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Abstract. Turcicum Leaf Blight (TLB) and Polysora Rust (PR) are two important diseases of maize in India. Through a collaborative project, we have identified resistant donors, identified/validated SSR markers linked to specific genes/QTLs conferring resistance to TLB and PR, and undertaken molecular marker-assisted pyramiding of the target genomic regions into five elite but susceptible inbred lines (CM137, CM138, CM139, CM140 and CM212). Seven different backcross populations were generated using four resistant donors with these inbreds as recurrent parents. An SSR polymorphism survey covering all ten chromosomes was undertaken on the selected recipient and donor parents. Foreground selection for different resistance gene combinations using polymorphic SSR markers was carried out on BC_1F_1 and BC_2F_2, and background selection for high recovery of recurrent parent genome was undertaken on BC_1F_1 and BC_2F_1 progenies. Phenotypic screening of BC_2F_1 and BC_2F_3 against TLB was undertaken at Nagenahalli and Hawalbagh (Almora) and against PR at Nagenahalli, under artificial inoculation with local isolates. The study revealed differential responses of the genotypes to TLB at Naganahalli and Hawalbagh. Specific backcross progenies manifesting resistance to TLB at Nagenahalli were also found resistant at Hawalbagh although the converse was not true, suggesting possible differences in the virulence of the isolates as well as some common resistance mechanisms. Genetic background of the recurrent parents was found to influence the expression of TLB/PR resistance significantly. The study led to development of BC_2F_4 progenies with resistance to both the diseases, and shall further lead to development of TLB- and PR-resistant maize hybrids in India.

Key words: Maize, Turcicum leaf blight, Polysora, resistance, SSR markers, MAS

Introduction

Turcicum leaf blight (TLB), caused by *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs, and Polysora Rust (PR) caused by *Puccinia polysora* Underw., are two of the major diseases of maize. TLB is prevalent in peninsular, North-Eastern and Northern hill regions of India. Extensive defoliation due to TLB particularly during the plant's grain filling period can result in grain yield losses of 27 to 90% (Chenulu and Hora, 1962). Most of the early maturing and local maize cultivars are susceptible to this disease. Temperatures of 23-28^{o}C and high relative humidity as often found in sub-tropical and tropical environments favours Polysora rust which can occur simultaneously with TLB in some regions (Melching, 1975). Damages caused by PR include reduction of vigour, grain size and yield in addition to lodging (Leonard, 1974). Strong breeding efforts are required to address the problem of TLB, and PR as mechanical and chemical control methods, have either practical limitations or economic and ecological drawbacks.

Growing resistant cultivars is the most effective means of controlling both TLB and PR diseases. The presence of both qualitative and quantitative resistance to TLB and PR has been demonstrated. TLB has a number of pathogenic races that can overcome major resistance genes. Physiologic races of PR are also known (Pratt and Gordon, 2006). Diverse sources of qualitative and quantitative resistance are available for TLB, but qualitative resistances (*Ht* genes) could be often unstable (Welz and Geiger, 2000). Particularly in the tropics, these are either overcome by new virulent races or suffer from climatically sensitive expression. Quantitative resistance is expressed independently of the physical environment and has never succumbed to TLB pathotypes in the field. Recent studies have led to identification of specific regions of the maize genome carrying resistance genes/QTL to several diseases including PR and TLB in multiple mapping populations (Freymark et al., 1994; Holland et al., 1998, Chen et al., 2004, Wisser et al., 2006) and are therefore, good candidates for utilization in molecular marker-assisted breeding.

DNA-based markers offer significant advantages in genetic dissection of quantitative traits, such as plant yield, disease or insect pest resistance, and abiotic stress tolerance (Prasanna and Hoisington, 2003). Pyramiding several resistance genes in an elite genotype is a more effective measure in combating diseases such as TLB and PR than relying on a single resistance gene. The objective of the present study is to undertake molecular marker-assisted pyramiding of specific genes/QTLs for resistance to TLB and PR (using SSR markers validated at Maize Genetics Unit, IARI) into five elite but susceptible maize inbred lines (CM137, CM138, CM139, CM140 and CM212) which are parental lines of some popular single-cross maize hybrids in India.

Materials and methods

A large number of maize inbreds were phenotyped for disease resistance at UAS-ARS Naganahalli and VPKAS (Hawalbagh, near Almora) during *Kharif* (monsoon season) 2005 season. Based on disease reaction responses, seven different backcross populations were generated using five susceptible inbreds as recurrent parents and four donors (Table 1).

An SSR polymorphism survey was undertaken on the selected recipient and donor parents. Foreground selection for different resistance gene combinations using polymorphic SSR markers tagging specific genes/QTLs was carried out on BC_1F_1 and BC_2F_2 progenies. Background selection for high recovery of recurrent parent genome was undertaken on BC_1F_1 and BC_2F_1 progenies using polymorphic SSR markers covering the maize genome. This was coupled with rigorous selection at each generation (BC_1F_1 to BC_2F_3) for conformity of the plant and ear traits to those of the recurrent parent. Phenotypic screening of BC_2F_1 and BC_2F_3 progenies against TLB was carried out at Naganahalli (Karnataka) and Hawalbagh (Uttarakhand), and against PR at Naganahalli, under artificial inoculation with local isolates. Altogether 146 heterozygotes were selected among 840 BC_1F_1 progenies, and 16 BC_2F_1 populations were further developed which were advanced up to BC_2F_3 through the above strategy.

Disease scoring was done by visual estimation of disease severity on a 1-5 scale where a score of 1 indicated no disease or least severity while a score of 5 indicated severe disease symptoms. Lines with a score of 0-<2.5 were considered as resistant; a score between 2.5-<3.0 as moderately resistant; a score of 3-<4.0 as moderately susceptible; and a score from 4.0-5.0 as susceptible.

Table 1. Recipients and donors of backcross populations, and target genomic regions for foreground selection using SSR markers.

Recipient	Donor	Target bin locations for foreground selection
CM 137	NAI 147	1.01, 9.07, 10.00
CM 138	SKV 21	7.05, 8.04
CM 138	NAI 112	3.00, 9.03
CM 139	NAI 112	3.00, 9.03
CM 139	NAI 147	1.01, 5.04, 10.00
CM 140	SKV 21	7.05, 8.04
CM 212	SKV 18	1.01, 5.04

Results and Discussion

In this study, we adopted an integrated strategy of MAS and phenotypic selection in different backcross generations. Although there are several sources of resistance to TLB, availability of sources of resistance to PR is relatively limited. Some donors like NAI147 carry resistance to both TLB and PR. A major gene *RppQ* for PR resistance (at bin 10.00; Chen et al., 2004) has been validated using SSR markers and further utilized in this study.

Foreground selections (Fig. 1) aided in deriving BC_2F_3 lines offering resistance to TLB at Naganahalli and/or Hawalbagh, and PR. Besides foreground selection for target genes at specific bins using flanking SSRs, high recovery of recurrent parent genome was obtained through background selection using polymorphic SSRs (Fig. 1), coupled with phenotypic selection for plant and ear features (Fig. 2).

Mean disease severity scores among 13 selected BC_2F_1 populations tested for TLB ranged from 1.43-4.74 at Nagenahalli and 1.07-2.71 at Hawalbagh. Mean PR scores for 10 selected populations ranged from 1.93 to 4.08 while the individual scores ranged from 1 to 4.5. The BC_2F_3 lines were evaluated in trials under artificial inoculations at Naganahalli and Hawalbagh (against TLB) and at Naganahalli against PR during *Kharif*-2008. Twelve different BC_2F_3 lines have been identified to show resistance to TLB at both locations, in various recurrent parent backgrounds (except CM140). Several other combinations (R-MR; R-MS; MR-MR etc.) have also been derived. Two CM137-based lines recorded resistance to both TLB (at Naganahalli and Hawalbagh) as well as PR at Naganahalli.

Specific populations manifesting resistance to TLB at Nagenahalli were also found resistant at Hawalbagh although the converse was not true, suggesting possible differences in the virulence of the isolates as well as some

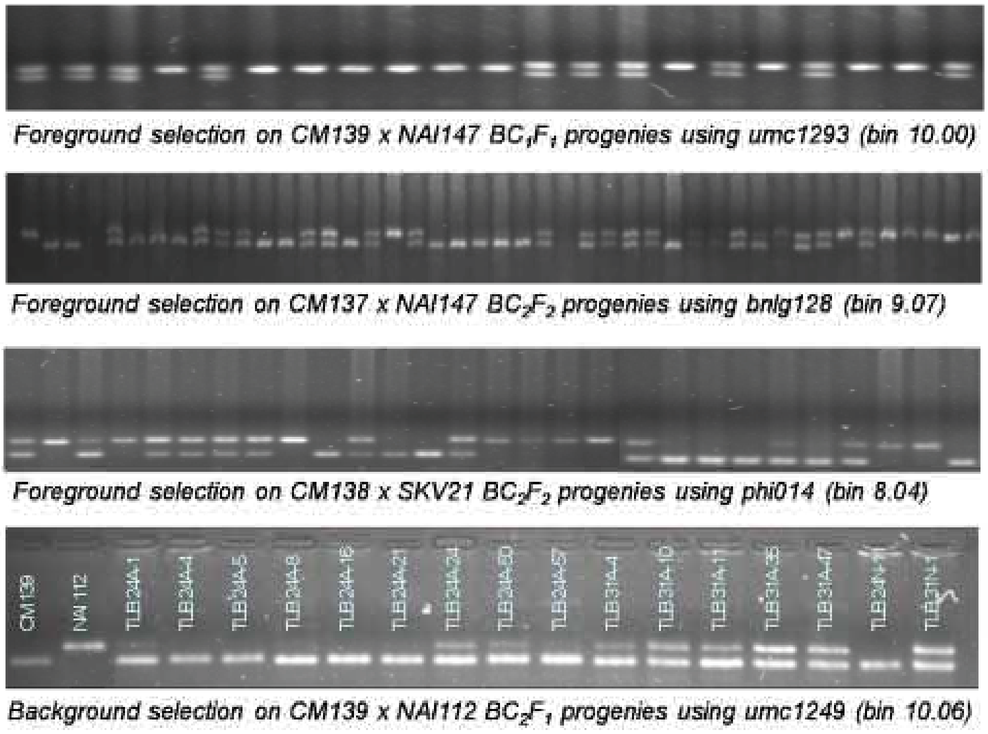

Fig. 1. Foreground and background selections of backcross progenies using SSR markers.

Fig. 2. Ears derived from selected BC_2F_1 plants of different recurrent parents.

common resistance mechanism(s). Overall, there was a moderate correspondence (R^2=0.57) of BC_2F_3 TLB scores between Naganahalli and Hawalbagh (Fig. 3). It was also evident that the genetic background of the recurrent parent had a significant effect on the TLB/PR resistance, even when the same donor parent was involved. Selected BC_2F_3 lines offering resistance to TLB/PR were selfed during *Kharif*-2008 for deriving BC_2F_4 lines. From these, a subset of 100 BC_2F_4 lines were selected showing not only resistance TLB and/or PR, but also morphological similarity with the respective recurrent parents.

Thus, the study resulted in derivation of a series of BC_2F_4 lines (Fig. 4) with resistance to both TLB isolates at both Naganahalli and Almoa, besides PR resistance in some of these lines. These MAS-derived lines are being further evaluated for development of TLB- and PR-resistant hybrids.

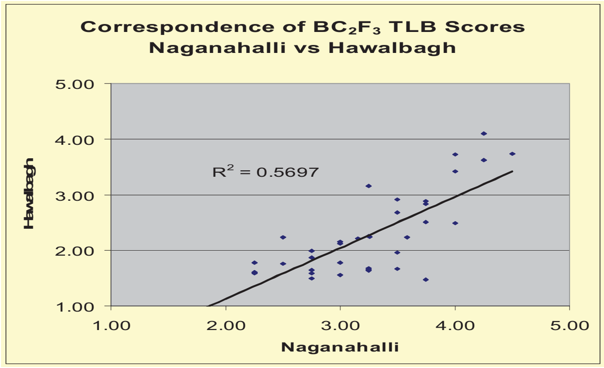

Fig. 3. Correspondence of TLB scores of BC2F3 families at Naganahalli and Hawalbagh (*Kharif*-2008).

Fig. 4. TLB responses of some MAS-derived TLB-resistant lines (BC_2F_4) in comparison with the recurrent parents (Naganahalli; *Kharif*-2008)

Acknowledgement

The study was undertaken with financial support from the ICAR Network Project on Gene Pyramiding [Sub-project: Pyramiding genes for resistance to Turcicum leaf blight and Polysora rust in maize], awarded to Drs. Sangit Kumar (DMR), BM Prasanna (IARI), KTP Gowda (UAS-ARS) and SK Pant (VPKAS).

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Identification of quantitative trait loci for drought tolerance at seedling stage by screening a large number of introgression lines in maize

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Abstract. The maize genome hosts tremendous phenotypic and molecular diversity. Introgression lines (ILs), developed by continuous backcrossing to recurrent parents, could provide a unique genetic stock for quantitative trait locus (QTL) mapping. Using maize lines from six heterotic groups of different ecological zones, we developed > 500 BC_2F_2 IL sets by crossing 11 inbred lines (as recurrent parents) with > 200 local maize inbred lines (as donor parents). Of them, 34 IL sets were selected as a subset for drought tolerance screening and a total of 417 ILs survived under severe water stress at seedling stage. One set of 32 surviving ILs, derived from Chang7-2/DHuang212, was used for QTL mapping with SSR markers covering the whole genome, with seven QTL detected. Furthermore, investigating all surviving ILs, we identified two common regions in bin 3.04, corresponding to marker intervals bnlg1904–umc1772 and umc1223–bnlg1957, respectively, which shared high genetic variation in three IL sets. Our results indicated that selective genotyping can be used to identify genetic loci for complex traits. The ILs, highly selected for drought tolerance in this study, provide a unique set of materials for both genomic studies and development of enhanced germplasm resources.

Key words: Maize (*Zea mays* L.) ¯ Drought tolerance ¯ Quantitative trait loci (QTL) ¯ Seedling stage ¯ Introgression line (IL)

Introduction

Maize is increasingly important for staple food and renewable energy resources. In the past 50 years, the domestication, artificial selection and intensive breeding for high yield in maize has narrowed the genetic base, rendering modern cultivars more vulnerable to biotic and abiotic stresses (Ali *et al.*, 2006). Unfavorable environmental conditions cause a large loss of crop yield in the world every year. Among such environmental abiotic stresses, drought is one of the greatest worldwide environmental constraints to agriculture. Therefore, genetic improvement of drought tolerance in crop plants has huge economic importance (Umezawa *et al.*, 2006).

To develop crop plants with enhanced drought tolerance, it is essential to understand physiological, biochemical and gene regulatory networks associated with drought response (Valliyodan and Nguyen, 2006). The tremendous worldwide efforts in molecular genetics and functional genomics have advanced our understanding of the molecular basis of drought tolerance in plants. Analysis of molecular markers and quantitative trait loci (QTL) were intensively employed to dissect the genetic basis of crop complex traits including drought tolerance, and then, marker-assisted selection (MAS) was proposed as a means of molecular breeding for crop improvements (Agrama and Moussa, 1996; Salvi and Tuberosa, 2005; Bernardo, 2008). However, the inherent limitations of narrow genetic backgrounds in classical QTL mapping based on F_1-derived mapping populations and the challenging task of cloning individual QTL has slowed effective molecular breeding for drought tolerance (Flint-Garcia *et al.*, 2003; Li *et al.*, 2005). The two parents used to construct mapping populations are usually widely adapted lines with characteristics of good quality or high yield rather than abiotic stress tolerances, which make it difficult to identify the functional loci expressed under stress. Moreover, the statistical methods to identify QTL still require a large population and a dense map to precisely locate QTL through the methods and software developed and implemented in past decades. To overcome these limitations to QTL mapping for abiotic stress tolerances, an alternative but powerful approach is to develop an introgression line (IL) mapping population consisting of a series of lines harboring a single homozygous donor segment introgressed into a uniform cultivated background produced by backcrossing. One key advantage of IL mapping populations is reducing the complexity of polygenic traits by separating them into a set of monogenic loci (Peleman and van der Voort, 2003), which makes QTL identification much more accurate. This approach has given promising results describing QTL related to stress tolerance in rice (Li *et al.*, 2005; Ali *et al.*, 2006). Szalma *et al.* (2007) developed a set of nearly isogenic maize lines that were used for QTL mapping.

Drought tolerance in maize has received great attention in QTL mapping in the past decade. Most efforts have focused on traits that are scored at reproductive stage or for final grain yield, and few reports have described drought tolerance at seedling stage. Early seedling growth is a very important for maize establishment and for upward growth and development, as well as biomass and grain yield production. It is necessary to identify the genes/ QTL conferring drought tolerance of maize at seedling stage, which will contribute to the profile of drought tolerance throughout the maize life cycle and help understanding of the complex mechanism and genetic network of this important agronomic trait. In this study, we present the development of maize ILs and their use at seedling stage in identifying QTL for drought tolerance that are originally hidden in different maize inbred lines.

Materials and methods

Development of large-scale IL sets and screening for drought tolerance

To develop IL sets of high genetic diversity for elucidating the genetic mechanism controlling maize tolerance to various stresses, 11 inbred lines that are widely used as testers for hybrid development in Chinese maize breeding were selected as recurrent parents. These lines were crossed with > 200 local elite inbreds (as donor parents) that were collected from different ecological zones and six heterotic groups. Then, two generations of backcrossing and one generation of selfing were used to produce > 500 BC_2F_2 IL sets, each derived from one parent set and consisting of multiple ILs. These maize IL sets were used in various types of biotic and abiotic stress screenings. Of them, a subset containing 34 IL lines was selected for drought tolerance screening. This subset of ILs was derived from crossing, backcrossing and selfing using three recurrent parents (Ye478, Chang7-2 and Qi319) and 30 donor lines (Table 1). Among donor parents, U8112, Zhongzi01 and Dan599 were used twice by crossing with both Ye478 and Qi319 recurrent parents, and K12 was crossed twice with both Chang7-2 and Qi319 recurrent parents, respectively.

A screening trial was conducted for water stress in the greenhouse of the Institution of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, in the summer of 2006. A total of 200 seeds per IL set were randomly selected and planted in 450-mL pots with one seed per pot. For recurrent parent controls, ten pots per parent were randomly placed among the pots of IL sets. Each pot was filled with 350 g of mixed nutrient soil (5 field soil: 1 grassland soil). After seeding, 100 mL of water was added to each pot for germination. Then 50 mL of water was applied every 7 d until the maize plants reached the four-leaf stage, and the pots were then subjected to water stress by withholding water. The watering was resumed when the recurrent parents died. Leaf samples from surviving plants were taken for DNA extraction. The sampled plants were then transplanted from pots to the field for continuous growing, pollination and seed harvest.

Genotyping of ILs for drought tolerance

Genomic DNA of the leaf samples was extracted following the CTAB procedure (Saghai-Maroof *et al*. 1984). One set of ILs derived from Chang7-2/DHuang212, which

Table 1. 30 donor inbred lines used in backcrossing program and their pedigree/origins

ID	Donor lines	Pedigree/source
1	U8112	Pioneer
2	488	8112 × 5003
3	8002	Pineer
4	48-2	Selected from a synthetic
5	Zhongzi01	Selected from Pioneer hybrid “PN78641”
6	Dan599	Selected from Pioneer hybrid “78599”
7	Mo17	C103 × 187-2
8	Qi319	Selected from Pioneer hybrid “78599”
9	V9	
10	K14	5005 × 6917
11	DHuang212	D729 × Huangzaosi
12	502	Dan340 ×Huangzaosi
13	Ye515	(Huafeng100 × AiC103)S2×Huangzaosi
14	CN962	Huangzaosi improvement
15	Shuang105	
16	K12	Huangzaosi × Huaichun
17	832	Selected from foreign hybrid
18	PI41	
19	Hua160	Hybrid “Huadong 2”
20	Jinhuang96B	Selected from Zhongzhong3
21	Dan340	Baigulv9 × *Z. mays* - tunicata
22	K22	Ye478 × K11
23	8065	
24	Chuan321	Selected from Pioneer hybrid “78599”
25	Zi330	Oh43 × Keli67
26	Ji477	Selected from foreign hybrid “HC777”
27	178	Selected from foreign hybrid
28	Tie7922	Separated from American hybrid “3382”
29	B73	Lowa Stiff Stalk Synthetic (BS13C5)
30	Shen118	Zhao23 × Super sweet

had the highest number of surviving plants after drought treatment, was firstly used for genetic mapping with SSR (Simple Sequence Repeats) markers across the whole genome. A total of 93 SSR polymorphic loci between the two parental lines were selected from the Maize Genetics and Genomics Database (http://www.MaizeGDB.org), which are randomly distributed across the genome, and used for a whole genome scan of QTL for drought tolerance. Fine mapping around major QTL regions was then conducted using all surviving plants. The SSR genotyping and silver staining procedure followed the CIMMYT manual of laboratory protocols with modifications (http://www.cimmyt.org). The amplified bands of the same sizes as the recurrent parent were scored as A, those with the same size as the donor parent were B, and the heterozygotes were H.

QTL identification using selected drought-tolerant ILs

Clear genotypic graphs for the introgression segments of each IL were constructed using GGT32 software (http://www.dpw.wau.nl/pv/pub/GGT). In specific random BC populations, the allelic and genotypic frequencies at genetic markers are known. By comparing observed and expected frequencies, a significant deviation of donor allele frequency at single loci in ILs from the expected implies a positive selection favoring the donor allele (in excess), or negative selection against the donor allele (in deficiency). A standard $\div^2$ test on the allelic (1 df) or genotypic (2 df) frequencies was performed to identify QTL and markers that were significantly associated with drought tolerance as measured by the rate of introgression segments of these surviving ILs. To minimize the probability of false positives, we used a strict threshold of *P***d”**0.0001 for a putative QTL.

Results

Plant survival rates among IL sets derived from different recurrent and donor parents

There were significant differences in plant survival rates after water stress at seedling stage among ILs derived from different recurrent and donor parents (Table 2). A total of 417 ILs survived, showing stronger drought tolerance than their recurrent parents. The IL sets derived from the recurrent parent Ye478 had the lowest average plant survival rate (3.4%), and its nine IL sets had a total of 55 surviving plants, with range 0–29 per IL set. The IL sets derived from Chang7-2 had the highest average plant survival rate (11.6%), and its ten IL sets had 195 surviving plants with 2–32 plants per set. The average plant survival rate from IL sets derived from recurrent parent Qi319 was intermediate (6.2%). Comparison of different donor parents showed that V9, K14, DHuang212, CN962, Hua160, 178 and B73 had plant survival rates > 10%. The IL sets from Qi319/Shen118, Qi319/Zhongzi01, Chang7-2/DHuang212, Chang7-2/K14 and Chang7-2/Hua160 had significantly higher plant survival rates (13.0, 14.0, 17.2, 18.4 and 20.5%, respectively) than other crosses.

There was different germination rates among IL sets, possibly since most donor parents were inbred lines used for breeding over a range of years and were collected from different ecological zones. However, germination rates were not significantly correlated with plant survival rate (r = –0.17), indicating that the seed vigor had no significant effect on the phenotyping of drought tolerance at maize seedling stage.

Table 2. Germination & plant survival rates among different recurrent & donor inbreds

	Name	Germination rate (%)	Average survival seedling sper IL	Plant survival rate (%)
Recurrent parents	Ye478	89.6	6.1	3.4
	Chang7-2	83.5	19.5	11.6
	Qi319	90.3	11.1	6.2
Donor parents	U8112	88.5	7	4.1
	488	88	3	1.7
	8002	87	0	0
	48-2	91.5	0	0
	Zhongzi01	90.5	14	7.6
	Dan599	89.3	3.5	2
	Mo17	90.5	11	6.2
	Qi319	90	3	1.7
	V9	90.5	29	16
	K14	81.5	30	18.4
	DHuang212	93	32	17.2
	502	87.5	8	4.6
	Ye515	88.5	17	9.6
	CN962	90.5	22	12.2
	Shuang105	92	13	7.1
	K12	80.5	13.5	9.3
	832	72	2	1.4
	PI41	79	13	8.2
	Hua160	78	32	20.5
	Jinhuang96B	88	8	4.6
	Dan340	84	6	3.6
	K22	94	7	3.7
	8065	95	20	10.5
	Chuan321	89	5	2.8
	Zi330	95	3	1.6
	Ji477	89	4	2.3
	178	91	21	11.5
	Tie7922	97.5	8	4.1
	B73	93	22	11.8
	Shen118	84.5	22	13

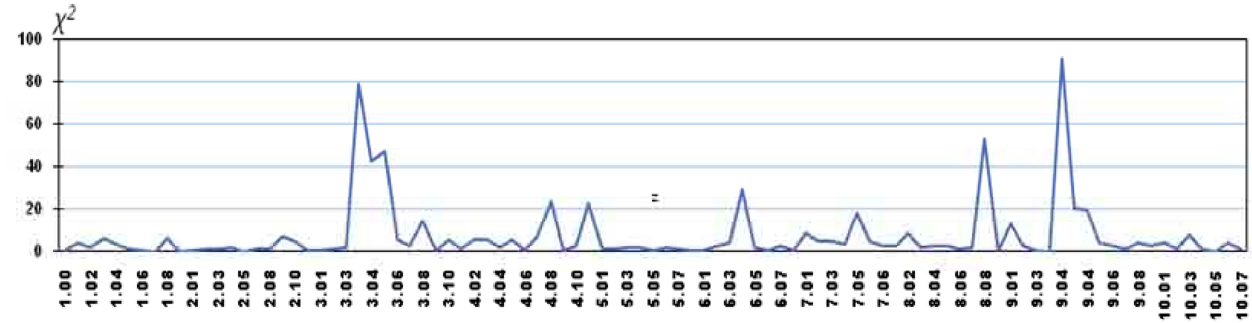

Fig. 1. χ^2 profiles across the maize genome showing bin 3.04 and bin 9.04 regions with genes related to drought tolerance at seedling stage. Y-axis represents the χ^2 value and X-axis shows the bins of each chromosome in maize (÷^{2} = 18.4 at *P* = 0.0001).

QTL for drought tolerance across whole genome at maize seedling stage

Chang7-2/Dhuang212, a population with more surviving plants, was selected for whole genome QTL mapping. A total of 32 surviving ILs were genotyped with 93 polymorphic SSR markers, which were evenly distributed on the maize genome. By comparing with the threshold ÷^{2} = 18.(*P* = 0.0001), a total of seven molecular marker intervals/ markers were identified as significantly deviating from normal segregation, indicating their association with drought tolerance at seedling stage. These markers located on bins 3.04, 4.08, 4.11, 6.04, 7.05, 8.08 and 9.04. Of them, bins 3.04 and 9.04 had the highest level of segregation distortion with ÷^{2} value of 78.5 and 90.4, respectively (Fig. 1).

Fine mapping of the drought-tolerant QTL region in bin 3.04

To fine map the drought tolerance QTL by adding more SSRs to the bin 3.04, 63 SSRs from this region were used to screen polymorphism between recurrent and donor parents. As a result, 30, 30, 29, 27 and 29 SSR markers were found polymorphic, respectively, for five IL sets, Chang7-2/K12 (26 ILs), Chang7-2/DHuang212 (32 ILs), Chang7-2/PI41(13 ILs), Chang7-2/V9 (29 ILs) and Qi319/B73 (22 ILs). There was significant deviation among those polymorphic markers. Each polymorphic SSR markers was located according to its position on IBM2 2008 Neighbors (http://www.MaizeGDB.org). By comparing genotypic frequencies of introgression and heterozygous segments with the expected frequencies within the surviving ILs, 1–8 markers /marker intervals were identified for each IL set (Table 3). Of these, marker interval umc1717–bnlg2047 and markers umc1351, umc1810, umc1773 and umc1087 were shared by two IL sets. Two chromosomal regions, bnlg1904–umc1772 and umc1223–bnlg1957, showed significant deviation (threshold ÷^{2} e” 18.4 at *P* d” 0.0001) and were shared by three IL sets derived from Chang7-2/K12, Chang7-2/Dhuang212 and Chang7-2/PI41 (Table 3 and Fig. 2).

Discussion

QTL discovery for drought tolerance at maize seedling stage using ILs

Maize is a widely adapted crop and is grown in tropical, subtropical and temperate areas worldwide. Although there is a huge genetic diversity among different types of maize germplasm, intensive breeding has narrowed the genetic base within specific regions. In rice, IRRI (International Rice Research Institute) initiated a large-scale backcrossing breeding effort in 1998, to construct a primary gene pool, to exploit hidden diversity for QTL mapping and to develop promising lines with significantly improved tolerance to various abiotic stresses (Li *et al*., 2005; Ali *et al*., 2006). An IL library was constructed, consisting of a series of lines harboring a single homozygous donor segment introgressed into a uniform cultivated background.

The selective genotyping strategy has been used to select extreme phenotypes from a large number of ILs derived from different donor parents, and should be further exploited for genetic mapping of quantitative traits, particularly for stress tolerance as suggested by Xu and Crouch (2008) and Xu *et al.* (2008). Simulation results indicated that selective genotyping would significantly increase mapping power by requiring much fewer plants or smaller population size; 30 individuals selected from each distribution tail provided strong mapping power (Xu

Table 3. χ^2 values for SSR markers in the bin 3.04 for five IL sets with significant deviation in bold

SSR markers	Marker positions on IBM2	Chang7-2/ K12	Chang7-2/ DHuang212	Chang7-2/ PI41	Chang7-2/ V9	Qi319/B73
bnlg1904	127	17.1	**35.0**	15.8	6.7	-
phi099	159	**23.6**	**35.0**	8.3	6.7	**72.0**
phi036	159	**41.5**	**35.0**	**29.1**	6.7	14.2
umc1012	159	**29.1**	**35.0**	3.0	4.8	-
umc1772	164	**19.7**	7.4	**25.7**	6.7	-
umc1729	167	3.9	7.4	7.5	6.7	-
phi029	168	16.5	**35.0**	3.0	1.9	5.1
nc030	168	12.0	**35.0**	3.0	-	5.1
umc1965	168	8.0	**35.0**	10.7	6.7	-
umc2158	177	2.6	5.2	11.3	6.7	-
umc1351	177	**49.5**	**34.8**	1.2	6.7	-
umc1392	181	1.8	2.3	3.0	-	7.1
umc1425	191	6.0	5.5	3.0	6.7	-
umc1717	191	**27.6**	**34.8**	6.6	6.7	-
bnlg1113	191	**26.8**	0.9	**18.9**	5.1	-
bnlg1638	193	**23.6**	0.8	3.0	6.7	-
bnlg2047	203	**43.7**	**34.8**	3.0	6.7	-
umc1908	214	12.6	5.5	**38.4**	6.7	-
umc1810	223	**41.1**	**28.6**	-	-	-
umc1347	228	3.9	7.4	6.4	6.7	13.3
umc1504	228	16.0	8.5	10.7	6.7	13.3
umc1223	234	**23.6**	**78.5**	**23.6**	6.7	-
bnlg1957	247	**21.7**	**42.3**	7.5	6.7	-
umc2264	261	6.0	7.4	3.0	4.8	5.1
umc1449	269	12.0	7.4	3.0	4.5	-
umc1386	276	1.8	7.4	3.0	6.7	5.1
umc1527	279	6.0	2.6	2.0	4.8	-
umc1773	280	**27.6**	7.4	**23.6**	0.6	-
bnlg1022	299	1.8	7.4	3.4	**45.8**	-
umc1087	365	**37.2**	**53.8**	1.4	6.7	-

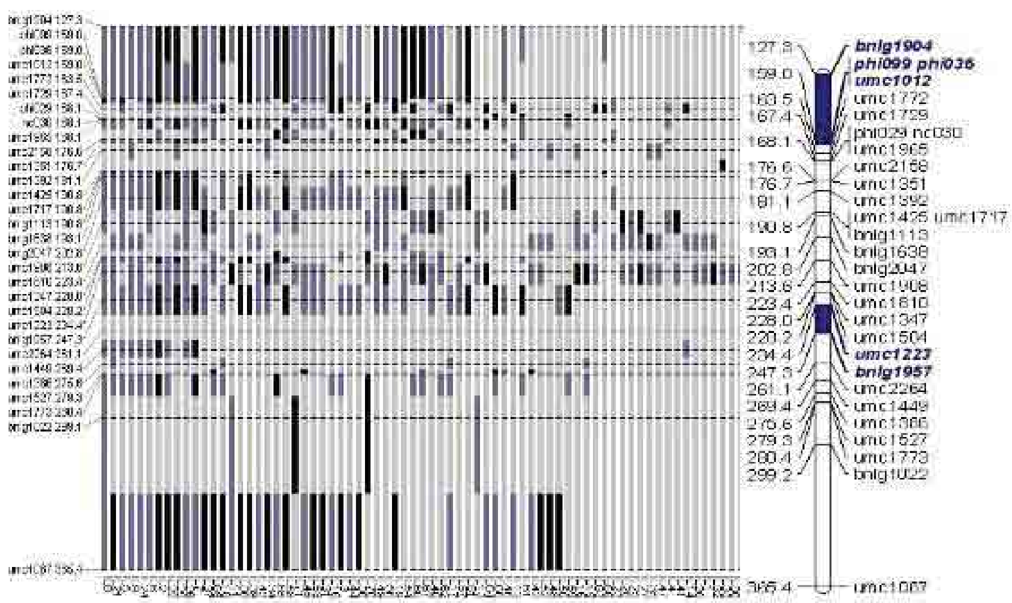

Fig. 2. Left: Graphical introgression pattern and ÷^{2} profile for 30 SSR markers within bin 3.04 for 71 surviving ILs derived from three IL sets, Chang7-2/K12 (IL1-IL26),Chang7-2/DHuang212 (IL27-IL58) and Chang7-2/PI41 (IL59-IL71). The black part represents donor introgressions, the gray represents heterozygotes and the rest has the recurrent parent background. Right: Genetic map of bin 3.04 and two important intervals related to drought tolerance at maize seedling stage marked with dark color and their linked markers with bold text.

et al., 2008). As an extreme case of selective genotyping, only plants surviving severe stress are used for genetic mapping. At marker loci that are significantly associated with the target trait selected under the stress condition, allele frequencies should be significantly different from those expected.

Abiotic stresses are often complex in nature and their genetic bases, as for most quantitative traits, remain poorly understood. Among stresses, drought tolerance is the most difficult to study because the drought environment is difficult to control or simulate, particularly in most rainfed environments as multiple abiotic stresses coexist. It can be expected that the genes/loci responsible for drought tolerance in maize reproductive stage and seedling stages are distributed across the whole genome, and trait expression regulated by a complex genetic network. Developing IL, combined with selective genotyping, provides a powerful tool to identify QTL with large effects on phenotype, and to dissect the associated genetic networks for the complex traits (Li *et al.*, 2005; Ali *et al.*, 2006). Maize drought tolerance at seedling stage is highly related to mature vigor and development of a dense population. However, this has not received much attention. In the present work, we developed a maize IL library with unique characteristics for each IL set, and these IL sets are suitable for this investigation. Based on the genetic analysis of the selectively genotyped ILs and molecular mapping, we detected several major QTL for maize drought tolerance at seedling stage. Particularly, QTL mapped on maize bins 3.04 and 9.04 showed high correlations with seedling drought tolerance. Using the surviving ILs of the Chang7-2/K12 set, seven marker intervals or markers (or QTL) were further identified as significantly deviating from normal segregation in the surviving plants. By two backcrosses, the ILs recovered most regions and chromosomes of the recurrent parent genome. This showed that some important loci/genes inferred by these seven QTL were linked in controlling maize drought tolerance. The fine mapping showed that two chromosomal regions on bin 3.04 had high deviation for three IL sets. A number of disease resistance genes were identified within bin 3.04, such as *scmv2* (resistance to sugarcane mosaic virus 2), *rp3* (resistance to *Puccinia sorghi* 3), *mdm2* (maize dwarf mosaic resistance 2), *cip1* (cytokinin inducible protease 1) and *aba1* (abscisic stress protein homolog 1). However, no genes or distinct QTL on this bin are known to be related to maize drought tolerance. Some candidate genes related to drought tolerance were highly correlated with our detected QTL in this bin, indicating that some genes on bin 3.04 may control the upstream function of plant response to survival under abiotic stresses. Two genome regions in bins 3.04 and 9.04 may contain some important regulatory factors controlling maize tolerance to abiotic stresses. By association mapping based on the candidate genes and our developed ILs, the functional factors controlling drought tolerance and functional markers may be identified and used for molecular breeding.

Use of the identified markers for MAS of drought tolerance

While many QTL have been reported in the literature, very few with large effects have been adequately exploited in crop breeding programs. The majority of the favorable alleles at these identified QTL reside in journals on library shelves rather than in crop cultivars improved by introgression or selection of these favorable QTL alleles. How can molecular markers be best used to improve (rather than simply study) a complex trait in crops? It is a serious question that scientists working on molecular breeding try to solve (Bernardo, 2008; Xu and Crouch, 2008). Use of identified QTL for MAS faces many challenges and must deal with many constraints. For MAS of drought tolerance, an approach using selectively phenotyped ILs is promising for breeding application. The selection efficiency for drought tolerance is greatly affected by the level of stress applied. In our screening program for drought tolerance in maize, the BC_2F_2 populations were developed and used to minimize the heterotic effect. The range in number of surviving plants was 0–32 in different populations. The ILs highly selected for drought tolerance showed stronger tolerance to drought stress and higher yields than the control, as shown in water-stressed field environments of Hainan (in 2006) and CIMMYT (International Maize and Wheat Improvement Center), Mexico (in 2007) (data not shown). Well-selected drought tolerant ILs confer some genes/loci that are hidden in donor parents, which have made them more drought tolerant. By fine mapping on bin 3.04, markers highly correlated with the recombinant segments, such as phi036 and umc1223, might be directly used for MAS and drought-tolerant screening in maize breeding.

Acknowledgements

The authors gratefully acknowledge the reviewers for their valuable comments and corrections to the earlier draft of this manuscript and the funds of the National Natural Science Foundation of China (30600394; 30721140554) and supporting program from the Chinese Ministry of Science and Technology (2006BAD29B04).

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Characterization of drought stress tolerant and susceptible maize lines using SSR markers and transcriptome profiling under drought stress conditions in India

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Abstract. Drought stress is the most important factor limiting maize productivity in India, with ~80 per cent of maize acreage under rainfed conditions. Analysis of drought stress responses of 24 selected CIMMYT-DTP inbred lines during *Kharif* (rainy season)-2006 and 2007 at DMR rain-out shelter at IARI, New Delhi, led to identification of promising drought tolerant genotypes. Molecular characterization of 24 selected inbreds differing in drought stress responses using 50 SSR markers resulted in effective differentiation and analysis of genetic diversity in this important germplasm. Transcriptome profiling of five selected CIMMYT-DTP genotypes was further undertaken by sampling of ear leaf, tassel leaf and silk tissues in replicates from control and drought stress blocks of experiments carried out in DMR Rain Shelter during 2006 & 2007. Transcriptome analysis using 'one cycle eukaryotic target labeling assay' and the GeneChip® Maize Genome Arrays (Affymmetrix) and statistical analysis of microarray datasets using ARRAY ASSIST software for various parameters, led to identification of a large set of genes that are up-regulated or down-regulated, including those associated with osmoprotection, chaperones, oxygen radicals scavenging, defense-related proteins (PRP), photosynthesis and starch metabolism. K-means clustering led to grouping of the differentially expressed genes. Validation of a selected set of putative candidate genes using RT-PCR is in progress.

Introduction

Drought stress is the most widespread abiotic constraint for maize production in the tropics, where most maize is grown under rainfed conditions. Annual loss due to drought across the tropical maize growing environments was estimated to be about 19 m.m.t., representing 17% loss in production (Edmeades et al., 1992). As per a study in CIMMYT (1994), India registered the highest absolute drought prone area (2.5 m.ha.) followed by Indonesia (2.2 m.ha.) and South China (1.15 m.ha.), respectively. In India, nearly 80 percent of maize crop is grown under rainfed conditions of which nearly 30-50 percent is prone to drought stress (Singh et al., 2004). The genetic complexity of drought tolerance makes this trait difficult to analyze using traditional genetic and physiological methods. Although major progress, to date, has been achieved through conventional breeding (Edmeades et al., 1999; Banziger et al., 2004), but this approach remains slow and time consuming.

Microarrays have become an important technology for the global analysis of gene expression in diverse organisms (Wullschleger et al., 2003). Computational analysis of microarray data allows identification of genes that are induced or repressed by only a few-fold, and are up-regulated or down-regulated. The genes with affected expression can also be located on the genetic map and when they appear to co-localize with a QTLs for drought tolerance, thus identifying candidate loci. Sawkins et al. (2004) used microarray analysis to provide a global picture of changes in gene expression in ears and silk of maize under water stress during flowering. The objective of the present study was to combine the molecular markers approach with that of functional genomics for analysis of drought stress tolerance of maize in the Indian context.

Materials and methods

A set of 24 inbred lines, including 16 CIMMYT-DTP lines and 8 Indian inbreds (HKI and CM lines), that were characterized for their responses to drought stress, were selected for molecular profiling using a set of 30 polymorphic SSR markers covering different bin locations of the maize genome. These lines include DT20, DT32, DT50, DT62, DT71, DS85, DS24, DTPYC9-F46-1-2-1-2-B, K64R, SCMALAWI, MAS[206/312]-23-2-1-1-1-B-6-B-B, CMLP1, CMLP2, CML91, CML247, CML360, HKI209, HKI335, HKI586, HKI1011, HKI1025, CM138, CM139, CM140.

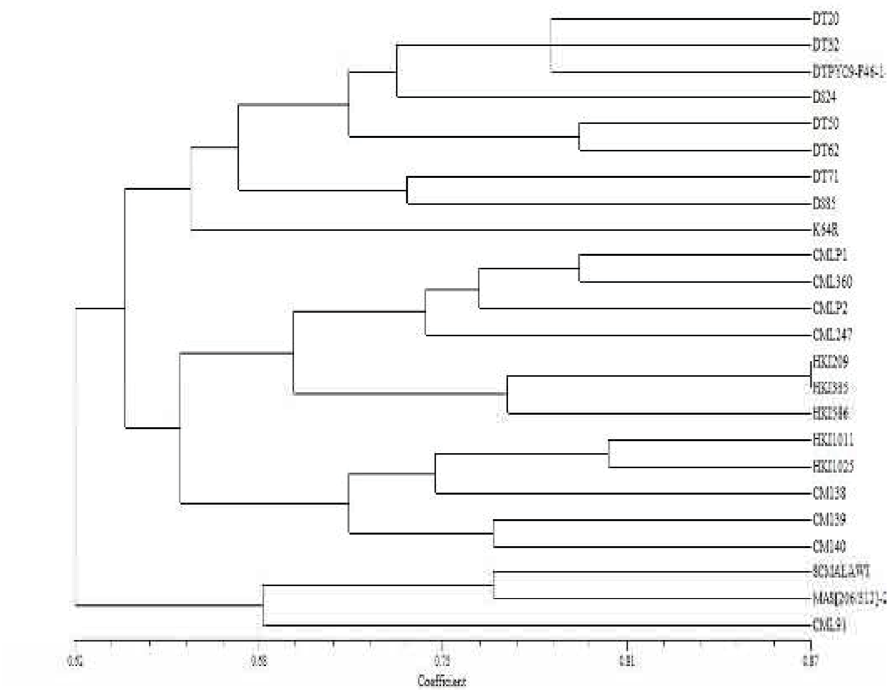

Fig.1. Dendrogram depicting genetic relationships among selected drought tolerant and susceptible lines from CIMMYT and India, based on SSR data analysis.

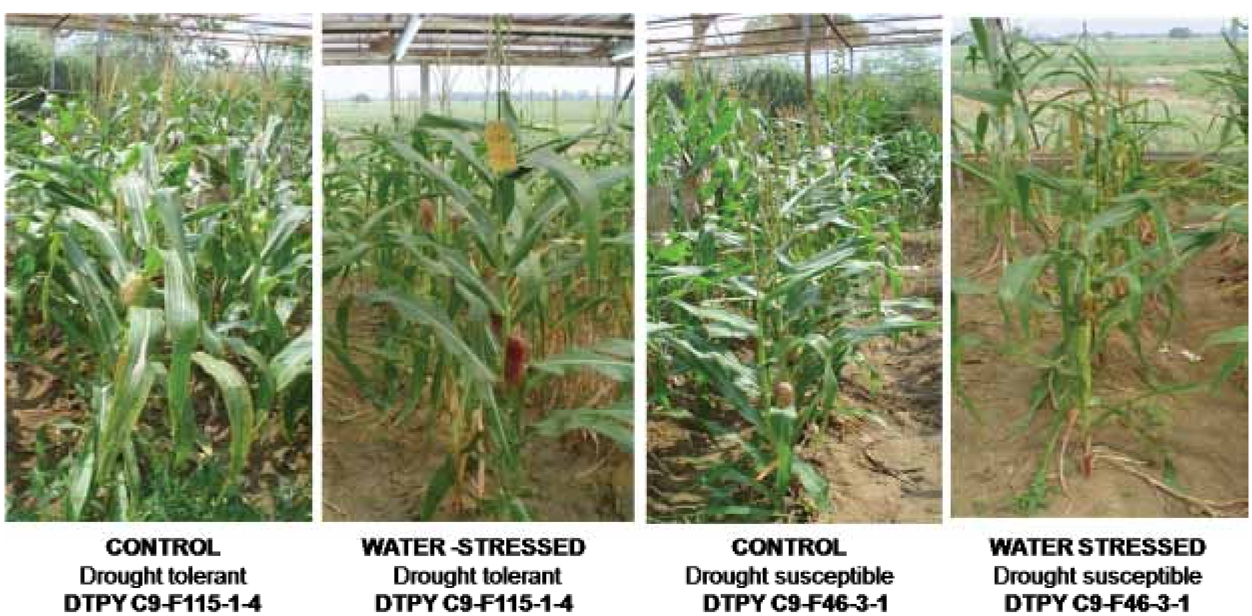

Fig.2. Responses of the selected drought tolerant and susceptible lines (for trascriptome profiling) in the experiment conducted at the Rain-Out Shelter, New Delhi (*Kharif*-2007)

Transcriptome profiling of two CIMMYT-DTP genotypes (DTPW C9-F115-1-4 & DTPY C9-F46-3-1) differing with respect to drought stress tolerance in India was undertaken by sampling of tassel leaf, ear leaf, and silk tissues in three biological replicates collected during *Kharif* (rainy season) 2006 in a drought stress experiment undertaken at DMR Rain-out Shelter at IARI, New Delhi (Fig. 2). The experiment was also repeated at DMR Rain-out Shelter during *Kharif* 2007. RNA isolations from the target tissues were undertaken from both control and drought-stress treatments. The total RNA was quantified using Nanodrop (Model ND-1000, v.3.3.3), and cDNA synthesis was performed by 'One-Cycle Target Labelling' method (Affymetrix). The biotin-labeled cRNA was obtained which was hybridized to the Affymetrix maize chips. After hybridization, chips were scanned and raw data was obtained. The two microarray datasets were normalized and further analyzed using ARRAY ASSIST software for various parameters, including internal and external controls, heat maps, Scatter plots and Box whisker plots. The data were normalized and used for further statistical analysis for identification of differentially expressed genes.

Results and discussion

SSR analysis

A set of 24 inbred lines, including 9 CIMMYT lines and 15 Indian inbreds, were analyzed using 30 polymorphic SSR markers. The study revealed a total of 101 alleles, with 3.36 alleles per locus. The PIC value ranged from 0.23 (*umc1627*) to 0.89 *(umc1569)* and with a mean value of 0.58. Genetic similarity matrix was computed using Jaccard's similarity coefficient and cluster analysis was carried out using UPGMA. The pair-wise genetic similarities ranged from 0.51 to 0.87. Three major clusters were observed. All the DT lines clustered together indicating their genetic relatedness, while the Indian maize lines (CM and HKI) were grouped in different clusters (Fig. 3).

Microarray analysis

The homology between the source cRNA and the maize probes on the gene chips ranged from 50% to 60%, with a mean of 54% in the Expt. I (Delhi *Kharif*-2006), while the same ranged from 59% to 72%, with a mean of 66% in Expt. II (Delhi *Kharif*-2007). The array diagnostic features indicated excellent quality of raw microarray data from both experiments. Differentially expressed genes were identified by simultaneously using three different criteria, namely *P*-value d" 0.01, adjusted FDR for fold change (less than -1 or more than +1) and average expression values. The analysis led to identification of a large set of statistically significant differentially expressed genes up-regulated or down-regulated and their putative proteins. The differentially expressed genes included those associated with osmoprotection, chaperones, oxygen radicals scavenging, defense-related proteins (PRP), photosynthesis and starch metabolism. K-means clustering led to grouping of the differentially expressed genes. Further annotation and validation of a selected set of putative candidate genes using RT-PCR is in progress.

Acknowledgements

The study was undertaken as a part of the ICAR Network Project on Transgenics in Crops [Sub-project: Maize Functional Genomics], awarded to BMP. The technical help provided by Dr. Devarshi Selote for transcriptome profiling (in 2006) is gratefully acknowledged.

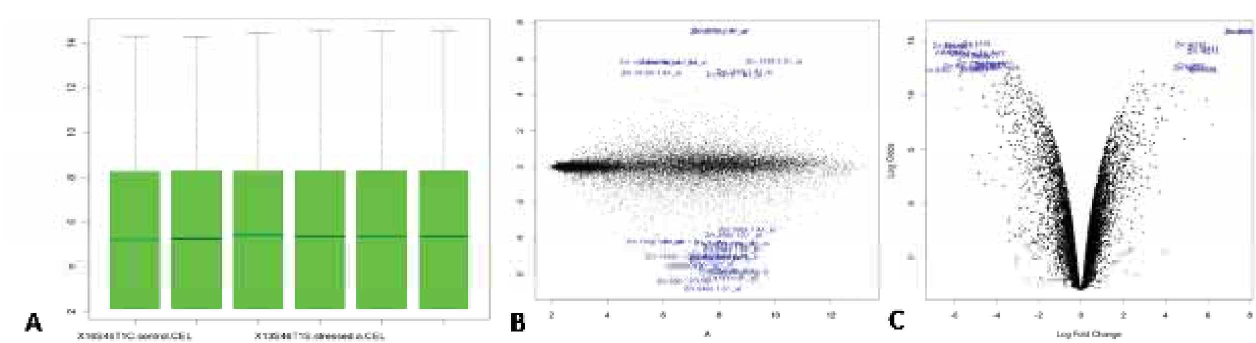

Fig. 3. (A) Normalized microarray data (using GCRMA); (B) MvA plot and (C) Volcano plot indicating the quality of data and indicating differentially expressed genes.

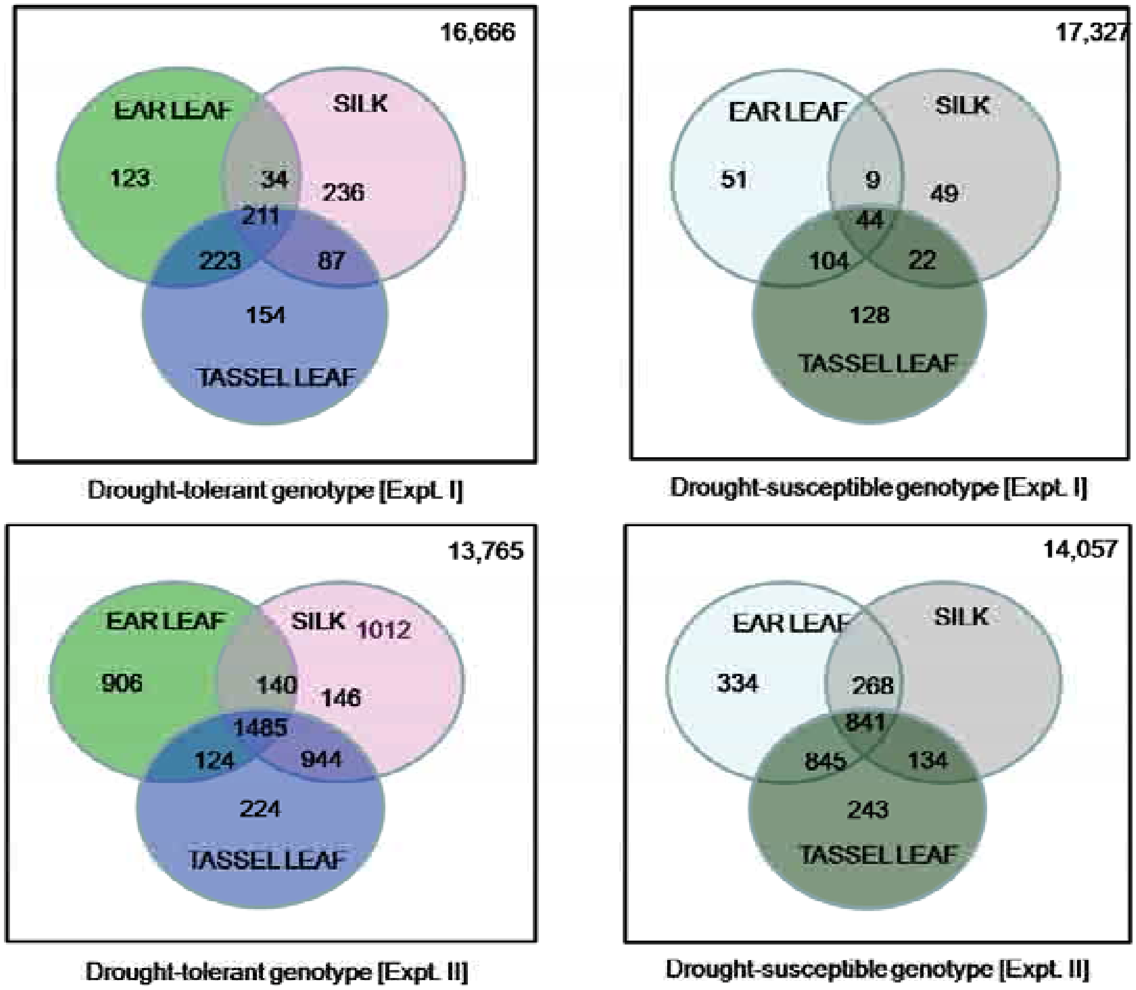

Fig. 4. Venn diagram depicting the commonly expressed and differentially expressed genes in different tissues (ear leaf, tassel leaf & silk) under drought stress and control conditions in two experiments.

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QTL mapping for downy mildew (*Peronosclerospora sorghi*) resistance in maize

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Abstract. Downy mildew (DM), *Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw, is one of the most destructive diseases of maize (*Zea mays* L.) in Thailand. Understanding the genetic basis of DM resistance could increase the efficiency of breeding for disease resistant germplasm. The objectives of this study were to determine the number, genomic positions and genetic effects of quantitative trait loci (QTL) conferring resistance to DM. The study included 251 $F_{2:3}$ families derived from a cross between resistant inbred, Nei9008 and susceptible inbred, CML289. Individuals in the population were genotyped for simple sequence repeat (SSR) and phenotypic resistance data were evaluated as percentage disease incidence in replicated field trials at three environments by Triple Lattice design. Heritability across environments was 94 %. Traits were analyzed within and across environment using composite interval mapping. Nine QTLs intervals were identified for resistance to DM, one QTL on chromosome 2,3,4 and 6, three QTLs on chromosome 5, and two QTLs on chromosome 9. All of the resistant alleles on each QTL came from the resistant parent, Nei9008, except the QTL on chromosome 5.07 which the resistance alleles came from the susceptible parent, CML289. The SSR markers associated with QTLs which produced major effects and consistent for all environments and combined over environments found in chromosome bin 2.09 at umc1736, 5.03 at bnlg1902, and 6.01 at bnlg1867 could be used to transfer resistance alleles to susceptible lines by marker-assisted selection.

Key-words: maize (*Zea mays*.L), Downy mildew, QTL mapping, quantitative trait loci, SSR, marker-assisted selection

Introduction

Downy mildew (DM), *Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw, family Peronosporaceae, order Peronosporales, is one of the most destructive diseases of maize (*Zea mays* L.) in Thailand. The DM infects its host soon after the seedling emergence until one month stage after planting, causing yield losses of 50-100 % for susceptible cultivars. Understanding of the genetic basis of DM resistance could increase the efficiency of breeding for disease resistant germplasm. Previous studies have identified QTLs for downy mildew resistance (DMR). Nair et al (2005) found three QTLs for DMR on chromosome 2, 3, and 6 in a back cross population derived from the cross, CM139 X NAI116. George et al. (2003) detected six QTLs for DMR on chromosomes 1, 2, 6, 7, and 10 in an RIL population from the cross of Ki3 X CML139 by the joint analysis across five environments in four Asian countries (India, Indonesia, Thailand, and Philippines). Agrama et al. (1999) found three QTLs for DMR resistant on chromosomes 1 and 9 in combined across two seasons of using RILs from the cross, G62 X G58. The objectives of this study were to determine the number, genomic positions and genetic effects of quantitative trait loci (QTL) conferring resistance to DM in the $F_{2:3}$ population from the cross, Nei9008 X CML289.

Materials and methods

Population development

A survey of DM disease in maize inbreds from three maize research program of the National Corn and Sorghum Research Center (Suwan Farm), Nakhon Sawan Field Crops Research Center (Nakhon Sawan), and International Maize and Wheat Improvement Center (CIMMYT) was carried out in the year 2000 at Suwan Farm, Nakhon Sawan, and Pacific Seed Company. Two inbred lines were selected for this study; Nei9008 from Nakhon Sawan had been identified as resistant, CML289 from CIMMYT was highly susceptible to *P. sorghi* pathogen. The cross of Nei9008 X CML289 was made at Suwan Farm during May-August 2001, and F_1 plants were selfed during August to November in the same year. In December 2001-March 2002, F_2 plants from one of F_1 ears were selfed to develop the 251 $F_{2:3}$ families. During November 2002-April 2003, seeds of 251 $F_{2:3}$ families were increased by sib-mating.

Experimental design

Field experiments for DM screening of 251 $F_{2:3}$ families were conducted in May to September 2003, the experiments were grown in three environments at Suwan Farm Early Rainy Season (SWE), Suwan Farm Late Rainy Season (SWL) and Nakhon Sawan Rainy Season (NSR). Two hundred fifty-one $F_{2:3}$ families, parents, F_1, and two checks were evaluated in a 16 X 16 triple-lattice design with two-row plots spaced 0.75 m apart and 5 m long. Each plot was planted two seeds per hill with a total of 104 plants.

Phenotypic data evaluation

The spreader-row technique was used for field inoculation. This technique consisted of planting a susceptible variety (Tuxpeno-1 Sel. Sequia C3) in thick stands every 16 rows, and in the alley-way. After emergence (two fully extended leaves stage of development), the seedlings were sprayed with a spore suspension of the pathogen. To obtain a spore suspension, infected leaves were collected, washed, and incubated in the dark for 8 hrs at 20-23ÚC to induce sporulation. Spores were then washed from the leaves and inoculated to the spreader rows. Artificial inoculation was performed at night with sufficient moisture to allow for good infection. Three weeks later, when spreader rows were showing systemic symptoms, the screening material $F_{2:3}$ families were planted. Systemic symptoms of infected plants were accessed three weeks after inoculation by stand-counting in the individual plants, and followed up with double checks one and two weeks later. Percentage disease was determined by the ratio of the total number of plants with systemic infection to the total number of plants multiplied by 100.

The initial lattice analysis of variance, including parents, F_1 and checks, was done by using SAS program. Lattice-adjusted means were used as input for analyses within and across environments, excluding parents, F_1 and checks. Variance components of the 251 $F_{2:3}$ families were estimated from linear functions of the mean squares. Broad-sense heritability (H_b) for the $F_{2:3}$ families on an entry-mean basis was calculated by dividing the genotypic variance (s^2_g) by the phenotypic variance (^2_p) (Hallauer and Miranda Fo., 1981). The distribution of the means of phenotypic traits for the $F_{2:3}$ families were checked for normality as described by Shapiro and Wilk (1965) using SAS PROC UNIVARIATE. We used the actual data for QTL analysis.

Molecular marker genotype analysis

Genomic DNA was extracted from bulked-leaf tissue of 20 plants of $F_{2:3}$ to reconstitute the F_2 genotype by modified method based on Rogers and Bendich (1982) and Saghai-Maroof et al. (1984). To identify simple sequence repeat (SSR) markers linked to a major QTL, 760 SSR markers were selected from the Maize Database of University of Missouri at http:/www.agron.missouri.edu and http:/www.maizegdb.org for a survey of polymorphism between parent Nei9008 and CML289. The 195 SSR markers that show polymorphism from parental screening were genotyped to $F_{2:3}$ population. The polymerase chain reaction (PCR) consisted of 0.5 mM each SSR primer, 50-100 ng genomic DNA, and 7.5 mL GoTaq® Green Master Mix (Promega, that contained GoTaqâ DNA polymerase, 1X Green GoTaq®Reaction buffer (pH8.5), 1.5 mM $MgCl_2$ and 0.2 mM dNTP) in a final volume of 15 mL. The thermocycling program pre–denatured DNA at 94ÚC for 2 min, followed by 30 cycles: denaturation at 94ÚC for 0.5 min, annealing at 50-65ÚC (depending on annealing temperature of each primer) for 1 min, extension at 72ÚC for 1 min. After the final cycle, the program was prolonged for a further 5 min at 72ÚC. Amplification products were resolved by electrophoresis on 4% (w/v) agarose gel (containing 2.5% Metaphor:1.5% Seakem agarose gel) in 1X TBE buffer at 120-150 volt for 2-3 hrs. The genetic linkage map was constructed using MAPMAKER/EXP3.0 (Lander et al., 1987; Lincoln et al., 1992).

Quantitative trait locus analysis

Quantitative trait loci analyses for individual and combined across three environments were performed by composite interval mapping using Windows QTL Cartographer 2.5 (Basten et al., 2003). A QTL was considered significant when the LOD ($\log_{10}$ of the likelihood of odds ratio) value was larger than 4 that derived from permutation analysis. Additive and dominance effects for detected QTLs were estimated using the Zmapqtl procedure of QTL Cartographer. The R^2 value, the percentage of the phenotypic variance explained by marker genotype at the QTL, (coefficient of determination) was taken from the peak QTL position as estimated by QTL Cartographer. Gene action was determined by the ratio of the absolute value of the estimated dominance effect divided by the absolute value of the estimated additive effect |d| / |a| following Stuber et al. (1987); (additive = 0 to 0.20; partial dominance = 0.21 to 0.80; dominance = 0.81 to 1.20; and overdominance > 1.20).

Results

Phenotypic data analysis

The distribution of phenotypic means, within and across three environments, deviated a significant from normal distribution (Shapiro and Wilk, 1965). No significant transgressive segregation was observed. Trait means, standard errors, heritabilities, and coefficients of variation for 251 $F_{2:3}$ families from the cross of Nei9008 x CML289, parents and F_1 from individual environments and combined across environments were shown in Table 1 and Table 2.

SSR linkage map and QTL analysis

A molecular linkage map of 195 SSR markers was shown in Figure 1., the percent of polymorphism was 29.53. The results of quantitative trait loci analyses for individual and combined across three environments were shown in Table 3. Nine QTLs intervals for resistance to DM were identified on chromosomes 2, 3, 4, 5, 6 and 9. For chromosome 2, consistent QTLs across three environments and in the combined analysis were detected in chromosome region 2.09 at SSR markers umc1736. Peak LOD scores ranging from 12.47-16.49, and R^2 values were between 18.09-26.67 %. For chromosome 3, the QTL was detected in two environments (SWE, and in the combined analysis) at SSR marker umc2002, in chromosome region 3.04, with peak LOD scores 6.44 and 5.39, and R^2 values were 4.76 % and 5.62 %. On chromosome 4 the QTL was detected only one environment, SWL, at SSR marker umc1051, in chromosome region 4.08, with peak LOD score 4.44 and R^2 value was 3.06 %. For chromosome 5, consistent QTLs across two environments (SWE and NSR) and in the combined analysis were detected in chromosome region 5.03, at SSR markers bnlg1902. Peak LOD scores ranging from 12.90-14.70, and R^2 values were between 8.88-13.32 %. The others QTLs were found in environment SWL, in chromosome region 5.03, at SSR marker umc1447, with peak LOD score 12.46, and R^2 value was 10.47%, and in environment NSR, chromosome region 5.07, at SSR marker umc1072, with peak LOD score 4.53, and R^2 value was 10.59%. On chromosome 6, consistent QTLs across two environments (SWE and SWL) and in the combined analysis were detected in chromosome region 6.01 at SSR markers bnlg1867. Peak LOD scores ranging from 7.23-11.68, and R^2 values were between 6.80-13.07 %. For chromosome 9, consistent QTLs across two environments, NSR and in the combined analysis were detected in chromosome region 9.07 at SSR

Table 1. Trait means, standard errors, and coefficients of variation for 251 $F_{2:3}$ families from the cross of Nei9008 x CML289, parents and F_1 from individual environments and combined across environments.

Environment	Mean ± S.E.† $F_{2:3}$ Families	Nei9008	CML289	F_1	CV‡
%					
SWE§	64.97±1.32	18.33±2.48	98.19±1.81	67.89±2.75	15.66
SWL¶	62.48±1.34	6.80±2.22	99.00±1.01	86.60±5.25	13.11
NSR#	67.80±1.26	11.71±1.81	100.00±0.00	84.46±5.43	14.26
Combined Env.	65.09±1.23	12.29±2.12	99.06±0.52	79.65±1.03	14.42

† S.E. = Standard error of mean
‡ CV was estimated from 256 entries in a 16 × 16 triple lattice designs
§ SWE = Suwan Farm 2003 Early rainy season
¶ SWL = Suwan Farm 2003 Late rainy season
# NSR = Nakhon Sawan 2003 Rainy season

Table 2. Estimates of variance component and heritability based on entry means of Nei9008 x CML289 from individual environment and combined across environments (arcsine transformed value).

	Variance components† s^2_g	$s^2_{g \times e}$	s^2_e	Heritability H_{β}□
SWE	0.082	-	0.0225	91.61
SWL	0.0816	-	0.0199	92.45
NSR	0.0799	-	0.0221	91.55
Combined Env.	0.0748	0.0064	0.0215	94.29

† s^2_g, $s^2_{g \times e}$, and s^2_e are variance components for genotypes, genotypes × environments, and phenotypes, respectively
‡ H_b is broad-sense heritability

Table 3. QTLs for downy mildew resistance for individual environments and combined over environments for 251 $F_{2:3}$ families from the cross of Nei9008 x CML289.

Chrom./ bin†	Nearest markers‡	Position§ (cM)	Max. LOD¶	$R^{2\#}$ (%)	Genetic effect* Additive	Dominance	Geneaction¥	Donor DMR allele[d]
Suwan Farm Early Rainy Season								
2.09	umc1736	264.41	12.47	18.09	11.07	6.60	**0.60, PD**	**Nei9008**
3.04	umc2002	157.81	6.44	4.76	7.64	5.84	0.76, PD	Nei9008
5.03	bnlg1902	114.01	14.70	13.32	12.64	4.30	0.34, PD	Nei9008
6.01	bnlg1867	109.71	11.68	13.07	11.18	0.10	0.01, A	Nei9008
9.05	umc2343	80.91	4.87	0.69	4.85	11.06	2.28, OD	Nei9008
Suwan Farm Late Rainy Season								
2.09	umc1736	264.41	13.79	21.70	12.45	6.88	**0.55, PD**	**Nei9008**
4.08	umc1051	137.21	4.44	3.06	6.15	4.42	0.72, PD	Nei9008
5.03	umc1447	118.81	12.46	10.47	11.74	6.98	0.59, PD	Nei9008
6.01	bnlg1867	109.71	8.47	6.80	9.51	5.34	0.56, PD	Nei9008
Nakhon Sawan Rainy Season								
2.09	umc1736	260.41	13.70	24.06	11.51	8.67	**0.75, PD**	**Nei9008**
5.03	bnlg1902	114.01	12.90	8.88	11.49	9.51	0.83, D	Nei9008
5.07	umc1072	291.71	4.53	10.59	-8.10	8.13	1.00, D	CML289
9.07	dupssr29	90.41	4.07	0.01	2.75	14.92	5.42, OD	Nei9008
Combined								
2.09	umc1736	262.41	16.49	26.67	12.00	8.80	**0.73, PD**	**Nei9008**
3.04	umc2002	157.81	5.39	5.62	6.79	1.31	0.19, A	Nei9008
5.03	bnlg1902	114.01	14.70	10.61	11.52	7.48	0.65, PD	Nei9008
6.01	bnlg1867	109.71	7.23	8.37	8.30	0.37	0.04, A	Nei9008
9.07	dupssr29	90.41	4.84	0.02	2.70	14.97	5.53, OD	Nei9008

† Chromosome/ bin corresponded to UMC SSR map (University of Missouri–Columbia, USA).
‡ Nearest markers = Position of QTL peak as indicated by SSR primer.
§ Position = Position of QTL peak as indicated by cumulative distance from the end of the short arm.
¶ Max LOD = Likelihood of odds (LOD) scores = Likelihood ratios (LR)/4.6052. Critical thresholds of QTL were defined at LOD 4.
\# R^2 = Percentage of the phenotypic variance explained by genotype at Max LOD peak.
* Genetic effect, additive and dominance effect at QTL peak, +/- direction of additive value with reference to Nei9008.
¥ Gene action was established by |d| / |a|: A (additive) = 0 to 0.20, PD (partial dominance) = 0.21 to 0.80, D (dominance) = 0.81 to 1.20, and OD (over dominance) >1.20.
[d] Donor DMR allele detected by +/- of additive with reference to Nei9008. Positive values showed resistance alleles came from Nei9008, negative values showed resistance alleles came from CML289.

markers dupssr29. Peak LOD scores were 4.07 and 4.84, and R^2 values were 0.01 and 0.02 %. And one QTL was detected in one environment, SWE, at SSR marker umc2343, in chromosome region 9.05, with peak LOD score 4.87 and R^2 value was 0.69 %. The phenotypic valueexplains accounted for 46.48% of the total phenotypic variance by multiple regression analysis.

For gene action, from combined analysis (Table 3), we found two QTLs showed additive gene action in chromosome bins 3.04 and 6.01, two QTLs showed partial dominance in chromosome bins 2.09 and 5.03, and one QTLs showed over dominance in chromosome bin 9.07.

Discussion

The distribution of means of the $F_{2:3}$ families for DMR showed the quantitative genetic basis of this trait. No transgressive segregation was observed, suggesting that the resistant parent (Nei9008) contained most of the resistant alleles with major effects. The high heritability estimates (94%) indicated a strong genetic basis for DMR with correspondingly low environmental influence. The heritability of DMR that was reported by Agrama et al. (1999) was 70.6%, George et al. (2003) reported that heritability estimates of individual environments ranged from 59% to 75%, and 50% across all environments, and Nair et al (2005) reported the heritability value was 74 %. The high heritabilty estimates in our study suggested that DMR variation was largely controlled by major genetic

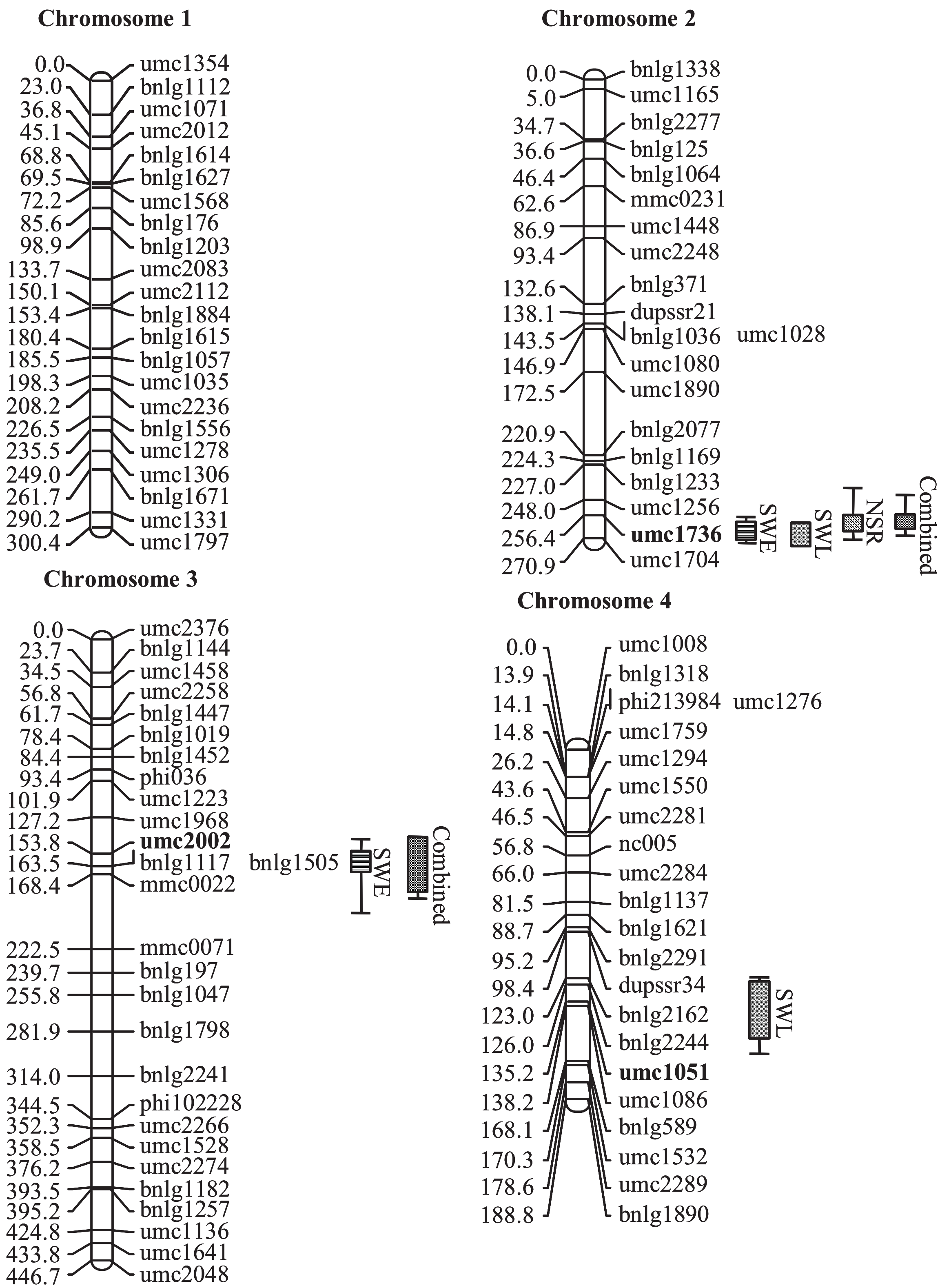

Figure 1. **Molecular linkage map of 195 SSR loci and location of QTLs in the 251 $F_{2;3}$ population from the cross, Nei9008 x CML289, for three individual environments and combined over environments. Numbers to the right of the chromosomes indicate the cumulative distance in cM. The boxplots along the chromosome indicate the QTLs region in which the LOD scored exceed 4.**

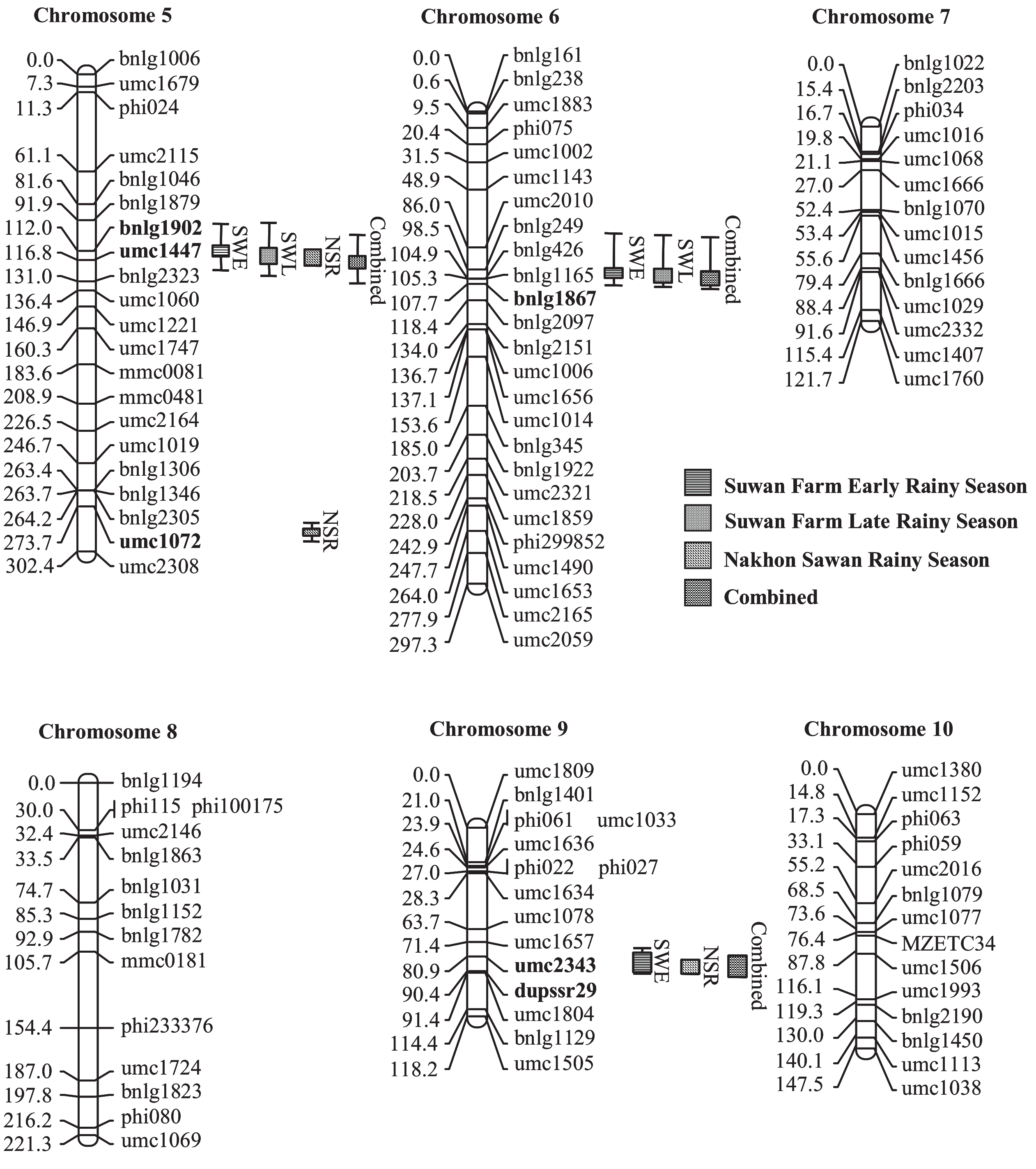

Figure 1. (cont.) **Molecular linkage map of 195 SSR loci and location of QTLs in the 251 $F_{2;3}$ population from the cross, Nei9008 x CML289, for three individual environments and combined over environments. Numbers to the right of the chromosomes indicate the cumulative distance in cM. The boxplots along the chromosome indicate the QTLs region in which the LOD scored exceed 4.**

effects and that DMR was accurately measured over environments.

QTLs in this study were compared to QTLs from other studies and we considered QTLs from separate studies or traits to be potential matches if they occurred in the same or adjacent bin. Two QTLs in this study in chromosome bins 3.04 and 2.09 coincided with those previously reported. Seven QTLs in chromosome bins 4.08, 5.03, 5.07, 6.01, 9.05 and 9.07 were new QTLs which have not been previously reported by other researchers for DMR. Nair et al. (2005) reported three QTLs for resistance to DM in chromosome bin 2.04, 3.04, and 6.05 in back cross population of CM139 X NAI116. George et al. (2003) detected six QTLs in chromosome bins 1.07, 2.06/09, 6.05, 7.02, and 10.04 in an RIL population from the cross of Ki3 X CML139. Agrama et al. (1999) found three QTLs in an RIL population from the cross of G62 X G58 in chromosome bins1.03, 1.07, and 9.01. For gene action, the results indicated that additive and partial dominance gene action were most frequent for resistance to DM.

Quantitative trait loci for resistance to DM from this study were compared to disease and insect resistance loci reported by McMullen and Simcox (1995) and Jampatong et al. (2002). We found that QTLs for resistance to DM were in the same chromosome regions as some major maize disease resistances in chromosomes bin 2.09 (European corn borer); 3.04 (European corn borer, Fusarium stalk rot, Common rust, Maize mosaic, and Wheat streak mosaic); 4.08 (Gray leaf spot, Antracnose stalk rot); 6.01 (Wheat streak mosaic, Maize dwarf mosaic, Southern corn leaf blight); and 9.05 (European corn borer, Fusarium stalk rot). This result supports the concept that resistance genes for diseases and insects in maize are not randomly distributed over the genome, but located in clusters (Bohn et al., 2000).

One of the major goals of QTL mapping is to locate markers that can be used for marker assisted selection (MAS) in a breeding program. One major concern in using MAS for QTLs has been the lack of consistency of QTLs across environments. In this experiment, QTLs in chromosome bin 2.09 and 5.03 were detected in all three environments and the combined analysis, while QTLs in chromosome bin 6.01 were detected in two environments and the combined analysis. Stuber et al. (1992) found that QTLs detected in one environment were frequently found in the other environments, suggesting little QTL × environment interaction. Schön et al. (1994) reported similar results in that most likelihood peaks were identified in the same marker intervals for all environments and differed only in the level of significance and the size of estimated genetic effects.

Summary

(1) Nine novel QTLs for resistance to DMR were identified in the inbred Nei9008 that may be used for improving host-plant disease resistance. (2) The QTL intervals associated with resistance to DMR identified in this study have also been reported to be associated with resistance to diseases and insects, suggesting common genetic control for multiple species. (3) Markers linked to QTLs which produced major effects and consistent for all environments and combined over environments found in chromosome bins 2.09 at umc1736, 5.03 at bnlg1902, and 6.01 at bnlg1867 could be used for MAS in a breeding program for DMR.

Acknowledgments

We are grateful to the National Center for Genetic Engineering and Biotechnology (BIOTECH) for supporting this research. Our deeply acknowledge go to Dr. Surinder Kumar Vasal, Maize Breeder and Consultant, CIMMYT, and Sehgal Family Foundation, USA, for financial support. We would like to express our sincere thanks to Nakhon Sawan Field Crops Research Center and Pacific Seed Company for testing locations, Dr. Pongthep Akratanakul, the director of Center for Agricultural Biotechnology (CAB), Kasetsart University and Monsanto Thailand Limited for financial support of SSR primers. Our deeply thankful go to Mr. Tanee Sreewongchai, Rice Gene Discovery Unit, Kasetsart University, Kamphaeng Saen Campus, for assistance in preparing the genetic linkage map and QTL analysis.

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Phenotypic and molecular characterization of selected maize landraces from the non-NEH (North Eastern Himalayan) regions in India

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Abstract. Understanding of the extent and patterns of genetic diversity is important for effective utilization of genetic resources. The present study focused on intensive phenotypic evaluation and molecular analysis of a selected set of 48 landrace accessions of maize from the plains and non-NEH tribal hill regions in India. The multi-location evaluation of agronomic performances revealed considerable variations among the accessions for yield components, besides G x E interactions. Biplot analysis depicted that landraces can exhibit both location-specific and broader adaptations. Molecular characterization based on SSR loci revealed a total of 550 alleles, with a mean of 13.1 per locus, and an average PIC value of 0.60, indicating high genetic diversity among the tested accessions. Identification of 174 unique SSR alleles led to clear differentiation of 44 accessions. The study also revealed six highly frequent SSR alleles at different loci, which were previously identified to be associated with some important agronomic traits. Analysis of Wright's *F* statistics indicated high levels of genetic differentiation both between and within populations, showing a significant population substructure. Cluster analysis of the SSR allele frequency data showed eight clusters with no specific separation based on geographic origin. However, the cluster patterns largely matched with that derived through yield performance data.

Key words: Maize, landraces, genetic diversity, SSRs

Introduction

Maize has great economic significance worldwide as food, feed and source of industrial products. Due to its unparallel genetic diversity and adaptability, maize is grown in diverse environmental conditions both in tropical and temperate regions. In India, maize is grown in a wide range of environments extending from the extreme semi-arid to sub-humid and humid regions. It is imperative to identify and effectively utilize diverse genetic resources, particularly landraces, for enhancing productivity and for developing biotic and abiotic stress tolerances, especially in tropical maize.

In India, maize landraces are mostly prevalent in the areas of rainfed farming in NEH and non-NEH regions. Most of these locals have specific characteristic features and some of them are already utilized in maize improvement programs in India (Prasanna and Sharma, 2005). However, very limited were conducted to characterize, evaluate and analyze the diversity of maize landraces in India. Accurate assessment of the level and pattern of genetic diversity is invaluable in proper germplasm management and crop breeding for diverse applications (Mohammadi and Prasanna, 2003). This study focuses on intensive phenotypic evaluation and molecular analysis of a selected set of 48 landrace accessions of maize from the plains and non-NEH tribal hill regions in India, and formulates a 'minicore' using both phenotypic and molecular datasets.

Materials and methods

A set of 48 landrace accessions from diverse agro-ecologies of non-North East Himalayan region (non-NEH) were selected as experimental materials for this study (Table 1). Multi-location evaluation of the 48 accessions was carried out at three locations; Almora (Uttrakhand) Bajaura (Himachal Pradesh) and Hyderabad (Andhra Pradesh) during 2006-2007. Important agronomic and yield component data were recorded. Individual location and combined data were analyzed. Biplot analysis was performed to identify the G x E interaction. Cluster analysis using performance data was undertaken.

DNA extraction of selected accessions was done using population bulk stratergy (Rebourg et al., 2001), and molecular characterization of these 48 accessions was done using 42 fluorescent-labeled microsatellite/SSR markers. PCR product was size separated by capillary electrophoresis using MegaBACE DNA sequencer 1000. SSR allele sizes were determined using the software FRAGMENT PROFILER Version 2.1 (Amersham Biosciences). Allele frequencies of each population were estimated using the 'FreqsR' software, and allele lengths for hypothetical individuals correspond to each bulk populations were estimated using 'FtoL(R)' software.

Diversity parameters were computed based on SSR data (marker-wise and population-wise) using POWERMARKER V3.0. Cluster analysis was done using UPGMA (Unweighted Pair Group Method using Arithmetic Means) algorithm. The genetic variability among and within accessions was tested using analysis of molecular variance (AMOVA) and deviation from HWE, if any, were analyzed using ARLEQUIN V3.01. The phenotypic and molecular datasets were analyzed using POWERCORE V1.0 for determining a minicore, using the advanced M (Maximization) strategy.

Results and discussion

Agronomic performance

Location-wise data analysis showed that the tested accessions showed significant differences with respect to yield performance, in comparison with the 'checks'. Combined ANOVA revealed highly significant effects of locations, genotypes as well as genotype x environment (G x E) interactions for different yield components. Grain yield showed significant location effect, genotypic effect and G x E interaction. Ear length and ear diameter showed non-significant variations over locations, and significant effects of genotypes as well as G x E interaction. 100-kernel weight showed non-significant effects over locations and significant genotypic variation and G x E interaction. Kernel rows, kernel number per ear row and kernel number per ear showed significant genotypic effect while showing non-significant location and G x E interaction effects.

Biplot analysis based on SREG model depicted that landraces could exhibit both location-specific as well as broader adaptation across locations. Some of the landrace accessions performed on par or superior to the improved varieties. Cluster analysis led to grouping of the accessions into four clusters based on performance data (Fig. 1). The study was successful in identifying 17 highly promising

Table 1. List of selected maize accessions with available passport data.

S.No.	Gene Bank No.	IML No.	State	S.No.	Gene Bank No.	IML No.	State
1	IC 77418	IML 118	Haryana	25	IC 262988	IML 404	Uttar Pradesh
2	IC 77433	IML 119	Haryana	26	IC 263215	IML 405	Uttarakhand
3	IC 77469	IML 123	Uttar Pradesh	27	IC 267464	IML 409	Jharkhand
4	IC 77477	IML 124	Uttarakhand	28	IC 273246	IML 413	Madhya Pradesh
5	IC 77604	IML 133	Rajasthan	29	IC 273281	IML 416	Madhya Pradesh
6	IC 77611	IML 134	Gujarat	30	IC 274642	IML 422	Madhya Pradesh
7	IC 82097	IML 143	Rajasthan	31	IC 279809	IML 426	Uttarakhand
8	IC 97997	IML 172	Himachal Pradesh	32	IC 282536	IML 431	Jharkhand
9	IC 108151	IML 185	Himachal Pradesh	33	IC 298558	IML 434	Gujarat
10	IC 199114	IML 288	Bihar	34	IC 309931	IML 436	Rajasthan
11	IC 199124	IML 294	Bihar	35	IC 332276	IML 452	Madhya Pradesh
12	IC 199157	IML 296	Bihar	36	IC 333247	IML 454	Madhya Pradesh
13	IC 251267	IML 297	West Bengal	37	IC 337439	IML 459	Gujarat
14	IC 283422	IML 321	Bihar	38	IC 342653	IML 462	Jharkhand
15	IC 319791	IML 324	Himachal Pradesh	39	IC 353812	IML 467	Tamil Nadu
16	IC 325937	IML 325	Maharashtra	40	IC 370494	IML 474	Rajasthan
17	IC 331544	IML 330	Bihar	41	IC 373213	IML 475	Orissa
18	IC 331594	IML 331	Bihar	42	IC 374676	IML 476	Jammu & Kashmir
19	IC 332300	IML 332	Madhya Pradesh	43	IC 385875	IML 480	Jharkhand
20	IC 395739	IML 339	Gujarat	44	IC 397864	IML 485	Himachal Pradesh
21	-	IML 340	Uttar Pradesh	45	IC 430635	IML 496	Andhra Pradesh
22	-	IML 341	Madhya Pradesh	46	IC 436850	IML 498	Andhra Pradesh
23	-	IML 342	Rajasthan	47	IC 565898	IML 499	Karnataka
24	-	IML 346	Karnataka	48	-	IML 505	Uttarakhand

IC: Indigenous collection; IML: Indian Maize Landrace/Local

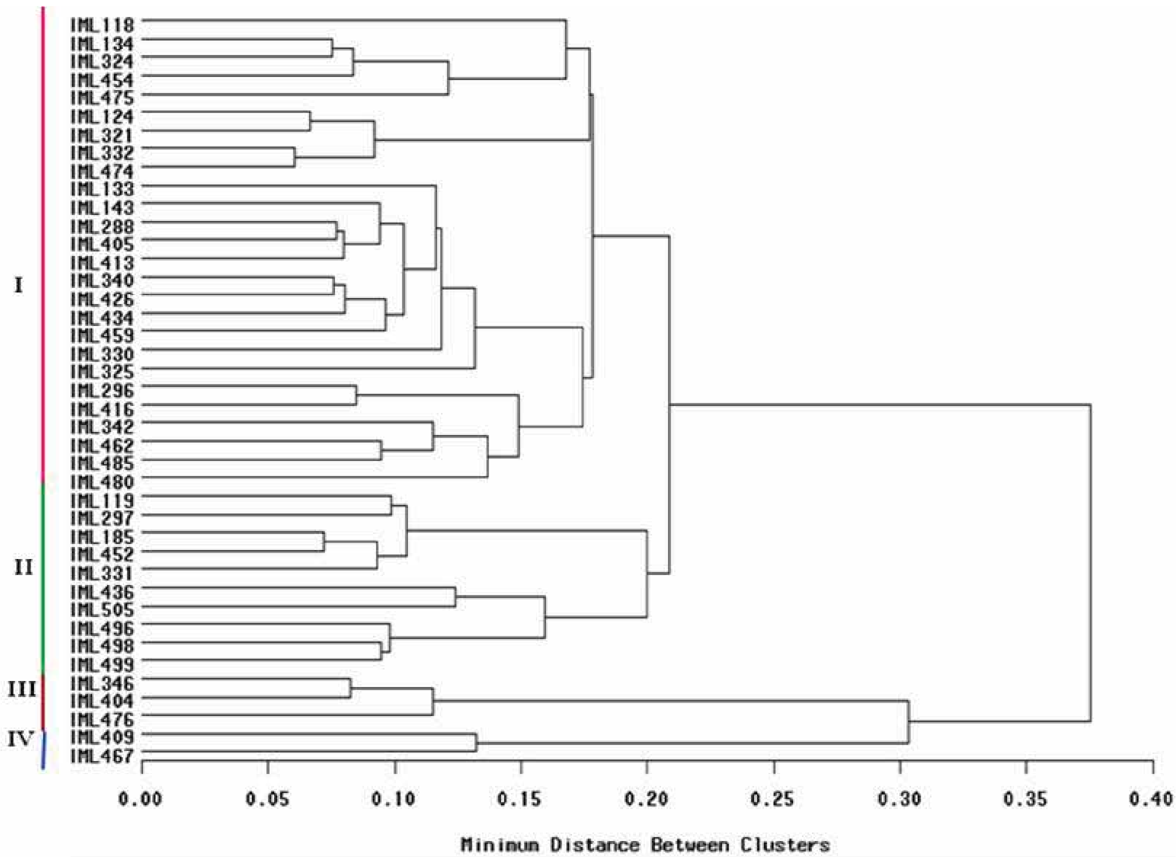

Fig. 1. Cluster analysis of maize accessions based on agronomic performance data, using Euclidean distance and Ward's clustering algorithm.

accessions on the basis of their performance for yield components and flowering (Table 2).

Molecular diversity

The total number of SSR alleles identified across the 42 SSR loci accessions was 550. The number of alleles detected at each locus varied greatly, ranging from 3 (*phi062*) to 26 (*phi083*), with a mean of 13.1 alleles per locus. PIC values of the loci ranged from 0.20 (*umc1367*) to 0.90 (*phi083*), with a mean of 0.60, reflecting the presence of high diversity among the tested marker loci. The observed heterozgosity (H_o) for different SSR loci was less than the expected heterozygosity (H_e). The allelic richness of the populations, ranged from 1.79 to 2.90.

Identification of 174 unique SSR alleles led to clear differentiation of 44 accessions. It is also an indicator of potential of these accessions as a reservoir of novel alleles for crop improvement. The study also revealed six highly frequent SSR alleles at different loci (*phi014*, *phi090*, *phi112*, *umc1367*, *phi062* and *umc1266*) with individual

Table 2. Promising accessions identified based on multi-location trials.

Accession	State	Specific traits*
IML119	Haryana	EL (A,H); ED (A,H); GY (H)
IML134	Gujarat	EL (A,H); KN (A,H); GY (H)
IML185	Himachal Pradesh	ED (A,H); KN (A,H); GY (A)
IML297	West Bengal	KPR (A,H); KN (A,H), GY (H)
IML324	Himachal Pradesh	EL (B,H); GY (A,B)
IML331	Bihar	100KW (A,B,H)
IML332	Madhya Pradesh	KR (A,B); GY (A,H)
IML409	Jharkhand	Extra early flowering (Almora)
IML431	Jharkhand	100KW (A,B); GY (B)
IML452	Madhya Pradesh	KR (A,B); KN (A,H); GY (A)
IML459	Gujarat	ED (A,B,H); GY (B,H)
IML462	Jharkhand	Extra early flowering (Almora)
IML475	Orissa	ED (A,B); GY (A)
IML496	Andhra Pradesh	KR (A,B,H); KN (A,B,H)
IML498	Andhra Pradesh	EL (B,H); ED (A,H); KR (A,B); KPR (B,H); KN (A,B,H)
IML499	Karnataka	KPR (A,H); KN (A,B,H)
IML505	Uttarakhand	KR (A,H); KN (A,H)

A: Almora; B: Bajaura; H: Hyderabad
EL: Ear length; ED: Ear diameter; GY: Grain yield; KR: No. of kernel rows per ear; KN: Kernel no. per ear;
KPR: No. of kernels per ear row

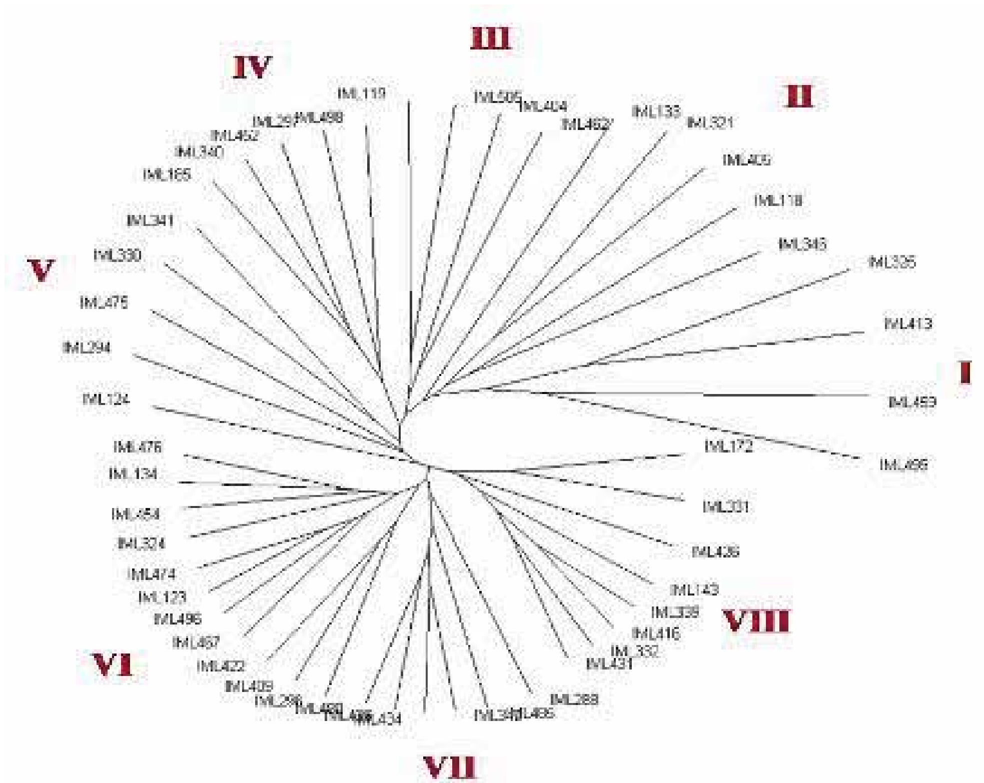

Fig. 2. UPGMA-based unrooted tree depicting the genetic relationships among the selected landrace accessions.

frequencies greater than 0.75. These six SSR loci were earlier reported to tag genes/QTLs associated with some important traits (Dubreuil et al., 2006; Reif et al., 2004). Thus, it is clear that the chromosomal regions harboring these SSRs are not selectively neutral.

Wright's *F* statistics revealed high levels of genetic differentiation both between (36.3%) and within (63.4%) populations, indicating significant population sub-structure. The test of Hardy-Weinberg law in this study describes that different levels of deviation, varying from 4.88 to 48.78%, occur in each population. The predominant type of selection expected on such locals/landraces can be due to human interference or selection pressures exerted by the farmers in favour of specific characteristics exhibited by these landraces. Cluster analysis of the SSR allele frequency data, using Nei's (1972) genetic distance and UPGMA, showed eight clusters with no specific separation based on geographic origin (Fig. 2). However, the clustering pattern largely matched with those derived through analysis of agronomic performance data.

The advanced M (Maximization or Marker allele richness) strategy was implemented for formulating the minicore based on both phenotypic and molecular datasets. The study was successful in identifying a minicore of 14 landrace accessions that had effectively captured ~93% of the phenotypic and molecular diversity among the tested 48 accessions.

Thus, the present study of molecular diversity coupled with analysis of agronomic performance multiple locations, analysis of G x E interactions, and identification of promising accessions could be of potential utility not only for applied research, but also for allele mining.

Acknowledgement

The study was carried out as a part of the ICAR National Fellow Project titled "Molecular characterization of Indian maize landraces and allele mining for agronomically important traits" awarded to BMP.

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In silico mining of EST-SSRs from putative drought stress-responsive candidate genes and polymorphism survey in selected maize inbred lines

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Abstract. Maize production and productivity is severely affected worldwide by water deficit stress, particularly in countries like India where nearly 80 percent of maize is cultivated under rainfed conditions. We have undertaken *in silico* mining of microsatellite/SSR markers from the EST sequences of putative drought stress-responsive candidate genes in maize. The EST sequences from the NCBI database were processed and clustered to reduce redundancy to develop consensus sequences. The consensus sequences were searched for SSR motifs. In total, 930 di- to hexa-repeat motif SSR markers were identified, including 132 from EST-SSR sequences that are homologs of functionally characterized genes involved in dehydration responses. The mined data was validated using tBLASTn and tBLASTx searches of the retrieved regions against the databases at NCBI, built inside the MaizeEST project. Validated sequences were used for designing primers (through PBC Bioinformatics) for an initial set of 132 EST-SSRs. These SSRs were used for analysis of SSR polymorphisms in a set of 24 maize inbred lines with significant differences in responses to drought stress in India. The genic SSRs, identified based on putative drought-stress responsive genes in maize, can be potentially for various purposes, including genetic diversity analysis, comparative mapping and marker-assisted selection (MAS).

Kew words: Maize, drought, EST-SSRs, bioinformatics, functional diversity

Introduction

Drought stress is a major constraint to maize production and yield stability in rained ecosystems. Drought spells across Asia have become more frequent and severe, leading to irregular and insufficient irrigation of the crop and depletion of groundwater resources, leading to 100% yield losses in certain areas. Insufficient water availability leads to a host of biochemical, physiological, and metabolic changes in plant. These changes, many apparently adaptive, include an array of pathways associated with signal perception, transduction, and regulation of gene expression in a temporal and spatial pattern. A significant number of genes, gene products, transcription factors and pathways associated with drought stress response have been identified in maize using functional genomic approaches. This provides powerful tools for identifying expressed genes.

The discovery of novel genes and its possible utilization in modern plant breeding continue to engage the attention of most plant biologists. Expressed Sequence Tags (ESTs) are short DNA molecules (100-500 nt) reverse-transcribed from a cellular mRNA population. They are generated by large scale single-pass sequencing of randomly picked cDNA clones and have proven to be efficient and rapid means to identify novel genes. ESTs thus represent informative source of expressed genes and provide a sequence resource that can be exploited for large-scale gene discovery.

A wealth of EST sequence information has been generated and deposited in online databases (Rudd, 2003). These can be scanned for identification of Simple Sequence Repeats (SSRs), which are typically referred to as 'EST-SSRs' or 'genic microsatellites'. Subsequently, locus-specific primers flanking EST- or genic SSRs can be designed to amplify the microsatellite loci present in the genes. EST analysis currently provides the most straightforward strategy to address the transcribed parts of genomes, thus rendering even complex and highly redundant genomes, such as that of maize and other cereals amenable to large-scale analysis. In the present study, we report on the utilization of bioinformatic approaches for scanning maize EST sequences based on drought stress-responsive candidate genes, leading to the detection of EST-SSR markers.

Materials and Methods

The EST sequences for maize available in the public domain were acquired from NCBI EST site (http://www.ncbi.nlm.nih.gov/sites/entrez). The identified ESTs were subjected to masking of repeat sequences, low-complexity sequences, including the SSRs, interspersed repeats and crossmatch, prior to clustering. CAP3 assembly algorithms (Huang and Madan. 1999) were used to assemble the individual ESTs into clusters of sequences deriving from the same transcript as tentative consensus sequences (TCs) and singletons representing unique transcripts.

The identification and localization of microsatellites in ESTs was accomplished by a microsatellite search module named PBC bioinformatics *(http://hornbill.cspp.latrobe .edu.au/cgi-binpub/ssrprimer/indexssr.pl)* Homology searches were performed against non-redundant (nr) nucleotide and protein sequence databases using BLASTN 2.2.2 and BLASTX 2.2.2 versions of the BLAST programs (Altschul *et al.*, 1997). The BLASTN program was used to identify maizeEST hits in the non-redundant (nr) nucleotide sequence database, High Throughput Genomic Sequences (HTGS) Division of GenBank.

The ESTs associated with drought stress responses were identified from multiple sources, based on the compiled list of stress-regulated genes documented in more than one plant species (http://stress-genomics. org/ stress.fls/expression/expression.html). In addition, data from microarray expression profiles of putative candidate gene sequences (Maize Genetics Unit, IARI) have been used. Based on above analyses, a local database was constructed and utilized for BLAST analysis.

Primer pairs for non-redundant EST-SSRs were designed using PRIMER3 *(http://www-genome.wi. mit.edu/cgi-in/primer/primer3.cgi)*. A set of inbred lines differing in responses to drought stress under Indian conditions were used for analysis of polymorphisms based on EST-SSRs identified in this study. These lines included genotypes from CIMMYT (Mexico), particularly these belonging to DTPY (Drought Tolerance Program - Yellow) and DTPW (Drought Tolerance Program - White) and some elite inbred lines of India. DNA extractions from the maize genotypes, PCR amplification using EST-SSRs primers, allele resolution and statistical analyses were carried out as described by Bantte and Prasanna (2003).

Results and discussion

From a total of 1200921 maize ESTs, accessed from the NCBI database (January, 2008), our search was restricted to EST sequences in putative drought stress-responsive genes. This led to the identification of 43083 ESTs, with lengths ranging from ~100 to ~700 bp. These sequences were base-called and the readable sequences of high quality were considered for further analysis. The sequences were trimmed off leading primer, oligoDT adapter, poly-A tail and poor-quality sequences. The greatest number of trimmed sequences had lengths between 100 to 500 nt.

Gene annotations were based on similarities to either known or putative ESTs in the public databases. All annotations were based on BLAST searches, with a score threshold of e” 100 for BLASTn. For tBLASTx, a score threshold of e”200 was set, as these generally had e-values $<10^{-5}$ with a minimum of 50% identity over at least 30% of the length of the protein, which are the commonly used thresholds for reliable sequence annotation (Quackenbush *et al*., 2000). Tentative functional annotations of EST sequences were determined through BLASTx with a threshold of e”200 and e-value 10^{-5}. The 43083 sequences were assembled into 5302 contigs which represent unigene sets.

Putative function could be assigned to 5302 sequences with all of these proteins represented in other plant species, assuming that in most cases each contig represents one gene (White *et al.* 2000). EST assembly with functional annotation leading to the generation of tentative consensus (TC) sequences has dramatically reduced the redundancy in this database, as reported earlier (Rounsley *et al.* 1996). The 3282 sequences with significant hits for water stress-responsive candidate genes were further used for SSR retrieval. Over three quarters of the 5302 consensus sequences (62.9%) were found to have significant similarity (BLASTx e”200) to sequences in public databases. As expected, a substantial number of the EST contigs and singletons had no significant homology to either nucleotide or protein sequences in public databases at the time of analysis. In addition, a substantial number of ESTs (approximately 11% of the unigenes) were found to have similarity with unannotated hypothetical, putative or unknown proteins contained in the public databases and were, therefore, placed in the unidentified function class.

Out of 3282 functional annotated sequences searched for the presence of SSRs, a total of 930 di- to hexa-repeat motif SSR markers were identified. These sequences were sorted out an initial set of 132 EST-SSR sequences were further selected for primer designing (Table 1); these represented putative genes involved in dehydration responses. SSR polymorphisms for the 132 EST-SSRs were analyzed in selected drought stress-tolerant and susceptible lines. Of the 132 EST-SSRs, 27 provided good amplification products. These markers succeeded to produce polymorphic and monomorphic alleles when applied on the 24 maize genotypes. The 27 EST-SSRs revealed a total of 100 polymorphic alleles. The number of polymorphic alleles generated by individual primer pairs

Table 1. Annotation of 132 EST-SSRs based on extensive BLAST search

S. No.	Gene/Pathway	Repeat	S. No.	Gene/Pathway	Repeat
1.	Actin	(GAG)$_{14}$	67.	DOF1	(GCGA)$_{17}$
2.	cysteine protease	(GTACA)$_{14}$	68.	tonoplast intrinsic protein1	(GCTGC)$_{17}$
3.	methyltransferase	(TCG)$_{17}$	69.	putative integral membrane protein	(GCAAC)$_{19}$
4.	ribosomal protein L25	(TGCA)$_{13}$	70.	yptm1 protein	(TTG)$_{20}$
5.	acidic ribosomal protein P2a-2	(GAG)$_{14}$	71.	spermidine synthase	(AGGA)$_{13}$
6.	40S ribosomal protein S14	(CCT)$_{14}$	72.	lipoxygenase	(ACGGA)$_{14}$
7.	cinnamoyl-CoA reductase	(TAGC)$_{13}$	73.	cinnamyl alcohol dehydrogenase	(CCG)$_{14}$
8.	ypt homolog2	(CGG)$_{14}$	74.	lipoxygenase	(ACGGA)$_{14}$
9.	Cytochrome c	(CAG)$_{13}$	75.	histone H2A	(CTG)$_{13}$
10.	class I acidic chitinase	(AAATC)$_{18}$	76.	ubiquitin / ribosomal protein S27a	(AACAA)$_{16}$
11.	profilin	(TTGCA)$_{15}$	77.	beta-glucosidase aggregating factor precu	(GCCCG)$_{17}$
12.	acidic ribosomal protein P2a-3	(GCG)$_{13}$	78.	putative cytidine deaminase	(CCCT)$_{13}$
13.	PL3K2 [Zea mays]	(TGCA)$_{13}$	79.	ascorbate peroxidase2	(GGCC)$_{13}$
14.	profilin	(TTGCA)$_{15}$	80.	xyloglucan endotransglycosylase homolog1	(ATACC)$_{14}$
15.	inorganic pyrophosphatase	(GCC)$_{14}$	81.	ribosomal protein S27	(GCG)$_{16}$
16.	ADP-ribosylation factor homolog1	(GAT)$_{19}$	82.	Histone H3.2	(GCTCT)$_{16}$
17.	class I acidic chitinase	(AAATC)$_{18}$	83.	ADP-ribosylation factor homolog1	(CAT)$_{13}$
18.	male-gametophyte specific1	(GAC)$_{14}$	84.	pathogenesis-related protein 1	(CGC)$_{15}$
19.	ferredoxin3	(CGC)$_{13}$	85.	mgp1 GTP-binding protein	(AAAAG)$_{14}$
20.	putative cytidine deaminase	(GTGC)$_{21}$	86.	trypsin inhibitor	(AGCTG)$_{14}$
21.	PL3K2	(TGCA)$_{13}$	87.	cytosolic 3-phosphoglycerate kinase	(AGC)$_{15}$
22.	early drought induced protein	(CCAAG)$_{16}$	88.	GR1a protein	(ACGT)$_{13}$
23.	Dip protein	(TGG)$_{14}$	89.	sucrose-phosphatase1	(TATTC)$_{16}$
24.	cysteine synthase2	(TAAAA)$_{15}$	90.	putative beta 4 proteasome subunit	(GGC)$_{14}$
25.	lipoxygenase	(ACGGA)$_{14}$	91.	physical impedance induced protein	(ACTG)$_{13}$
26.	putative GRF-interacting factor 2	(GCT)$_{14}$	92.	lipoxygenase	(ACGGA)$_{14}$
27.	heat shock protein 17.2	(TAAT)$_{13}$	93.	O-methyltransferase	(CAAG)$_{14}$
28.	benzoxazin6	(ACAG)$_{17}$	94.	tonoplast intrinsic protein1	(GCTGC)$_{17}$
29.	proton-exporting ATPase	(TAAA)$_{16}$	95.	beta-glucosidase aggregating factor precu	(GCCCG)$_{17}$
30.	tonoplast intrinsic protein1	(GCTGC)$_{17}$	96.	golgi associated protein se-wap41	(TGAGC)$_{17}$
31.	profilin	(CCAAG)$_{15}$	97.	GR1a protein	(ACGT)$_{13}$
32.	14-3-3-like protein	(GTAC)$_{13}$	98.	heat shock protein18c	(GCGCC)-$_{14}$
33.	Exopolygalacturonase precursor	(TCCG)$_{14}$	99.	cysteine synthase2	(TAAAA)$_{15}$
34.	Superoxide dismutase [Cu-Zn] 4AP	(CAT)$_{14}$	100.	putative oligouridylate binding protein	(GCT)$_{16}$
35.	Histone H2B.1	(GCAGC)$_{15}$	101.	ribosomal protein L30	(GCG)$_{14}$
36.	Histone H4	(TTAT)$_{14}$	102.	proton-exporting ATPase	(ACGT)$_{14}$
37.	Actin-1	(GCG)$_{13}$	103.	ustilago maydis induced11	(CGT)$_{14}$
38.	cinnamoyl CoA reductase1	(TCCA)$_{17}$	104.	ascorbate peroxidase2	(GGCC)$_{13}$
39.	Ran-related GTP binding protein	(TTTTA)$_{14}$	105.	isopentenyl pyrophosphate isomerase	(AACA)$_{17}$
40.	ribosomal protein L35A	(AGC)$_{13}$	106.	sopentenyl pyrophosphate isomerase	(GCA)$_{16}$
41.	lipoxygenase	(CCGT)$_{14}$	107.	proton-exporting ATPase	(ACGT)$_{14}$
42.	pollen-specific profilin3	(TCC)$_{20}$	108.	GR1a protein [Zea mays]	(ACGT)$_{13}$
43.	isopentenyl pyrophosphate isomerase	(AACA)$_{17}$	109.	beta-glucosidase aggregating factor precu	(GCCCG)$_{17}$
44.	tonoplast intrinsic protein1	(GCTGC)$_{17}$	110.	lipoxygenase	(ACGGA)$_{14}$
45.	ribosomal protein S11	(GGC)$_{13}$	111.	putative beta 4 proteasome subunit	(GGC)$_{14}$
46.	40S ribosomal protein S4	(AGC)$_{13}$	112.	ustilago maydis induced11	(CGT)$_{14}$
47.	histone H2A	(CGA)$_{13}$	113.	cysteine synthase2	(TAAAA)$_{15}$
48.	voltage-dependent anion channel protein	(CTGAT)$_{14}$	114.	tonoplast intrinsic protein1	(GCTGC)$_{17}$
49.	cytosolic 3-phosphoglycerate kinase	(GCT)$_{15}$	115.	sucrose synthase1	(AAGA)$_{14}$
50.	ascorbate peroxidase2	(CGGC)$_{13}$	116.	ferritin	(AAAGA)$_{22}$
51.	ribosomal protein S27	(CCG)$_{16}$	117.	heat shock protein18c	(GCGCC)$_{14}$
52.	sucrose-phosphatase1	(AGAAT)$_{16}$	118.	aquaporin ZmPIP2	(GATC)$_{13}$
53.	Histone H3.2	(CAGAG)$_{16}$	119.	nucleosome/chromatin assembly factor	(AAC)$_{14}$
54.	flavonoid O-methyltransferase	(TGCT)$_{14}$	120.	cytokinin response regulator1	(AAC)$_{18}$
55.	ZmGR1b	(TACG)$_{13}$	121.	cytokinin response regulator1	(AAGA)$_{14}$
56.	acidic ribosomal protein P3	(CCG)$_{14}$	122.	benzoxazin6	(GCA)$_{13}$
57.	ribosomal protein S11	(CGC)$_{13}$	123.	ascorbate peroxidase2	(GGCC)$_{13}$
58.	beta-glucosidase aggregating factor precu	(GCCGG)$_{17}$	124.	GR1a protein	(ACGT)$_{13}$
59.	global transcription factor group C	(CTG)$_{13}$	125.	proton-exporting ATPase	(TAAA)$_{16}$
60.	S-adenosyl-L-homocysteine hydrolase	(CGC)$_{13}$	126.	lipoxygenase	(ACGGA)$_{14}$
61.	dehydrin	(AGG)$_{14}$	127.	heat shock protein18c	(GCGCC)$_{14}$
62.	DNA cytosine methyltransferase MET2a	(CGC)-$_{13}$	128.	spermidine synthase	(AGGA)$_{13}$
63.	cytokinin response regulator2	(GTT)$_{18}$	129.	spermidine synthase	(TACG)$_{17}$
64.	histone H2A	(GCC)$_{12}$	130.	ascorbate peroxidase2	(GGCC)$_{13}$
65.	transcription factor MYB8	(CGA)$_{13}$	131.	transcription factor MYB31	(CTC)$_{16}$
66.	DNA cytosine methyltransferase MET2a	(CATC)$_{16}$	132.	O-methyltransferase	(CAAG)$_{14}$

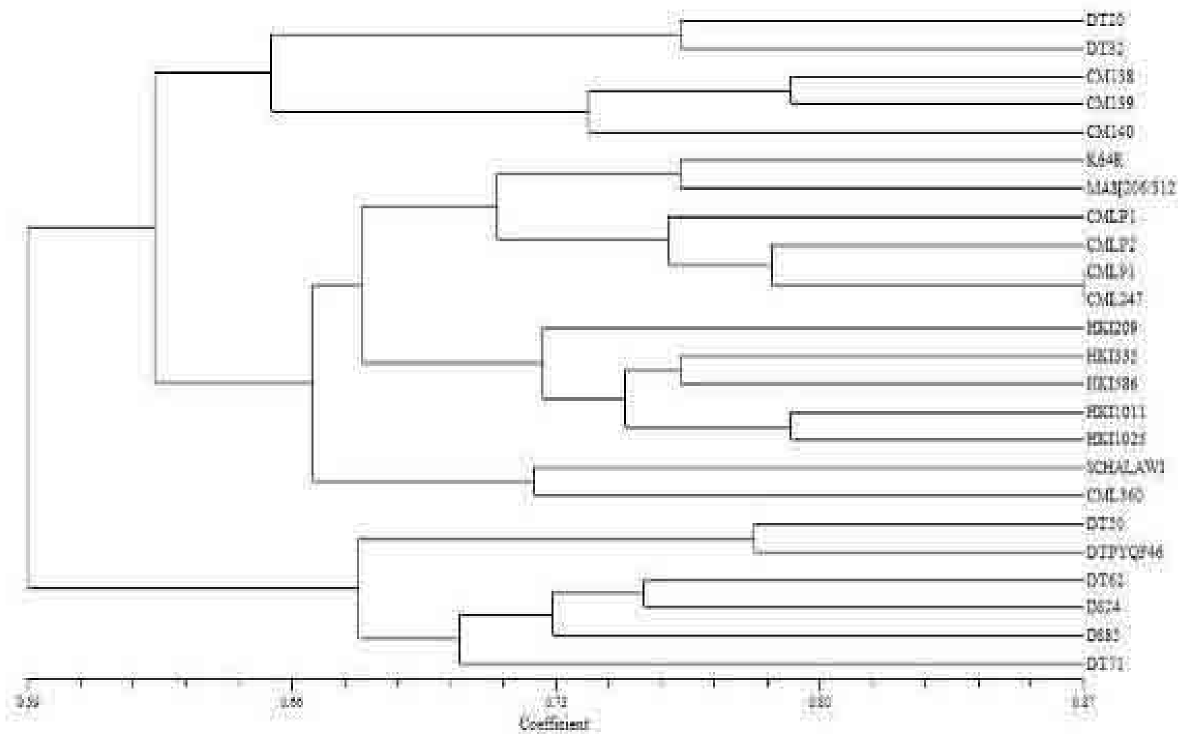

Fig. 1. Dendogram depicting genetic relationships among 24 inbred lines based on analysis using EST-SSR data.

ranged from two (MDE203, MDE210 and MDE229) to seven (MDE149) with an average of 3.74 alleles per locus. The PIC (Polymorphism Information Content) values for the EST-SSR markers ranged from 0.23 (MDE176) to 0.95 (MDE154) with a mean of 0.56 across the inbred lines assayed.

Cluster analysis based on EST-SSR data grouped the 24 lines into three main clusters, each comprising 5, 13 and 6 genotypes, respectively (Fig. 1). Cluster I included DT20, DT32, CML138, CML139 and CML140, Cluster II included K64R, SCHALAWI, MAS[206/312], CMLP1, CMLP2, CML91, CML247, CML360, HKI209 HKI335, HKI586, HKI1011 and HKI1025, and Cluster III included DT50, DT62, DT71, DS24, DS85, DTPYQF46. The pair-wise genetic similarity values (based on Jaccard's Coefficient) ranged from 0.43 (HKI335-CM140) to 0.87 (CML91-CML247).

Thus, the present study was successful in development and characterization of 132 EST-SSR markers from maize sequences with functional annotations, some of which contain SSR motifs. These markers have detected high levels of polymorphism amongst a selected set of inbred lines differing in drought stress tolerance. The EST-SSRs originate in transcripts with putative annotations for ion transports, major intrinsic proteins (MIP), osmolyte biosynthesis, heat shock proteins, LEA proteins, SP1 protein, ROS-scavenging enzymes, etc. These EST-SSR markers could be of potential value in diversity analysis, comparative mapping and MAS. We are currently exploring the drought microarray data recently obtained by our Unit.

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Analysis of genetic diversity within Nepalese maize populations using SSR markers

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Abstract. Information on genetic diversity and relationships among breeding materials is necessary for hybrid maize breeding. Four open-pollinated varieties were analyzed using SSR markers to determine the genetic diversity within the populations. In each population, 15 individuals were genotyped with 30 SSR markers. Average heterozygosity percentage of the population was 45.07%, ranging from 35.23% in Rampur Composite to 54.64% in Khumal yellow, indicating the higher level of heterozygosity found in these populations. An average PIC value across all the polymorphic SSR loci was 0.50; it ranged from 0.47 in Manakamana-2 to 0.52 in Khumal yellow and Arun-4. At the genotype level, the range was from 0.07 in umc1161 to 0.84 in umc1136. The total number of alleles detected was 415 for 30 SSR markers in 60 genotypes. The unique and common alleles detected, respectively, were 27 and 71 alleles. The average number of alleles per locus was 3.45 among the populations, ranging from 3.21in Manakamana-2 to 3.76 in Khumal yellow. Average gene diversity across the populations was 0.54 and ranged from 0.51 in Manakamana-2 to 0.56 in Khumal yellow and Arun-4. The genetic similarity coefficient of all individuals among the populations was seen at 0.35.The MRD values were high between Arun-4 and Manakamana-2 (0.290) and low between Khumal yellow and Rampur Composite (0.221).Estimates of genetic distances among the populations showed that Rampur Composite, Khumal yellow, and Manakamana-2 were closely related and they shared similar genetic backgrounds, whereas Arun-4 was genetically more distantly related.

Introduction

An analysis of genetic diversity in germplasm collections facilitates reliable classification of accessions and identification of subsets of core accessions with enormous utility for specific breeding purposes. Genetic diversity has a significant impact on crop improvement (Hallauer, 1988). This information is critical in hybrid breeding and population improvement programs in assessing the level of genetic diversity and characterizing and assigning germplasm into different heterotic groups (Reif et al., 2003).

Genetic diversity in crop plants can be analyzed at different levels?inbred lines/purelines, clones, populations, germplasm accessions, and species. The development of molecular markers has made it possible to analyze plants at the DNA level to achieve a high degree of precision (Melchinger and Gumber, 1998).

Genetic diversities among inbred lines in the past were studied only on the basis of pedigree records and the amount of heterosis expressed by the hybrids. The information is sometimes incomplete, unreliable, or unavailable (George et al., 2004). Moreover, the expression of morphological traits can be affected by environmental factors but this will not affect molecular analysis. Molecular analysis, therefore, provides reliable and complementary information.

SSR markers have been used effectively to measure genetic diversity in many crop plants, including maize. A number of molecular marker systems are currently available for molecular analyses. These include RFLPs, RAPDs, AFLPs, and SSR markers. These markers have been extensively used for genetic diversity analysis, heterosis, and combining ability studies in maize. Among the markers, SSRs have become the marker of choice for many genetic analyses because of their high level of polymorphism, repeatability, low cost, and amenability to automation. SSR markers are codominant in nature, which can detect genes in both heterozygous and homozygous states, an important feature in maize hybrid testing.

Molecular markers can help maize breeders to assign inbred lines efficiently to heterotic groups and to guide them in the choice of parents to develop new hybrids (George et al., 2004). In this context, there is a need to generate reliable information on the genetic diversity of Nepalese maize germplasm to achieve greater breeding efficiency. Therefore, this study was undertaken to analyze the genetic relationships among Nepalese maize germplasm and determine the breadth of genetic diversity.

Materials and methods

Four open-pollinated varieties?Rampur Composite, Khumal Yellow, Manakamana-2, and Arun-4?were used in this study. Rampur Composite and Arun-4 are the varieties recommended for both terai and hill environments, whereas Khumal Yellow and Manakamana-2 are suitable to the hill environments. Khumal Yellow and Rampur Composite are still very popular varieties in the terai and hills, even though they were released in 1966 and 1975, respectively. We obtained the seeds of these varieties from the National Maize Research Program (NMRP). Rampur, Chitwan, Nepal. Seeds were grown on plastic trays in the greenhouse for DNA isolation.

SSR markers

Thirty SSR marker loci, possessing a repeat unit greater than two nucleotides and representing six bins per chromosome, were selected based on bin location and PIC value for uniform genome coverage (Maize DB, www.agron.missouri.edu). Markers were chosen from the 43 priority markers used by the AMBIONET. Primers were synthesized through Research Genetics, Inc. (Huntsville, AL, USA). A laboratory protocol developed and used by the Asian Maize Biotechnology Network (AMBIONET) was followed for the molecular analysis of the populations.

Lyophilization and DNA isolation

Leaf samples were taken from 10-day-old plants. Plants were cut at the base of the second leaf from the bottom. Fifteen individual plants from each population were taken for sampling. These samples were kept in the silk bags and placed inside the condenser and run for 72 h at -75 ^{0}C for lyophilization. Grinding of the samples was done in a Spex Certi-prep Inc. Geno/grinder 2000® for 30 min. The DNAs were isolated/extracted following the CTAB procedure (CIMMYT, 2001).

SSR analysis

The protocol for DNA extraction; amplification, and detection, as described in George et al. (2004), was followed. PCR products were separated using 4.5% PAGE and visualized using silver staining (Promega). A laboratory protocol used by the AMBIONET Service Laboratory was followed for running the PAGE. Allele scoring was done based on standard allele markers with known molecular weights. Data were scored as present ("1") or absent ("o"), while bands that were diffused or too difficult to score were considered as missing data ("9"). In the case when a line has multiple bands of varying intensities, the most intense band is scored as ("1"). A matrix of binary data was analyzed with NTSYS-pc version 2.02 (Rohlf, 1999). We used Jaccard coefficient to calculate the matrix of genetic similarities among pairwise comparisons of genotypes. Cluster analysis was done with the unweighted pair group method using arithmetic averages (UPGMA) and relationships between populations were visualized in a dendrogram.

Statistical analysis

We measured the discriminatory power of each SSR locus using the polymorphism information content (PIC) The PIC values were calculated according to Smith et al. (1997). The number of alleles per locus, number of total unique alleles per population, size range, and number of missing data, percent heterozygosity, and gene frequencies were computed. Similarly, gene diversity, variance of gene diversity, and genetic distance were estimated. The genetic distance matrix was determined using the UPGMA and a dendrogram was prepared based on the matrix of genetic distances. Relationships between populations were visualized in a dendrogram. Modified Roger's distances (MRD) between two populations were calculated according to Wright (1978, p.91) and Goodman and Stuber (1983).

Results and discussion

Polymorphism information content

We estimated the PIC values of each of the polymorphic SSR locus detected in the study. The values ranged from 0.00 (tetra repeats SSR) in *phi089* to 0.84 (tetra repeats SSR) in *phi064* and *umc1136*. Average PIC value at the population level was 0.50 and the range was from 0.47 in Manakamana-2 to 0.52 in Khumal Yellow and Arun-4. SSR loci *phi064*, *phi109188,* and *phi053* showed the best average PIC values (0.76 and 0.67), respectively. SSR markers showing less than 0.50 PIC values were 14 loci with eight tri repeats, four with tetra, one with penta, and two with hexa repeats.

Number of alleles per locus

The total number of alleles detected was 415 for 30 SSR markers in 60 genotypes from the four maize populations. The alleles ranged from one in *phi089* to seven in *bnlg118, phi109188,* and *umc 1152* (Table 1). Loci *bnlg118, phi064,* and *phi109188* detected seven

Table 1. Mean PIC values, number of alleles per locus, heterozygosity percentage, gene diversity, and variance of gene diversity in four maize populations as revealed by SSR markers.

Population	PIC	Number of alleles per locus	Heterozygosity (%)	Gene diversity	Variance of gene diversity
Rampur Composite	0.48	3.41	35.23	0.52	0.06
Khumal Yellow	0.52	3.76	48.21	0.56	0.03
Manakamana-2	0.47	3.21	42.19	0.51	0.04
Arun-4	0.52	3.41	54.64	0.56	0.04
Across populations	0.50	3.45	45.07	0.54	0.04
Range	0.00-0.84	1.00-7.00	21.00-68.97	00.00-0.93	0.00-0.31
SE ±	0.35±0.64	3.05±3.84	44.23±45.90	0.39±0.68	0.02±0.10

alleles per locus, the highest number among those detected, and six alleles were detected by *phi065, phi034,* and *umc1136*. The average number of alleles among the populations was 3.45 (range: from 3.21 alleles in Manakamana-2 to 3.76 in Khumal Yellow). The unique alleles detected were 27, whereas the total common alleles were 71 in 60 genotypes from the four populations.

Percent heterozygosity

The average heterozygosity at the population level was 45.07%; the range was from 35.23% in Rampur Composite to 54.64% in Arun-4 (Table 1). *phi109188* showed the maximum heterozygosity (81.03%), while *umc1109* SSR exhibited the minimum (1.69%). Average heterozygosity was 45.45% among the markers used in the study. At the population level, Arun-4 showed the highest heterozygosity (54.64%), followed by Khumal Yellow (48.21%), Manakamana-2 (42.19%), and Rampur Composite (35.23%). Manakamana-2 and Khumal Yellow had higher heterozygosity percentage, reflecting greater gene diversity than the other two populations.

Gene diversity

Khumal Yellow showed the highest gene diversity (0.62) among the populations, followed by Rampur Composite (0.59), Arun-4 (0.57), and Manakamana-2 (0.56) (Table-1). The observed average gene diversity was 0.59 among the populations. The average variance at the genotype level ranged from 0.19 in *phi089* to 0.83 in *phi064* locus. The gene diversity in Rampur Composite ranged from 0.13 in *umc1304* to *0.93 in phi064* (Table 1). The minimum gene diversity was observed in *phi423796* (0.07) and the maximum was noted in *phi072* (0.83). The gene diversity at the population level ranged from 0.51 in Manakamana-2 to 0.56 in Khumal Yellow and Arun-4. It was 0.54 among the populations.

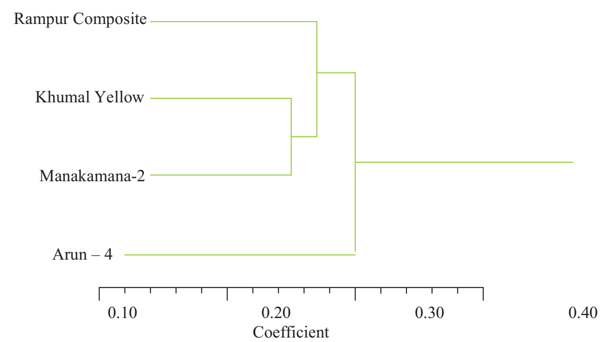

Fig. 1. Genetic distance of four Nepalese maize populations based on modified Rogers' distance.

Cluster analysis

A dendrogram of the four populations was constructed with UPGMA using NTSYS. The similarity coefficient of Manakamana-2 was observed at 0.45 coefficient value, whereas the values were 0.50, 0.42, and 0.40 in Arun-4, Khumal Yellow, and Rampur Composite, respectively. The overall similarity coefficient was 0.35 among the populations, indicating that gene diversity existed in these populations.

Genetic distance

Based on the MRDs, a dendrogram was constructed according to Wright (1978) and Goodman and Stuber (1983). The highest genetic distance (GD) observed was 0.290, between Manakamana-2 and Arun-4, followed by Rampur Composite and Arun-4 (0.271), and Khumal Yellow and Arun-4 (0.265) (Fig. 1). Khumal Yellow and Manakamana-2 displayed the lowest GD (0.220) among the four populations.

Based on GD, Rampur Composite, Khumal Yellow, and Manakamana-2 are considered closely related, sharing similar genetic backgrounds, whereas Arun-4 is genetically distant from the three populations.

Conclusions

The highest number of alleles per locus was observed in Khumal Yellow and Arun-4. Khumal had the highest percent heterozygosity. The results suggest that a considerable amount of allelic and gene diversity exists among the four populations.

Manakamana-2 and Arun-4 had the maximum MRD among the four populations. Arun-4 was more distantly related compared with the other populations. Rampur Composite, Khumal Yellow, and Manakamana-2 are closely related to each other and share a similar genetic background.

Simple-sequence repeats have proved to be accurate, reliable, and efficient tools in fine-scale genetic characterization of germplasm collections. Studies on the genetic diversity of Nepalese germplasm need to be carried out to analyze their genetic relationships and to assign them accurately into different heterotic groups.

Acknowledgment

The authors are very grateful to the Asian Maize Biotechnology Networks (AMBIONET), CIMMYT, Philippines, for providing the materials and technical support needed in the conduct of this study. They also like to thank Ms. Ellen S. Regalado and Meggy Lou Katimbang of AMBIONET, CIMMYT, Philippines, for their valuable help in the molecular analysis. NMRP, Rampur, Nepal is greatly acknowledged for providing the seeds of maize varieties used in the study.

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Mapping Quantitative Trait Loci for Nutritional Quality Traits in Maize

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Abstract. The maize genetic linkage map was constructed by using SSR markers based on $F_{2:3}$ population consisting of 233 family lines derived from the cross between inbred line 201 and 698-3, and QTL analysis of the major nutritional quality traits in maize was carried out with the interval mapping procedure. A genetic linkage map with 134 pairs of SSR markers was established, covering 1831.4 cM of the whole genome with average map distance of 13.67 cM. A total of 35 QTLs were detected for 16 nutritional quality traits, among which 6 QTLs identified on chromosome 1, 2, 4 and 8 influenced protein, starch and oil contents respectively, with 1-3 QTLs for each character and a single one accounting for 8.1% to 21.0% of phenotypic variation. There are 29 QTLs detected on chromosome 1, 2, 4, 8, 9 and 10, contributing to the contents of lysine and other 12 amino acids, with 1-5 QTLs for a single character and each QTL explaining 3.5% to 30.1% of phenotypic variation. The overdominant effects are primary for the QTLs of nutritional quality traits in the population tested, followed by the full dominant effects.

Key words: Maize (*Zea mays* L.); Nutritional quality; Genetic linkage map; SSR marker; QTL mapping

Introduction

Maize is an important crop with three functions as foods, animal feeds and economic uses in China. Improving nutritional quality in maize plays a key role in developing animal husbandry, food processing, and agricultural production of high quality. But for a long time, because of the great pressure of rapid growing population and food shortage in China, maize has long been regarded as a coarse food source, and the objective of maize breeding has been focused on the increase of grain yield, the study on quality improvement of maize, however, has been lagged due to the poor investigation and financial support. With the change from quantity-centered to quality- centered agriculture technology, especially with the rapid development of husbandry and processing industry, the market demands for quality maize are continuously increasing. Accordingly, the development of quality maize hybrid with high yield has become an important task in current maize breeding. To locate the maize quantitative trait locus (QTL) and identify their effects on the phenotype is of strategic importance to maize breeding by utilizing molecular marker assisted selection (MAS) and QTL Cloning.

In this study, the maize genetic linkage map was constructed by using SSR molecular marker technology, and the quantity, location and genetic effects of QTL controlling the content of protein, starch and oil, as well as 13 amino acids determining protein quality, were investigated. This work may lay a good foundation for molecular breeding of quality maize.

Materials and methods

Mapping Populations and Origin of Primers

The mapping population was the $F_{2:3}$ population consisting of 233 family lines derived from the cross between inbred line 201 and 698-3. The field trials were adopted with randomized block design in Ya'an and Deyang, Sichuan, China, from which the artifical selfing seeds obtained for the trait evaluation. The primer was designed according to http://www.agron.missouri.edu, and synthesized by Shanghai Sanggon Biological Engineering Technology & Services Co. Ltd. and Beijing SBS Genetech.

Measurement of Quality Traits

The content of protein, starch and oil was determined by near-infrared diffuse reflectance spectroscopy, and that of amino acids by acid hydrolysis method in an automatic amino acid analyzer (Hitachi L-8800).

DNA Extraction and Purification

Method of 2õCTAB was adopted in the genome DNA extraction and purification [2].

SSR Analysis

PCR reaction and extension were designed in accordance with http://www.burr.bio.bnl.gov/acemaz. html. The reaction products were electrophoresed on 3.5% agarose or 6% polyacrylamide gels before silver-stained. The results were examined by Gel Doc 1000 (BioRad) and analyzed by Quantity I software.

The construction of molecular genetic linkage map: Kosambi function was used to convert recombination ratio into map distance in centimorgans, and MAPMAKER/ EXP3.0b was used to construct the genetic linkage map. QTLs were mapped by MAPMAKER/QTL1.1b with LOD 2.0 set the threshold value for the existence of QTL. Gene action was determined according to Stuber (1987) [6].

Results

The Primer Screening for Parental SSR Polymorphism

A total of 520 SSR markers were used for the polymorphic screening and 134 primers with distinguishable polymorphism were selected. The frequency of polymorphism between two parents was 27.1%.

The Construction of SSR Molecular Marker Linkage Map

A total of 134 primers with distinguishable polymorphism were used to analyze the $F_{2:3}$ population and the mark genotype separation band were obtained. According to c^2 test, these markers fit Mendelian segregation ratio of 1:2:1 or 3:1. The genetic linkage map with 134 pairs of SSR markers was constructed, which covered 1831.4 cM of the whole genome with the average distance of 13.67 cM (Fig. 1).

The QTL of Nutritional Quality Traits in Maize

The statistical analysis on phenotypic value of nutritional quality traits

The phenotypic value and variation for 16 nutritional quality traits of the parents, F_1 and $F_{2:3}$ were listed in Table 1. It was shown that the skewness and kurtosis fit normal distribution. The variance analysis indicated that all traits

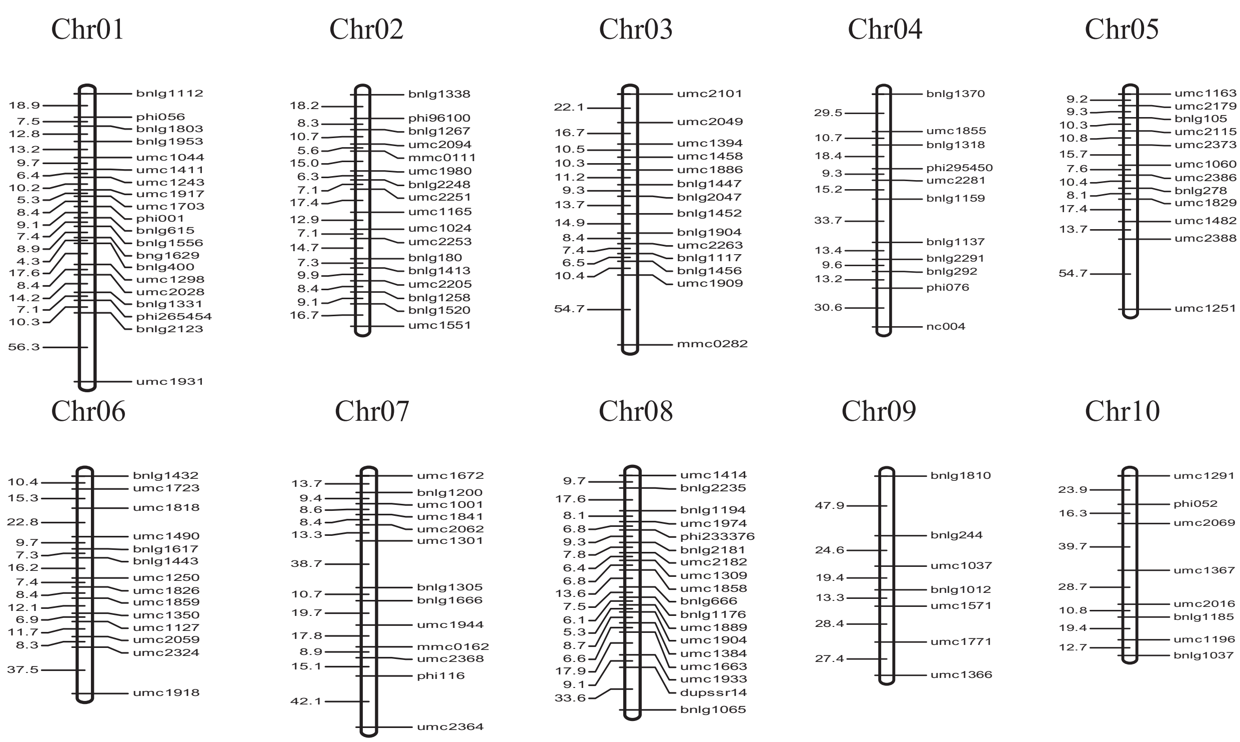

Fig.1 Maize genetic linkage map with SSR markers

Table 1 Estimates of phenotypic value and variation for nutritional quality traits in maize

Trait	Parent		F_1	$F_{2:3}$ population				
	201(P_1)	698-3(P_2)		Mean ± SE	Range	CV%	Skewness	Kurtosis
Protein	10.81	12.61	11.43	11.58 ± 1.45	9.47-13.59	6.37	-0.02	-0.18
Starch	71.09	69.65	70.15	69.98 ± 2.41	66.21-73.31	1.76	-0.12	0.03
Oil	3.11	3.97	4.26	3.54 ± 1.19	1.99-5.31	17.19	0.09	-0.27
Lys	0.3290	0.3086	0.3287	0.3159 ± 0.0746	0.2232-0.4718	12.05	0.11	0.36
Thr	0.4763	0.5225	0.4846	0.4833 ± 0.1069	0.3047-0.6232	11.29	-0.54	0.62
Phe	0.5502	0.7916	0.7318	0.6779 ± 0.1908	0.3544-0.9194	14.36	-0.37	0.53
Ile	0.3081	0.4370	0.4186	0.3871 ± 0.1161	0.2468-0.5574	15.30	0.16	-0.30
Leu	1.1498	1.6965	1.5739	1.3724 ± 0.4310	0.6399-1.8696	16.02	-0.49	0.54
His	0.2970	0.3700	0.2857	0.3094 ± 0.0632	0.1215-0.4059	10.43	-0.35	4.48
Arg	0.4469	0.5404	0.5043	0.4792 ± 0.0972	0.3345-0.6571	10.35	-0.13	0.60
Asp	0.7527	0.9109	0.7935	0.7789 ± 0.1813	0.3919-1.0238	11.87	-0.41	0.97
Glu	2.3277	2.9056	2.4282	2.4495 ± 0.5778	1.0661-3.1864	12.04	-0.51	1.96
Ser	0.5619	0.6865	0.5309	0.5708 ± 0.1336	0.3197-0.7549	11.94	-0.38	0.82
Ala	0.7316	0.7624	0.7698	0.6466 ± 0.2375	0.3851-1.1633	18.74	1.01	2.88
Tyr	0.4592	0.4981	0.7618	0.4487 ± 0.1080	0.3142-0.6317	12.27	0.19	-0.13
Pro	1.0918	1.2848	1.0283	1.0888 ± 0.2639	0.5366-1.4695	12.36	-0.42	1.24

of $F_{2:3}$ families were of significant difference, thus further QTL analysis could be performed.

The QTL analysis of nutritional quality traits

The results of QTL analysis indicated that among the 16 nutritional quality traits, QTLs were detected on all traits. QTLs, genetic effects and their contribution to the phenotype of 16 nutritional quality traits were shown in Table 2.

According to Table 2, we can see that a total of 35 QTLs were detected among the 16 nutritional quality traits in maize. There were 6 QTLs, located on chromosome of 1, 2, 4 and 8, affecting the protein, starch and oil content, with 1-3 QTLs detected for each character and a single one accounting for 8.1% to 21.0% of phenotypic variation. For example, the QTL adjacent marker phi96100 on the chromosome 2 and affecting protein content had positive additive effect and its action was to increase the protein content. Another QTL controlling starch content was found to have dominant effect while the remaining 4 QTLs had overdominant effects.

There were 29 QTLs, located on chromosome 1, 2, 4, 8, 9 and 10, respectively, affecting the content of lysine and other 12 amino acids, with 1-5 QTLs for a single character and each QTL explaining 3.5% to 30.1% of phenotypic variation. Among them, the QTL affecting proline content, adjacent marker phi052 on chromosome 10, had a partial dominant effect and its additive effect was positive, i.e. its presence could increase the proline content. Among the other 28 QTLs, 14 were disclosed to have full dominant or overdominant effects, respectively.

The 35 QTLs detected in this study distributed over 15 chromosomal regions that belong to 6 linkage groups. Among them, 7 regions affected one single trait while 4 affected two, two affected three, and two affected 7, respectively. For instance, the bnlg1331 region on chromosome 1 affected both starch and glutamic acid content; the phi96100 region on chromosome 2 affected the content of protein, starch and lysine. Interestingly, the number of traits affected by two chromosomal regions reached as many as 7, e.g. the bnlg292 region on chromosome 4 affected the content of 7 amino acids including lysine, threonine, ileucine, leucine, arginine, aspartate and phenylalanine; meanwhile, the bnlg666 region on chromosome 8 affected 7 amino acids including threonine, alanine, leucine, aspartate, glutamic acid, serine and phenylalanine. This phenomenon indicated the polypheny of chromosomal regions.

Discussion

The QTL Distribution, Type of Action and Heterosis of Maize Nutritional Quality Traits

In current study, there were 6 QTLs on chromosome 1, 2, 4 and 8 detected to affect protein, starch and oil content. This result differs from previous study [1,3-5]. This may be due to the different populations utilized in our study and needs further investigation. Meanwhile, 29 QTLs on chromosome 1, 2, 4, 8, 9 and 10 detected to contribute to

Table 2 QTL analyses for nutritional quality traits in maize

Trait	QTL	Nearest marker locus	Chr.	Dis. a) (cM)	LOD Score	Genetic effects b)			Gene action c)	Phenotypic variation %
						a	d	#”d/a#”		
Protein	1	bnlg1194	8	-7.9	2.53	-0.1135	0.5677	5.00	OD	16.4
	2	phi96100	2	3.5	2.05	0.5177	0.032	0.06	A	14.9
Starch	1	umc1889	8	-2.4	2.67	0.2352	-0.7726	3.28	OD	12.4
	2	bnlg1331	1	1.9	2.30	0.6127	-1.205	1.97	OD	9.1
	3	phi96100	2	-8.0	2.79	-0.552	0.6431	1.17	D	21.0
Oil	1	bnlg1159	4	12.4	2.03	0.031	0.329	10.62	OD	8.1
Lys	1	phi96100	2	2.0	2.69	-0.0323	-0.0529	1.64	OD	11.8
	2	bnlg292	4	0	2.88	-0.0042	-0.0246	5.86	OD	5.9
Thr	1	bnlg666	8	-1.4	2.93	-0.0559	0.0769	1.38	OD	19.6
	2	bnlg292	4	-1.5	3.76	-0.0004	-0.0362	90.50	OD	8.6
	3	phi052	10	-11.2	2.32	0.0628	0.0636	1.01	D	19.8
Phe	1	bnlg666	8	-6.2	3.28	-0.0385	0.1029	2.67	OD	22.0
Ile	1	bnlg292	4	-4.7	2.21	0.0035	-0.0384	10.97	OD	10.1
Leu	1	bnlg666	8	10.0	2.34	-0.1972	0.2993	1.52	OD	25.8
	2	bnlg292	4	-9.5	2.24	0.0311	-0.1145	3.68	OD	8.5
His	1	phi056	1	-1.2	4.52	0.0953	0.0927	0.97	D	3.5
	2	umc1974	8	-1.7	4.18	0.0931	0.0966	1.04	D	3.5
	3	umc1366	9	-1.6	4.86	0.0916	0.0985	1.08	D	3.5
Arg	1	bnlg292	4	-3.2	2.61	-0.0323	-0.0638	1.98	OD	16.5
Asp	1	bnlg666	8	-3.4	3.29	-0.0760	0.1179	1.55	OD	22.7
	2	bnlg292	4	-2.1	2.94	-0.008	-0.0714	8.93	OD	10.2
Glu	1	bnlg666	8	-3.4	2.77	-0.2613	0.3376	1.29	OD	20.2
	2	bnlg1331	1	-5.9	2.71	-0.3442	0.4397	1.28	OD	18.9
Ser	1	bnlg666	8	-3.4	2.51	-0.0636	0.0705	1.11	D	18.9
	2	phi052	10	6.0	2.53	0.0836	0.076	0.91	D	17.9
Ala	1	bnlg666	8	-5.4	3.97	0.1233	-0.1906	1.55	OD	28.1
	2	umc1551	2	-3.9	9.54	-0.2272	-0.2157	0.95	D	30.1
	3	bnlg1185	10	-4.7	6.63	-0.2110	-0.2232	1.06	D	30.1
	4	bnlg292	4	-4.9	7.17	-0.2199	-0.2242	1.02	D	29.7
Try	1	bnlg1159	4	-1.8	3.26	0.1396	-0.1288	0.92	D	14.0
	2	umc1551	2	-1.9	4.38	-0.1481	-0.1320	0.89	D	14.4
	3	umc1663	8	-2.3	3.56	-0.1320	-0.1221	0.93	D	4.7
	4	bnlg2235	8	2.0	3.32	-0.1431	-0.1363	0.95	D	13.2
	5	phi056	1	-1.2	4.22	-0.1610	-0.1432	0.89	D	14.3
Pro	1	phi052	10	5.6	3.45	0.1802	0.1072	0.59	PD	25.0

a) the distance between the nearest marker loci and the max *LOD* loci of the QTL, + : QTL loci below the nearest marker loci, ÿ: QTL loci above the nearest marker loci.. b) a: additive effect, d: dominant effect, | d/a |: dominant degree. c) A: additive (dominant degree =0-0.20); PD: partial dominance (dominant degree =0.21-0.80); D: dominance (dominant degree =0.81-1.20); OD: overdominance (dominant degree >1.20).

the content of amino acids were not reported before. Through studying the mechanism of heterosis in maize by using RFLP technique, Stuber et al. showed that most quantity traits supported the overdominance hypothesis [7], and our study supported this view. Among 35 QTLs of nutritional traits, overdominant effects played a leading role, followed by full dominant effects.

The Applications of QTLs of Maize Nutritional Quality Traits

Yousef et al.[8] demonstrated that MAS was more effective than phenotypic selection. The majority of the 35 QTLs detected in our study were within the distance of 10 cM to the nearest molecular marker or even less (< 5 cM), with relatively high contribution to phenotype. For example, on linkage group 1, QTLs near marker bnlg1331 could increase both starch and glutamine content, and on linkage group 2, QTLs near marker phi96100 increased protein and lysine content. This may offer the possibility for MAS breeding of the major nutritional quality traits and thus laying a good foundation for the transfer of nutritional traits of high quality through a relatively convenient and fast approach.

Acknowledgment

We thank Dr. Junpin Yang in Sichuan Academy of Agricultural Sciences and Prof. Shaojiang Chen in China Agricultural University for their technical support on the study.

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Genetic Diversity of Waxy Maize Landraces from Southwest and Northeast China Based on SSR Data of Chromosome 9

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Abstract: The genetic diversity of 35 maize materials, including 10 waxy maize landraces from the northeast of China, 16 waxy maize landraces from the southwest of China, 4 waxy maize from China, 2 waxy maize overseas, 2 waxy mutations of maize overseas, 1 domestic maize inbred line, was tested using bulk DNA samples and 56 microsatellite (SSR) loci distributed on the chromosome 9 of maize. A total of 158 alleles were detected. At each locus, the number of alleles varied from 2 to 6, with an average of 3.59. Average marker index (MI) was 2.17. 7 primers accounting for 15.9ÿ in the used primers could detect 2 alleles, 9 primers accounting for 20.5ÿ could detect 5 or 6 alleles. Other 28 primers accounting for 63.6ÿ could normally detect 3 or 4 alleles. On the basis of the genetic similarity coefficients, clustering analysis separated those materials into 5 groups and 6 sub-groups. Northeast and Southwest China waxy maize were clustered into the same group, which consisted of northeast sub-group and southwest sub-group. The landraces collected from the same region were mostly grouped together. In addition, the genetic diversity of landraces was lower than that of hybrid maize. The results suggested that the origin center of waxy maize from China was not only in the southwest of China but also in the northeast.

Keywords: waxy maize; genetic diversity; SSR marker

Introduction

Waxy maize (*Zea mays sinensis*) is a popular vegetable food in China and Southeast Asia. Its amylopectin has good characteristics, which is an important raw material for ferment, textile and feed industry. Though there is still controversy, most studies show that China is an original region of waxy maize. The first mention of it was found in the archives of the SAN NONG JI, written by Zhongfa Zhang in 1760. The germplasm resource of waxy maize of China is rich, and there are more than 900 species. The waxy maize distribution in China shows intensely regional. Waxy maize was mainly distributed in Southwest China, and secondly in Northeast. Recessive waxy (wx) gene was found from waxy maize introduced into America from China by Collins in 1909, which is located on the short arm of chromosome 9.03 and codes for the waxy endosperm of the kernel. Only amylopectin is accumulated in waxy endosperm, but about a quarter of the starch in mature endosperm remains amylase in normal (Nelson and Rines 1962; Nelson 1968; Tsai 1974; Wessler et al. 1986). Mostly varieties of waxy maize come from landraces. Maize is an open-pollination crop. Analysis of genetic diversity and origin of waxy maize were affected by genetic complexity, which was induced by artificial backcross, natural hybrid and genetic exchange.

Microsatellite (SSR) is a codominant marker with sufficient polymorphisms, multi-allele, codominant and reproducible, which is widely used in analysis of maize genetic resource diversity, construction of DNA molecular marker linkage map, genes location and classification of heterotic group. The SSR markers results revealed that waxy maize had rich genetic diversity, and a large genetic distance to normal maize (Huang 1998; Tian 2003; Wu 2004; Zhang 2007). The material of waxy maize was mainly concentrated in the area of Southwest China in previous research, but lack in Northeast. Analysis of genetic diversity of waxy maize landraces using SSR loci distributed on 10 chromosomes of maize cannot avoid the effect of chromosome background and genetic exchange.

The genetic diversity of waxy maize landraces, which can represent materials from Northeast and Southwest China in our previous research results, was analyzed by SSR primer chosen from chromosome 9ÿespecially chromosome 9.03. It can exclude the influence of chromosome background and genetic exchange effectively. The objectives of this study were to give reference for waxy maize germplasm enhancement, improvement and development, and to provide some useful information about origin of waxy maize in China.

Materials and Methods

plant materials

The genetic diversity of 35 maize materials, including 10 waxy maize landraces from the northeast of China, 16

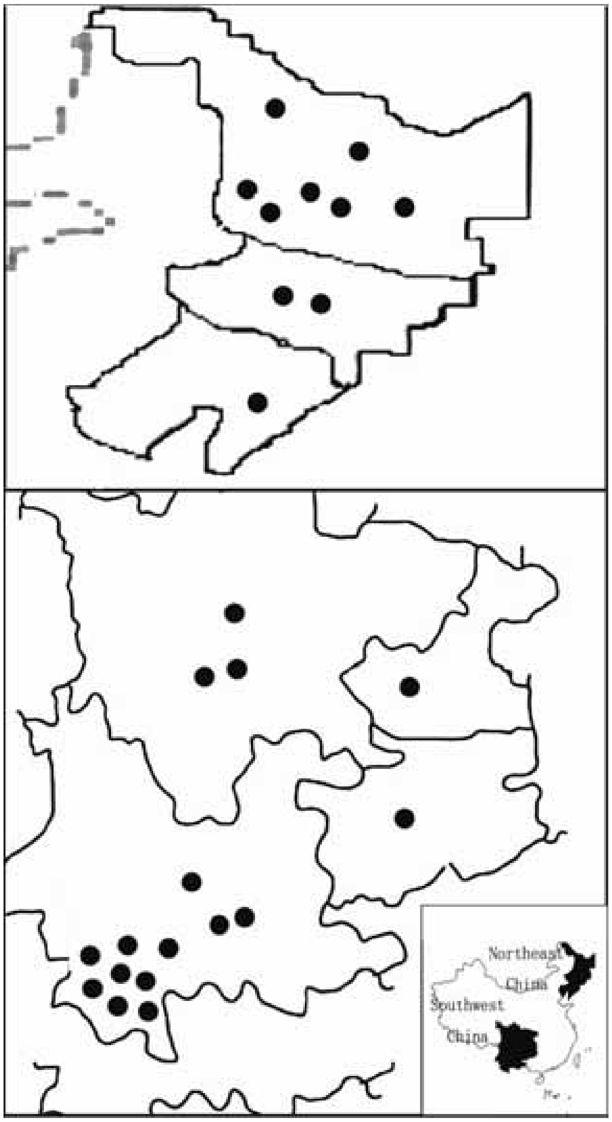

Fig. 1 Geographical distribution of the 26 waxy maize landraces sampled from Southwest and Northeast China.

Table 1. The sources of 35 materials

No.	Germplasm characteristics description	Origin (County, Province)
NE-1	landraces	Yichun, Heilongjiang
NE-2	landraces	Jixi, Heilongjiang
NE-3	landraces	Heihe, Heilongjiang
NE-4	landraces	Haerbin, Heilongjiang
NE-5	landraces	Haerbin, Heilongjiang
NE-6	landraces	Qiqihaer, Heilongjiang
NE-7	landraces	Daqing, Heilongjiang
NE-8	landraces	Dandong, Liaoning
NE-9	landraces	Changchun, Jilin
NE-10	landraces	Changchun, Jilin
SW-1	landraces	Kunming, Yunnan
SW-2	landraces	Yuxi, Yunnan
SW-3	landraces	Yuanjiang, Yunnan
SW-4	landraces	Yuanjiang, Yunnan
SW-5	Four row waxy, landraces	Jinghong, Yunnan
SW-6	Four row waxy, landraces	Jinghong, Yunnan
SW-7	Four row waxy, landraces	Mengla, Yunnan
SW-8	Four row waxy, landraces	Menghai, Yunnan
SW-9	landraces	Menghai, Yunnan
SW-10	landraces	Mengla, Yunnan
SW-11	landraces	Mengla, Yunnan
SW-12	landraces	Yaan, Sichuan
SW-13	landraces	Yaan, Sichuan
SW-14	landraces	Chengdu, Sichuan
SW-15	landraces	Chongqing, Chongqing
SW-16	landraces	Guiyang, Guizhou
AW-1	waxy maize hybrid Pioneer 33J58	America
AW-2	waxy maize hybrid Pioneer 5366	America
AW-3	waxy mutation of maize	America
AW-4	waxy mutation of maize	America
CW-1	waxy maize hybrid Fengnuoyihao	Beijing
CW-2	waxy maize hybrid Xiangnuo 618	Beijing
CW-3	waxy maize hybrid Huaxiangnuoyihao	Tianjin
CW-4	waxy maize hybrid Zixiangnuoyihao	Beijing
CK	domestic maize inbred line 18-599	Yaan, Sichuan

waxy maize landraces from the southwest of China, 4 waxy maize from China, 2 waxy maize overseas, 2 waxy mutations of maize overseas, 1 domestic maize inbred line, were used in this study (Fig 1, Table 1). They are planted in Plant Garden in Maize Research Institute of Sichuan Agricultural University at Ya'an of Sichuan Province, China. Genomic DNA was isolated from a bulk sample of 15 individual plants to analyze genetic diversity of waxy maize landraces.

SSR analysis

Genomic DNA was isolated from the third fresh leaf following CTAB procedure described by Scott with minor modifications (Scott 1988). PCR amplification was performed in a PTC-220 thermal cycler (Surplus Lab Inc, Michigan, USA) programmed at 35 cycles of 1 min at 95 !, 2 min at 55 !, and 2 min at 72 !, followed by a 10 min extension at 72 !. The PCR amplification products were separated on a 6% (w/v) denatured polyacrylamide gel and visualized by silver staining.

SSRs data scoring and analysis

The SSR bands were scored as present (1) or absent (0), each of which was treated as an independent character. Genetic diversity analysis was conducted on the basis of the score. The polymorphism information content (PIC) of each microsatellite was determined as described by Senior. PIC"ÿÿ

fi2, where fi is the frequency of the ith allele in the examined test lines. Marker index (MI) was determined as described by Weir (1996). MI=Allele×PIC, Allele is the allele number of the primer pairs. PIC was determined by the number of detected alleles, and allele frequency in particular. It can reflect significant stability different in a molecular level, and is an important parameter in estimating genetic diversity. The index of genetic similarity (*GS*): GS==$2N_{ij}/(N_i+N_j)$, where N_{ij} is the number of SSR alleles common to landrace *i* and *j*, while N_i and N_j are the total numbers of SSR alleles observed for landraces *i* and *j*, respectively. The dendrogram were constructed by the unweighted pair-group method with arithmetic mean (UPGMA) clustering using the computer software NTSYS-pc version 2.10.

Results

Genetic diversity among 35 maize materials

56 primer pairs of maize were used to amplify the DNA of 35 maize materials. 44 primers whose amplified bands were clear have been analyzed and genetic distances of different kinds of waxy maize landraces have been calculated (table 2).

44 SSR loci were distributed on chromosome 9 of maize, among which 13 SSR loci were distributed on chromosome 9.03. A total of 158 alleles were detected (table 2). At each locus, the number of alleles varied from 2 to 6, with an average of 3.59. Average marker index (MI) was 2.17. Seven primers accounting for 15.9ÿ in the used primers could detect 2 alleles, Nine primers accounting for 20.5ÿ could detect 5 or 6 alleles. Other twenty-eight primers accounting for 63.6ÿ could normally detect 3 or 4 alleles. The most polymorphic loci umc2168 revealed a high level of genetic variation, but had a lower allele number and marker index.

As the measure index of primer polymorphic, allele number, PIC and MI were inconsistent. The experimental results agree with Wang's report.

Genetic diversity within maize landraces

The genetic similarity coefficients of 35 maize materials varied from 0.507 (SW-12^ÿAW-3) to 0.945 (SW-5^ÿSW-6) and averaged 0.671. The genetic similarity coefficients among waxy maize varieties of China ranged between 0.534

Table 2 Genetic variation detected by 44 SSR primers in materials

Primer	Genomic position	Alellic number	PIC	MI
umc1279	9.00	4	0.52	2.08
umc1647	9.00	3	0.53	1.59
umc1957	9.00	3	0.72	2.16
umc2393	9.00	4	0.62	2.48
phi033	9.01	4	0.45	1.80
umc2084	9.01	6	0.43	2.58
phi067	9.01	3	0.49	1.47
bnlg1724	9.01	4	0.56	2.24
umc1040	9.01	5	0.69	3.45
umc2213	9.02	2	0.74	1.48
umc2336	9.02	4	0.62	2.48
umc1430	9.02	3	0.53	1.59
bnlg1082	9.02	4	0.47	1.88
umc1519	9.04	3	0.78	2.34
umc2121	9.04	3	0.70	2.10
umc1120	9.04	3	0.76	2.28
phi016	9.04	2	0.60	1.2
umc2371	9.05	3	0.54	1.62
umc2344	9.05	4	0.81	3.24
umc2342	9.05	3	0.49	1.47
Umc1519	9.05	5	0.51	2.55
umc2346	9.06	4	0.62	2.48
bnlg1191	9.06	5	0.86	4.3
umc1366	9.06	4	0.48	1.92
bnlg128	9.07	5	0.64	3.2
umc1804	9.07	3	0.55	1.65
bnlg1588	9.07	3	0.54	1.62
umc1366	9.07-9.08	4	0.68	2.72
umc1137	9.08	5	0.90	4.50
umc1505	9.08	4	0.81	3.24
bnlg1129	9.08	3	0.62	1.86
phi065	9.03	3	0.78	2.34
bnlg1687	9.03	5	0.7	3.5
phi027	9.03	3	0.86	2.58
umc2087	9.03	2	0.08	0.16
umc1586	9.03	2	0.94	1.88
umc1599	9.03	6	0.58	3.48
umc1743	9.03	5	0.37	1.85
umc1258	9.03	4	0.11	0.44
umc2168	9.03	2	0	0
umc2340	9.03	2	0.88	1.76
umc2394	9.03	4	0.58	2.32
phi042	9.03	3	0.69	2.07
bnlg1730	9.03	2	0.75	1.5

and 0.945. The genetic similarity coefficients of waxy maize landraces from Northeast China varied from 0.616 to 0.932. The genetic similarity coefficients of waxy maize landraces from Southeast China varied from 0.603 to 0.945. The genetic similarity coefficients of four-row waxy maize landraces varied from 0.822 to 0.932. The genetic similarity coefficients of waxy maize hybrid varied from 0575 to 0.918. The genetic similarity coefficients of waxy maize hybrid of China varied from 0.836 to 0.904.

clustering analysis

The dendrogram based on SSR markers distinguished 35 maize materials, which were distinctly clustered into 5 groups and 6 sub-groups (Fig 2). The biggest group (Group '!) consisted of 9 and 16 waxy maize landraces from northeast of China and southwest, respectively. Northeast and Southwest China waxy maize landraces were clustered into the same group, which consisted of northeast sub-group and southwest sub-group. The landraces collected from the same region were mostly grouped together. Group a! included 1 domestic maize inbred line 18-599. Two waxy maize hybrids from America were clustered into Group b!. Group c! included a waxy maize landraces from Heilongjiang. In addition, Two waxy mutant maize from America and four-row waxy maize hybrids from China were less related to others genetically, and formed grouped d!.

Discussions

Effect of chromosome background on genetic diversity

The type of maize landraces of China mainly is flint type. The result showed that waxy maize of china was originated from flint corn (Cao 1990; Zen 1981; Yang 1998). The type of waxy maize, first introduced to America from China by M. W. Farnham in 1909, was flint type waxy maize. The dent corn of America formed lately was introduced to China in the 1920s. Maize is an open-pollinated crop, so part of waxy maize landraces of China was dent corn, which might be the result of natural hybrid. It can be concluded that there were greater differences among waxy mutation of maize overseas, waxy maize landraces of China and domestic maize inbred lines. There was a remarkable similarity in the waxy maize materials from the same region.

Different from the former study on genetic diversity of waxy maize, average allele number, average polymorphism information and Average marker index are lower (Liu 2005; Yang 2006; Zhang 2007) (Table 3). The method, SSR loci distributed on chromosomes 9 of maize, is better to eliminate interference, and can accurately analyze the genetic diversity of waxy maize landraces of China.

Waxy maize inbred lines were mainly originated from waxy maize landraces of China, Most of which had not pedigree information. The genetic relationship of waxy maize inbred lines was still unclear. Heterotic Grouping of waxy maize inbred lines could not be classified based on pedigree data, which usually results in a low efficiency of breeding. Analysis of genetic diversity of waxy maize materials by SSR markers located on chromosome 9 is not only useful for enhancement, improvement and development of waxy maize germplasm resources, but also can provide theoretical guidance for breeding.

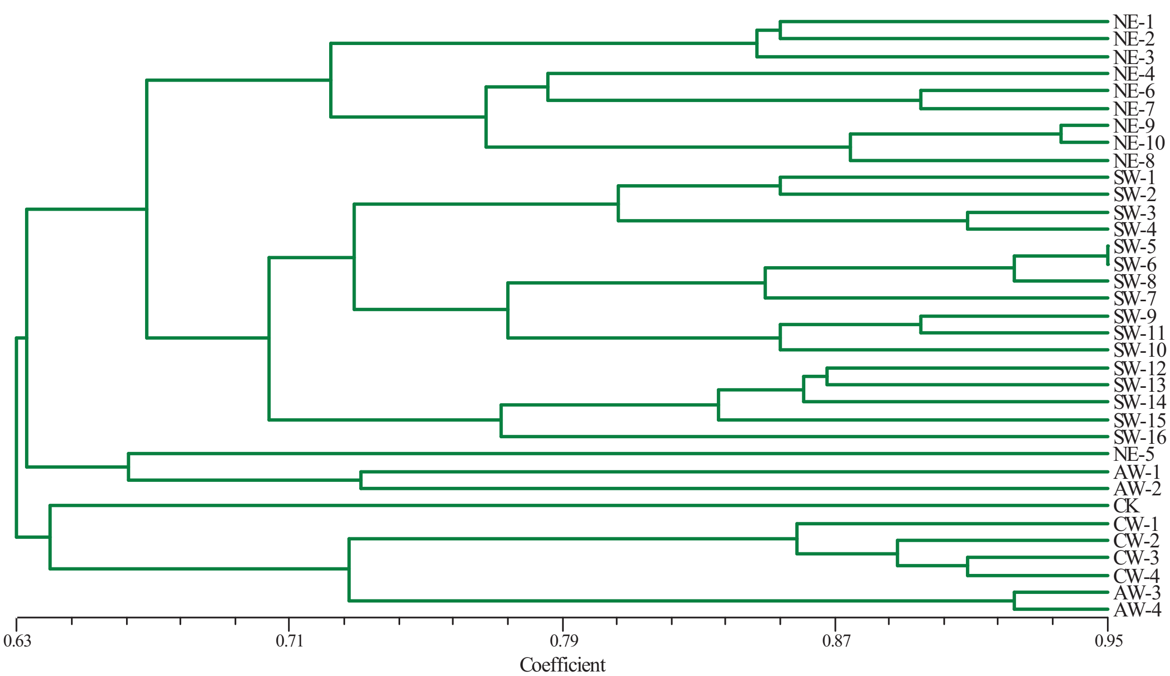

Fig. 2 UPGMA dendrogram by cluster analysis for 35 materials based on genetic similarity by means of 44 SSR markers

Effect of genetic exchange on genetic diversity

According to the genetic map (IBM2) from MaizeGDB, the relative coordinate value of waxy gene on chromosome 9 is 191.70. The relative coordinate value of primer umc1258 nearest to waxy gene is 195.70. The relative coordinate value of primer umc1505 farthest to waxy gene is 635.20 (Coe et al. 2002; Cone et al. 2002). The allele number, polymorphism information and marker index of primer umc1505 are more than that of primer bnlg1258. The Marker Index of primer umc1137 far from waxy gene is the highest than others. The allele number, polymorphism information and marker index of primer umc2168 near to waxy gene are the lowest. In addition, average of allele number, polymorphism information and marker index of the primers distributed on chromosome 9.03 are lower than the rest (table 4). With occurs of genetic exchange in long-term evolution, the genetic diversity of germplasm resources become richer and richer. The crossing-over value increases with the increasing distance from objective traits. The genetic exchange has certain effect to analysis of origin of waxy maize. Better results will be obtained by choosing the primer on chromosome 9.03.

Origin of waxy maize in China

The genetic diversity between waxy maize and normal maize mainly manifested as the difference between waxy character and non-waxy, which might be the difference between maize materials introduced firstly into China in the New World and maize materials recently introduced from overseas (Tian 2003).

Waxy maize landraces of China were clustered into the same group based on SSR marker. The group '! consisted of waxy maize landraces from China, which consisted of northeast sub-group and southwest sub-group. The landraces collected from the same region were mostly grouped. A waxy maize landraces from Heilongjiang formed grouped c!, which maybe introduced from overseas. The results show inconsistent results about origin center of waxy maize in China. It suggested that the origin center of waxy maize from China was not only in the southwest of China but also in the northeast. Whether there is an origin center of waxy maize of southwest and northeast in China or there are even other center, and needs further study.

Although morphological difference between four-row waxy maize and waxy maize landraces from Southwest China, they are grouped together. The experimental results are in agreement with previous research, which waxy maize landraces from Southwest China have the same origin.

Table 3. Comparison with previous research

Primers	Average allele number	Average polymorphism information	Average marker index
Chromosome 9	3.59	0.59	2.17
Zhang jinyu 2007	9.75	0.82	7.92
Yang yong 2006	4.80	0.60	—
Liu yongjian 2005	4.13	—	—

Table 4. The mean value of genetic variation detected by primers in bin 9.03 and in chromosome but not in bin 9.03

Primers	Average allele number	Average polymorphism information	Average marker index
The rest	3.71	0.62	2.31
On chromosome 9.03	3.31	0.56	1.84

Researcher consider that morphology of four-row waxy maize have a series of primitive characteristics such as hermaphrodite flower, multiple ear, tiller and so on. That is same with the characters of maize ancestor described by Weatherwax. According to the historical documents, four-row waxy maize had been planted in China about 250 years. There was no significant difference in the time from the first mentions of maize were found in the archives. The original order between four-row waxy maize and waxy maize of Southwest China cannot be distinguish by this research, and needs further study.

Acknowledgements

This work was supported by the Sichuan Youth Science and Technology Foundation (08ZQ026-011), and the Program for Changjiang Scholar and Innovation Research Team in University of China (IRT0453).

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Section 4

Stress Tolerant Maize

Expression of Dehydrin Gene in Maize Inbred Lines fromVietnam

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Abstract. The protein dehydrin (DHN, LEA-D11) is often synthesized under drought conditions. It plays a role in important functions including tolerance of water loss and low air humidity and in the regulation of ABA concentration in plant cells. To determine the presence of the dhn1 gene in the maize germplasm of Vietnam, young leaves of 40 maize inbred lines, H1 to H40, were subjected to genomic studies, in which the DNA was extracted and the dhn1 gene amplified through PCR. The results showed that the dhn1 gene encoding for the protein dehydrin 1 was present in 30 of the lines. The band was clearer in H1 and H36, which were subsequently cloned. The size of the amplified gene sequence of H1 was found to be 599 bp and that of H36, 597 bp. The dhn1 gene of H1 and H36 had 99.99% and 99.66% homology with the sequence published in the Gene Bank (accession number AM495895). Moreover, the gene sequences of H1 and H36 had an intron region of 124 and 121 bp. To determine the presence of the DHN1 protein in the maize lines of Vietnam, embryos of H36 were subjected to protein extraction from which the DHN1 protein was purified. The extracted protein solution, after temperature shock and centrifuging, was first purified using an ion-exchange chromatography column (CM-52) and then a gel column, Sephadex G-75. SDS-polyacrylamide electrophoresis showed that the purified protein had a molecular weight equal to 22 kDa. This was reconfirmed through an immunoblotting procedure by detecting the protein with a DHN1 antibody.

Key words: Dehydrin1 protein, dehydrin1 gene, drought-tolerant, inbred line, maize

Introduction

One of the strategies for increasing maize production is improvement of the germplasm for resistance to biotic and abiotic stresses. The biotic factors to be considered include drought, cold and salty and acidic soil. The role of drought as a biotic stress is well-known: it severely affects physiological and biochemical systems in the maize plant and reduces yield.

Dehydrin (DHN, LEA-D11) is adissolvable protein which is often synthesized in plants under drought conditions. It has important functions such as tolerance of water loss and low air humidity and regulation of ABA concentration in plant cells (Ceccardi *et al.* 1994). Therefore, the presence of the *dhn1* gene and its expression in the form of the DHN1 protein was determined to evaluate variability for drought resistance among 40 inbred maize lines of Vietnam.

Materials and Methods

Materials

- Forty maize inbred lines from the National Maize Research Institute of Vietnam.
- A pCR 2.1 plasmid of *E. coli* DH-5a was used in the Recombination Protein Lab of the College of Sciences of the Vietnam National University ((VNU).

Methods

- The maize DNA genome was isolated following the method of Saghai-Maroof *et al.* (1984). The dehydrin1 gene was amplified through PCR using specific primer pairs ZM1 and ZM2 (Close *et al.* 1989). The PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide and detected with a UV transilluminator.
- The PCR products were cloned using the method of Sambrook *et al.* (2001)
- The DNA fragments were sequenced following the CEQ-8000 system of Beckman Coulter. The dehydrin gene sequences were then aligned with those published and maintained in the Gene Bank.
- The total protein was extracted from maize embryos using the method of Ceccardi *et al.* (1994). Protein concentration was assessed by the Lowry method. The protein was then electrophoresed on a polyacrylamide gel.

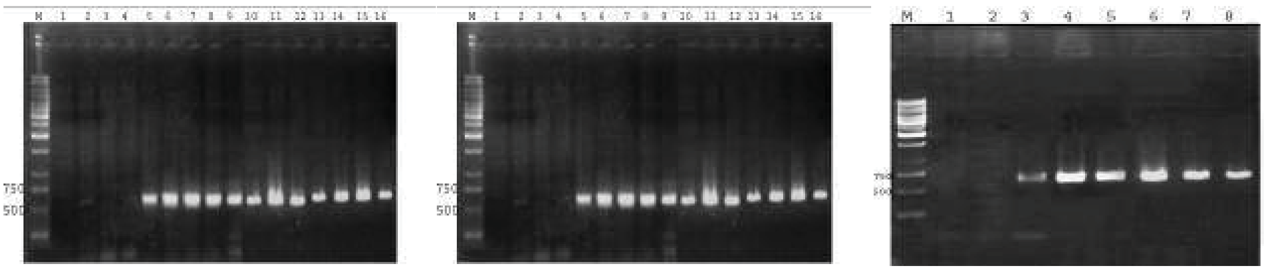

Figure 1. The PCR products of the *dehydrin1* gene on 1% agarose gel.
Lane M: 1 kb DNA leader Lanes 1-4 (A): H18, H19, H20, H21Lanes 5-16 (A): H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H12, H13Lanes 1-4 (B): H26, H27, H33, H11Lanes 5-16 (B): H14, H15, H16, H17, H22, H24, H25, H28, H29, H31, H32, H34Lanes 1-2 (C): H23, H30Lanes 3-8 (C): H35, H36, H37, H38, H39, H40

- The extracted protein was first purified through ion-exchange chromatography using a CM-52 column first and then a gel column– (Sephadex G-75).
- The Western blot method was used to detect and confirm DHN 1 with a DHN 1 antibody.

Results and Discussion

Cloning the dehydrin gene of 40 maize inbred lines

The total DNA genome of 40 maize inbred lines was used as the template to amplify *dhn1* in PCR. The results, presented in Figure 1, show the presence of *dhn1* in 30 lines (H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H12, H13, H14, H15, H16, H17, H22, H24, H25, H28, H29, H31, H32, H34, H35, H36, H37, H38, H39, H40) and its absence in the rest of the lines (H18, H19, H20, H21, H26, H27, H33, H11, H23, H30).

The results of electrophoresis showed the clarity level of the *dhn1* gene of the inbred lines. The *dhn1* gene bands of H1 and H36 were the clearest and, were therefore chosen for cloning. These products were inserted into a pCR 2.1 plasmid. The recombined plasmid was cut with a restricted enzyme *Eco*RI, to obtain two DNA bands, one of 600 bp (similar to the size of the PCR products) and another of 3900 bp (size of the pCR 2.1 plasmid) (Fig. 2).

Sequencing of gene encoded for dehydrin1

To ensure that the obtained gene was the *dhn1* gene, the PCR products of H1 and H36 were sequenced and compared with the nucleotide sequence of *dhn1* published in the Gene Bank (accession number AM495895) using the software Genetyx.

Figure 2. Result of electrophoresis of a recombined plasmid cut by the restricted enzyme *Eco*RI.
Lane M: 1 kb DNA leaderLane 1: PCR productLane 2: DNA plasmid isolated from white colony of H36 line treated with *Eco*RILane 3: DNA plasmid isolated from blue colonyLane 4: DNA plasmid isolated from white colony of H36 line

The size of the amplified gene sequences of H1 and H36 were 599 bp and 597 bp respectively (Fig. 3). The similarity of the H1 gene sequence to the published *dhn1* sequence was 99.99% and that of H36 99.66%.

The *dhn1* gene sequences of H1 and H36 were also compared with the mRNA (504 bp, coded X15290) and the *dhn1* gene sequences (628 bp, coded AM495895) in the Gene Bank using BioEdit. This indicated that the *dhn1* gene of H1 and H36 lines each had an intron fragment of 124 bp and 121 bp respectively(Fig. 4).

As per the *dhn1* gene sequence published in the Gene Bank (AM 495895), the intron region of the *dhn1* gene of maize is located from nucleotide 250 to 370 and lies between 2 extrons (exon 1: 1-129; exon 2: 371-628). In the H36 line,

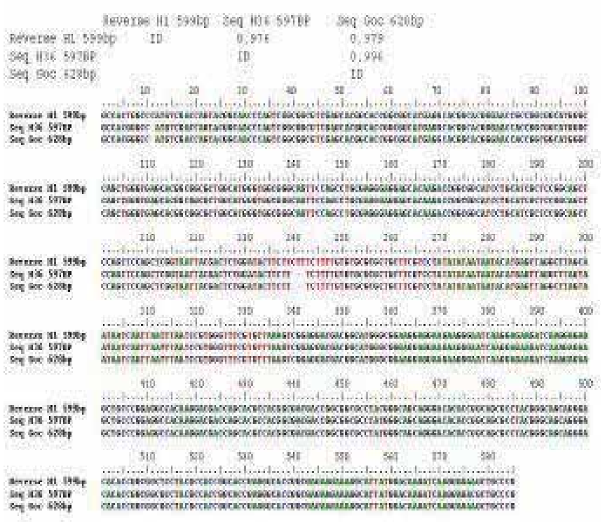

Figure 3. Comparison of the gene sequences of H1 and H36 lines with the *dhn1* gene sequence published in the Gene Bank (AM495895) using the software Genetyx.

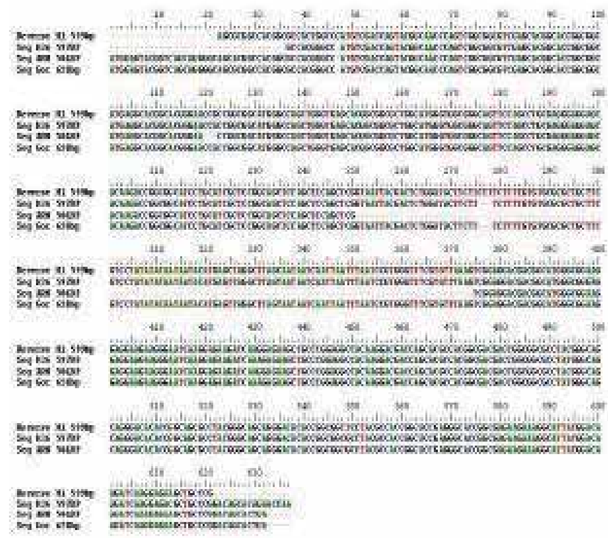

Figure 4. Comparison of the *dhn1* gene from the H1 and H36 lines with the mRNA (X15290) and DNA sequence of the *dhn1* gene published in the Gene Bank.

theexon 1 is located from nucleotide 1 to 220 and exon 2 from nucleotide 342 to 597; and the intron is flanked by the two exons just as in the *dhn1* gene in the Gene Bank; its size is 221 bp (from base pair 121 to 341). The intron region in H1 has the same structure as in H36 and the Gene Bank, but its size is 124 bp and longer than the intron fragment in H36 three nucleotides (CTT). These nucleotides are inserted at nucleotide 253-256. The first extron of the H1 line is located from nucleotide 1 to 228 and the second from 453 to 599. The intron region in plants often has a high percentage of the AT component (60%) and a GT-AG cutting sequence at the two ends of the intron region (Locrkovic *et al.* 2000). Accordingly, the intron regions of H1 (124 bp) and H36 (121 bp) were found to have a high percentage of AT, 64.48% and 64.46%, respectively. They also have a GT-AG cutting sequence at their ends. These results allow us to conclude that these intron regions are indeed introns of the *dhn1* gene in maize.

Extraction of total protein from maize embryo

Protein was extracted from H36 embryos using the extraction method of Ceccadi *et al.* (1994). The extracted solution had a protein content of 987.56 mg. This was treated at 90^0C for 10 minutes and centrifuged at 3000 rpm for 1 hour to remove the precipitated proteins. The resulting solution had a protein content of 462.76 mg, making up 46.85% of the crude extraction solution.

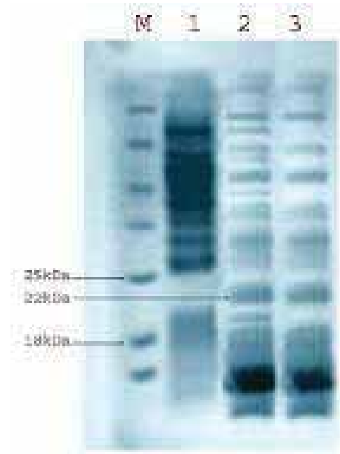

Figure 5. Electrophoresis of the protein solution on 12% SDS-PAGE.
Lane M: Protein molecular weight leader; Lane 1: Protein of the H33 line (nontolerant); Lane 2: Protein of the H36 line (drought-resistant); Lane 3: Protein of the H36 line (drought-resistant line) after heat treatment

As per published research studies (Close 1997; Rorat 2006), the molecular weight of the protein DHN1 is 22 kDa. Consistent with this finding, the protein solution from the H36 line (a drought-resistant line) contained a 22 kDa band (DHN1 protein band) which, in contrast, was absent in the H33 line (a nontolerant line) (Fig. 5).

Primary purification of DHN1 protein with ion-exchange chromatography using a CM-52 column

To purify DHN1, the extracted solution was passed over a CM-52 (size 3 x 10 cm) column preequilibrated with 25 mM MES buffer (pH = 6) and 20 mM NaCl under which conditions all dehydrin is bound. Protein separation was achieved by increasing the concentration of NaCl from 20 mM to 600 mM. Fractions of 2 ml were collected on ice. The protein collected from different fractions was assessed for DHN1 concentration at 280 nm (A_{280}).

The results showed single-protein peaks at fractions 22-36. This meant that the crude protein solution was not much different from the heat-treated solution in terms of the electric charge. To estimate the efficiency of chromatography using a CM-52 column, the protein from fractions 22-36 (with an equivalent NaCl concentration of 200-300 mM) was collected, condensed and analyzed on 12% SDS-PAGE. The protein content thus determined was 36.23 mg, making up 3.68% of the crude protein solution.

Estimation of the purity of DHN1 after filtration through a CM-52 column using 12% SDS-PAGE (Fig. 6) showed that the achieved protein was pure DHN1 (a single band compared with the multiple bands seen after the heat treatment). It size was 22 kDa.

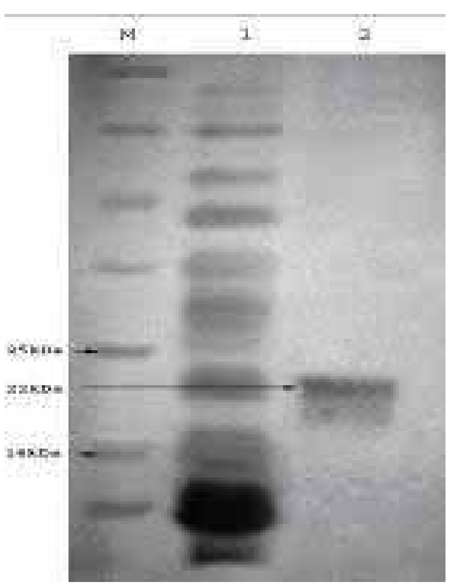

Figure 6. Estimation of the purity of the protein DHN1 after being passed over a CM-52 column on 12% SDS-PAGE.
Lane M: Protein molecular weight leaderLane 1: Protein solution after heat treatment (at 90^{0}C)Lane 2: DHN1 after being passed over a CM-52 column

Purification of DHN1 protein using a Sephadex G-75 column

The DHN1 protein obtained in the previous step was further purified over a Sephadex G-75 column continuously. The protein content was determined at 280 nm (A_{280}). There were two main peaks but only the peak relating to fractions 22-39 was clearer. These fractions were condensed by a centricon. The amount of DHN1 thus obtained was 6.03 mg (0.61% of the crude extracted solution).

Table 1 shows the amount of protein obtained after each step of the purification procedure. More than 50% of the soluble proteins were removed by heating. Following the final chromatographic fractionation, approximately 0.61% of the total protein was recovered as pure dehydrin.

Determination of the DHN1 protein by a DHN1 antibody using the immunoblotting technique

The Western blot technique was used to verify that the purified protein was indeed the DHN1 protein encoded by the *dhn1* gene. The protein collected in the above step was condensed and examined with a DHN1 antibody in an immunological reaction. The resultsshowed (Fig. 7) that the DHN1 band was present in lanes 3 and 4 of the protein solution extracted from the drought-resistant H36 line while the nontolerant H33 line (did not contain this band (lanes 1 and 2). The DHN1 protein was also seen singly and clearly in the purified protein solution (lanes 5 and 6). The DHN1 antibody detected the 22 kDa band exactly in the cross membrane. The results of this study are consistent with the studies of Close (1989) and Ceccadi (1994). These results also demonstrate that the DHN1 protein was obtained through two chromatographic steps and the chromatographic methods were applied efficiently. However, the DHN1 protein should be purified further to achieve high clarity. The properties of this protein and its physiological role related to drought resistance in maize need to be studied.

Table 1. Summary of the steps for purification of protein DHN1.

Purification step	Total protein (mg)	Protein yield (%)
Crude extract	987.56	100.00
Heat-treated extract ($t^0 = 90^0C$)	467.76	46.85
Ion-exchange peak (using CM-52 column)	36.23	3.67
Ion-exchange peak (using Sephadex G-75 column)	6.03	0.61

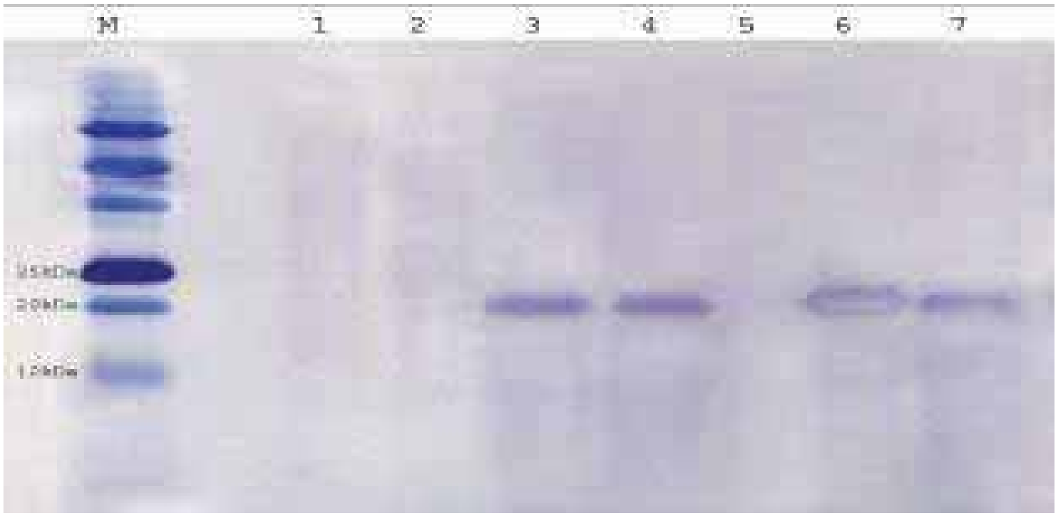

Figure 7. DHN1 protein detected by a DHN1 antibody.
Lane M: Prestained markersLane 1, 2: Total protein of H33 lineLane 3: Total crude protein of H36 lineLane 4: Total protein of H36 after heat-treatment at 90^0CLane 5: Protein not bound in the CM-52 gel columnLane 6: Purified protein using a CM-52 columnLane 7: Purified protein using a Sephadex G-75 column

Conclusions

This study determined the presence of the *dhn1* gene which encodes the DHN1 protein in 30 maize inbred lines of Vietnam. The *dhn1* gene found in the lines H1 and H36 was cloned and sequenced. The size of the amplified gene sequence of the H1 line was 599 bp and that of H36 597 bp. The homological percentage of the gene found in H1 and H36 was 99.99% and 99.66% respectively in relation with the *dhn1* gene sequence published in the Gene Bank (AM495895). The gene sequences of H1 and H36 had intron regions of 124 bp and 121 bp respectively.

The DHN1 protein was purified from the extracted protein solution of H36 embryos using CM-52 and Sephadex G-75 columns. The obtained protein was found to have a molecular weight equal to 22 kDa after analysis by SDS-PAGE. It was detected by a DHN1 antibody in an immunoblotting procedure.

Acknowledgment

We thank Prof. Timothy J. Close of the University of California (USA), for his support to this study.

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Development of A Rapid Screening Technique for High Nitrogen-Use Efficiency in Maize in Normal and Drought Environments

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Abstract. The damage to corn production due to drought caused by the El Niño episodes that hit the Philippines from 1990 to 1992 was estimated to be as much as PhP 3.2 billion. Such large losses can be avoided by developing varieties with the ability to produce relatively high yields under water-limited conditions. To produce good yields, varieties must also be able to absorb and utilize nutrients efficiently, especially nitrogen. Experiments were conducted using different levels of nitrogen fertilizer (0, 60 and 120 kg N ha^{-1}) and different water stress conditions (irrigated and nonirrigated). Corn seedlings were grown for 3-4 weeks in the greenhouse in trays using a sand culture. Field experiments were then conducted in Laguna and Ilocos Norte using selected genotypes to measure their grain yields and validate the results of the greenhouse experiments. Genotypes with seedlings that have greater shoot length, shoot weight, root length, root weight, total N uptake and total dry matter yield after drought treatment were found to give better grain yields and to have higher nitrogen-use efficiency potential when grown under intermediate water stress in the field. These genotypes were identified asCML 8, P6-1-3, (P6-1-3 x CML 337), (CML 8 x CBR/PDC 2-5-2-4-5) for white corn and SMCE, (95-6 x Pi 23), (CLOO 331 x Pi 23) for yellow corn. A protocol for rapid greenhouse screening at the seedling stage is presented here. The performance of these drought-tolerant F_1 hybrids will be validated on-farm before recommendation for variety release.

Key words: Corn genotypes, water stress, nitrogen-use efficiency, drought, diallel

Introduction

The two major thrusts of the Philippines government in agriculture are food security and global competitiveness. As an initial step toward this goal, the Department of Agriculture has identified key production areas for selected crops, and launched a program to sustain self-sufficiency in rice and corn with a target national production of at least 12 million t and 7 million t, respectively. However, in regions where farmers largely depend on rainfall as a source of water or in areas without supplemental irrigation, yield reduction may range from 10% to 75% (Logroño and Lothrop 1996). In fact, under severe water-deficit stress, corn yield may even be zero corn. The El Niño episodes that hit the country particularly in 1990 and 1991 caused crop damage worth P3.2 billion and P2.1 billion, respectively (DA Report 1994). Total corn losses due to drought in 1992 amounted to P2.1 billion, affecting about 281 349 ha.

Normal rainfall in the Philippines ranges from 1000 mm to 5000 mm. Drought occurs in an area when rainfall is erratic or falls below 1000 mm or when there is almost no rain during the normal growing season. When water becomes available, water-deficit stress is relieved but other factors limiting plant growth assume a greater role, such as soil nutrient availability/utilization. Nitrogen is the fourth most abundant nutrient in plants, and an essential component of proteins, nucleic acids, hormones chlorophyll and primary and secondary metabolites. In general, plants obtain the bulk of their nitrogen from the soil in the form of either nitrate or ammonium. However, supply of nitrogen in the soil is limited (Power 1990) and plants must compete with soil microorganisms for it. As a result, nitrogen is often a limiting nutrient for plants in both natural and agricultural ecosystems.

The occurrence of water and nitrogen stress may increase, due partly to global changes, partly to the displacement of corn to more difficult production environments by higher-value crops and partly to the decline in soil organic matter which may result in reduction of soil fertility and water-holding capacity. At the micro level, fertility and water availability vary greatly in farmers' fields. This means that a single variety must be able to withstand a wide range of drought stress and nitrogen availability – conditions that are more pronounced in the tropics than in the higher input fields of the temperate areas. In order to maximize grain yield under conditions of nonlimiting and limiting water supply, it is equally important to select corn genotypes with high nitrogen-use efficiency under such environments.

The general objective of this study was to increase (by 10%) corn production in the Philippines through selection of genotypes with high nitrogen-use efficiency under normal as well as water-limiting conditions with emphasis on the development of a rapid screening technique for high nitrogen-use efficiency for corn in normal and drought environments.

Materials and Methods

Greenhouse screening

Experiments were conducted in the greenhouse at the Institute of Plant Breeding (IPB) using the sand culture technique to evaluate the nitrogen-use efficiency (NUE) and productive efficiency of applied nitrogen (PENA) of white and yellow maize inbreds and diallel crosses. The water regimes were designated as irrigated and limiting water. The nitrogen levels were (a) control (no added N); (b) 60 kg N ha^{-1}; and (c) 120 kg N ha^{-1}. Phosphorus and potassium fertilizer in the form of solophos (0-18-0) and muriate of potash (0-0-60) were added at the rate of 120 kg ha^{-1}. The entries were grown at field capacity under both water regimes. To effect the drought treatment, water was withheld 7 days after seedling emergence.

A split-split-plot design in an RCB with two replications was used with the water regime as the main plot, N level as the subplot and the variety as the sub-subplot. Each main plot used styrofor trays containing 17 kg of sand previously analyzed for nutrient composition and water-holding capacity. Seeds were drilled into 35 cm rows to serve as sub-subplots. Twelve entries were initially evaluated per set-up but as the project progressed more were screened. Nutrients were added at sowing time. Equal volumes of water were used to irrigate the different treatments at planting to allow seed germination and seedling establishment. One week after germination, irrigation was withheld in the drought and recovery treatments. Visual ratings were made and soil moisture status was monitored. Plant sampling for N analysis commenced when visible differences were observed among the entries (leaf rolling). The entries were evaluated for the rate of recovery after resumption of watering. Plant samples were collected when differences due to rewatering became visible among the entries. Dry matter accumulation (shoot and root dry weights), shoot and root length and nitrogen contents of the plants were determined. N-efficient genotypes are entries with the ability to produce a high total dry matter yield, high plant N-accumulation and rapid root and shoot growth.

Field trials

To verify and validate the results obtained from the sand culture, field experiments were conducted at Laguna and Ilocos Norte using the following levels of N: no addition of N (control), 70 kg ha^{-1} and 140 kg ha^{-1} under water-limiting and normal water regimes. Phosphorus and potassium were applied at the rate of 120 kg ha^{-1} in all the plots. The recommended cultural management for corn was adopted except N-fertilization. The experiment was laid out using a randomized complete block design (RCBD) in a split-split plot with the water regime as the main plot, N level as the subplot and the variety as the sub-subplot. Five to eight potentially drought-tolerant and susceptible white and yellow corn genotypes were tested separately. To effect the drought treatment, water was withheld one month after planting, and resumption of watering was done to field capacity at the grain-filling stage. The sample plots or harvest area consisted of the three inner rows in the five-row plots which served as the basic experimental unit. Soil samples were collected for moisture and N analyses.

Samples were collected at physiological maturity for N analysis, dry matter accumulation, grain yield and its components. N-use efficient (NUE) genotypes and those with high PENA were identified. Comparison was done between the morphological and physiological traits of plants grown in the greenhouse and their yield performance in the field to determine whether genotype response under field conditions would be similar to genotype response in sand culture. Moreover, the responses of the genotypes at seedling stage were compared with their responses at maturity. As the ability of a plant to recover rapidly from stress is an important drought-tolerance mechanism, the entries were evaluated visually for the rate of recovery after resumption of watering.

Results and Discussion

Initial soil characteristicsThe sand used in the greenhouse experiment had a strongly acidic reaction (pH 6.90), very low nitrogen content (0.009 %) and high phosphorus (25 ppm) and potassium (0.50 cmol(+) kg^{-1}) levels. The Cervantes clay loam soil used in the field experiments at Piddig in Ilocos Norte had a moderately acidic reaction (pH 5.95), medium nitrogen content (0.155 %), high available P (16.75 ppm) and K (0.65 cmol(+) kg^{-1}) content. The Alipit clay soil used in the field experiments at Bay, Laguna had a moderately acidic reaction (pH 5.90), low nitrogen content (0.116%), high available P (18.47 ppm) and very high K (1.70 cmol(+) kg^{-1}) content. To mask the possible effect that may be caused by the problems of P and K availability to plants in the experiment, 120 kg each of these two nutrients were added to the soil at planting.

Greenhouse results

The greenhouse experiments involving white and yellow corn inbreds and diallel crosses were conducted to determine the plant parameters that could be used in the development of a rapid screening technique that will limit the number of entries to be tested in the field for grain yield determination and verification trials under waterlimiting conditions.

Based on the data obtained from the experiments, the parameters that showed significant differences across treatments (water regime, nitrogen levels and varieties) were shoot length, shoot weight, root length, root weight, total N uptake and total dry matter weight. The soil moisture content after drought imposition based on oven dry weight was 3.74 % while the moisture content of irrigated sand was 9.34%. The moisture content of sand at field capacity is 6-12% while at permanent wilting point it is 2-6 % (Hansen *et al.* 1980).

Field verification trial under normal and water-limiting conditions

Promising drought-tolerant white and yellow corn genotypes which showed good performance in the greenhouse and those susceptible to water stress were tested under field conditions during the dry cropping season at Ilocos Norte and Laguna to see whether they would produce similar results. One month after drought imposition, soil sampling was done to determine the amount of soil moisture in the field. The moisture content of plots under drought treatment decreased from 29.09% at 5 weeks after planting (WAP) to 19.31% at 9 WAP which coincided with the grain-filling stage. The moisture content for a clay loam soil at field capacity is 23-31% while for permanent wilting point it is 11-15% (Hansen *et al.* 1980). At this moisture level, the plants were under moisture stress. Corn plants undergo water stress prior to the flowering stage which is the most crucial time for the plant to produce grains. After this stage, the corn plants were irrigated to induce recovery.

Among the white corn inbreds and parentals tested, P6-1-3 and CML 8 produced the highest absolute yield at 140 kg N ha^{-1} under drought stress (Table 1). Their grain yields ranged from 2.79 t ha^{-1} to 3.53 t ha^{-1} with a 26-33% corresponding yield reduction due to water stress. CML 377 and Lagkitan gave the lowest yields of 1.44 t ha^{-1} and 1.58 t ha^{-1}, respectively, equivalent to a yield reduction of 58-63%. On the other hand, higher grain yield levels were shown by white diallel crosses developed for stress when compared to their respective parentals. At 140 kg ha^{-1} N fertilization, the high-yielding diallel crosses were (CML 8) x (CBR x PDC2-5-2-4-5) and (P6-1-3 x CML 377) with grain yields of 3.17 t ha^{-1} and 3.04 t ha^{-1}, respectively. In general, yield increased with increasing levels of nitrogen in most entries. The same results were observed in the experiments conducted in Laguna.

For the yellow corn entries, the highest yielder among the inbreds was SMCE 9-18 with a yield of 2.57 t ha^{-1} at high N fertilization under water-limiting condition, shoping a yield reduction of 30.91% in comparison with control. The entry with the lowest yield was CL00331 with a yield reduction of 49.41%. Three diallel crosses of yellow corn showed higher grain yields than their parent materials. CLOO331 x Pi 23 recorded the highest yield of 3.72 t ha^{-1} at a high N rate and under drought conditions followed by 95-6 x Pi 23 with 3.17 t ha^{-1}. The reduction in yield ranged from 42% to 50 %. It was also noted that there is higher yield reduction in yellow corn compared to white corn due to the fact that the yellow corn materials showed higher yield potential under irrigated conditions.

Table 1. Grain yield (t ha^{-1}) of selected white and yellow corn entries under irrigated and drought conditions at 140 kg N ha^{-1}.

Yellow corn	Nitrogen rate (140 kg N ha^{-1})	
	Control	Drought
SMCE	3.72	2.57
TUPI S3-5-18 (TUPI 1YL7)	3.79	2.25
IPB Var 1	5.42	2.28
IPB Var 7	5.03	2.37
(Pi17) x (Pi23)	5.99	2.60
(95-6) x (Pi23)	6.29	3.17
(CLOO331) x (Pi23)	6.41	3.72
CLOO331	1.70	0.86
White corn	**Nitrogen rate (140 kg N ha^{-1})**	
	Control	Drought
CML 8	4.19	2.79
P6-1-3	4.77	3.53
CML 151-32-1-4-2	3.43	2.69
P6-1-3 x CML337	5.50	3.04
(PK2 x Var 1-12-2-1-1) x (CML337)	5.53	2.93
CML 8 x (CBR x PDC2-5-2-4-5)	5.73	3.17
CML 337	3.91	1.58
Lagkitan	3.82	1.58

Table 2. Productive efficiency of nitrogen applied (PENA) of selected corn entries under irrigated and drought conditions at different N levels.

White Corn	Productive Efficiency of Nitrogen Applied (PENA) (kg grain yield per kg N applied)			
	70 kg N ha^{-1}		140 kg N ha^{-1}	
	Control	Drought	Control	Drought
P26-4-1	7.57	10.86	3.57	6.71
P6-1-3	10.14	3.43	8.64	13.14
CML 151-32-1-4-2	5.57	3.14	2.07	9.14
(P6-1-3) x (CML337)	19.00	24.86	8.29	8.79
(PK2 x Var1-12-2-2-1-1) x (CML 337)	12.71	8.14	8.21	8.43
(CML8) x (CBR x PDC2-5-2-4-5)	13.43	16.00	11.79	10.66
Lagkitan	9.71	4.00	5.07	1.36

Yellow Corn	Productive Efficiency of Nitrogen Applied (PENA) (kg grain yield per kg N applied)			
	70 kg N ha^{-1}		140 kg N ha^{-1}	
	Control	Drought	Control	Drought
95-6	11.71	5.43	3.71	2.07
TUPI S35-1B (TUPI 1YL7)	7.71	7.43	2.71	6.50
(95-6) x (Pi23)	19.86	5.57	13.79	8.93
(CLOO331) x (P123)	20.43	5.43	13.86	12.36
CLOO331	5.71	2.29	1.71	0.71

In testing for the correlation of grain yield obtained from the field with the parameters used in the greenhouse for white corn, correlation coefficients greater than 80% were obtained for parameters like shoot length, shoot weight, root length, root weight, total dry matter weight, shoot N and total N uptake under the water-stressed condition. The test of correlation for yellow corn, however, showed correlation coefficients less than 50% for root weight and root length while the rest of the parameters had correlation coefficients greater than 80%.

Table 2 depicts the NUE of white corn entries under irrigated and drought conditions. P6-1-3, which had the highest yield under water-limiting conditions, gave a PENA conversion of 13.14 kg grain yield per kg of N applied under the fertilization rate of 140 kg N ha^{-1} while the diallel cross (CML 8) x (CBR x PDC2-5-2-4-5) was also noted to have a PENA equivalent to 10.66 kg grain per kg of added N fertilizer. At the lower rate of N application (70 kg ha^{-1}), the entries with high PENA and high NUE under drought conditions were: (P6-1-3 x CML 337), (CML 8) x (CBR x PDC2-5-2-4-5) and (CML8 x P6-1-3). This revealed that these entries can thrive and produce good yields under water-limiting conditions and low N fertilization.

Among the yellow corn entries, Tupi 1YL7, which is one of the highest yielders under drought conditions, was also observed to have high PENA under low and high N fertilization. Compared with the yellow diallel crosses, CLOO331 x Pi 23 performed well under drought conditions in converting applied N into grain yield at 12.36 kg grain per kg N applied when the fertilization rate was 140 kg N ha^{-1}.

In field tests of the nitrogen-use efficiency of white and yellow corn, high PENA values were shown at fertilization rates of 70 and 140 kg N ha^{-1}. This suggests that some corn entries can make use of the available native or indigenous N in the soil and applied fertilizer N better than others, and this could result in less need for N inputs, lower production costs and higher returns/benefits to farmers.

These results indicate the validity of the protocol or methodology that was developed for greenhouse studies. The entries that were identified in the greenhouse as tolerant or susceptible to water stress and with high N-use efficiency showed similar responses or results in the field. Thus, rapid screening for the selection of drought-tolerant corn genotypes can be conducted during the seedling stage under greenhouse conditions.

Conclusions and Recommendations

The different objectives of this work were answered by conducting greenhouse and field experiments involving yellow and white corn genotypes and several developed diallel crosses. The greenhouse experiments were able to determine the parameters that could be used for the development of a rapid screening technique for the selection of genotypes with strong potential for high N-use efficiency under water-limiting conditions. Plant parameters such as shoot length, shoot weight, root length, root weight, total N uptake and total dry matter weight were found to significantly affect the growth of corn genotypes under water stress conditions. This was verified by the field experiment which showed that the identified potentially tolerant and susceptible entries and those with high N-use efficiency under water-limiting conditions in the greenhouse also correspond to the best and least performing corn genotypes, respectively, in the field experiment. Based on greenhouse and field experiments, the corn genotypes which possessed potential drought tolerance and high N-use efficiency were P6-1-3 and (CML 8) x (CBR x PDC2-5-2-4-5) for white corn and (CLOO331 x Pi 23) and (95-6 x Pi23) for yellow corn. The generation of diallel crosses that was initiated and the results obtained showed good promise in the development of these materials in terms of higher grain yield and high N-use efficiency under water-limiting conditions.

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Path Analysis of Yield and Yield Components of Synthetic Maros FirstCycle Population (MRSS-1 C0) under Low N

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Abstract. Around 50% of the maize crop grown in tropical regions including South East Asia suffers significant yield penalties due to drought stress and low nitrogen. Continuous monoculture of maize exhausts soil nitrogen rapidly. Furthermore, economic constraints often restrict farmers from applying the recommended fertilizers. Under such conditions, the best choice for farmers is to grow varieties with tolerance for low nitrogen. This study was aimed at measuring the correlation between the yield and yield components of the maize Synthetic Maros First Cycle (MRSS-1 C0) population under optimum and low N conditions. The experiment was conducted in the Muneng experimental farm, Probolinggo, East Java, Indonesia. The experimental material consisted of 200 S_1 families in four sets planted under optimum (recommended fertilizer: 300 kg urea ha^{-1}) and low N (50 kg urea ha^{-1}) conditions. Bisma, Bisi-2, SATP-1 (S2) C6 and S_0 families were used as controls in these sets. All experimental sets were replicated two times. The data were subjected to correlation and path analyses, which revealed that plant height and wet peeling weight were effective parameters correlating with yield under low N conditions.

Key words: Path analysis, Maros synthetic population, low N.

Introduction

Around 50% of the maize crop grown in tropical regions including South East Asia is reported to be affected by drought stress and low N, causing significant yield reductions (Eck 1984). Maize planted continuously under monoculture rapidly depletes soil nitrogen. Dry soil conditions can restrict the transformation of soil nutrients into available ions and decrease the availability of mobile ions to roots. On the other hand, economic constraints often restrict farmers from applying the recommended fertilizer dosages. Under such conditions, the best choice for farmers is to grow varieties that are tolerant of low soil nitrogen.

Budiarti *et al.* (2004) reported that yield is a quantitative character that is significantly affected by environmental factors; so other characters having functional relations (for instance, yield components) with yield need to be predicted for ascertaining their contribution to overall yield variance.

Path coefficient analysis is a statistical tool that is used to indicate the quality relations between predictor variables and responsive variables in a path diagram based on experimental results. The advantage of path analysis is that it can divide coefficient correlations into their components. The components of path coefficients measure the direct effect of predictor variables and other components, and the indirect effect of predictor variables through other predictor variables (Samonte *et al*. 1998; Board *et al*. 1999).

Path coefficient analysis has been widely used in plant breeding to determine the relationship between yield and yield-attributing traits. This helps the breeder in identifying characteristics that are useful in making an effective selection (Samonte *et al*. 1998; Muhammad *et al.* 2003).

This study was aimed at obtaining information about the relationship between the yield and yield components of the Maros Synthetic First-Cycle Population Family (MRSS-1 C0) under optimal and deficient N conditions.

Materials and Methods

The genetic materials used in this study were S_1 family maize seed of Maros synthetic (MRSS-1 C0). The comparison varieties were Bisma, Bisi-2, SATP-1 (S2) C6 and family S0. The fertilizer used was urea. Carbofuran 3G was used to prevent infestation of corn pests.

The field experiment was carried out at the experimental farm in Muneng, Probolinggo, East Java, Indonesia. It was conducted under two environments: low N (N_0) with addition of 50 kg urea ha^{-1}, and high N with addition of 300 kg urea ha^{-1}. In each environment, the field was divided into four sets and four controls. Each set was planted with 50 S_1 families. Every set was replicated twice.

Computation of path analysis was done by the matrix method, and the following characters were considered: X1 = tasseling; X2 = silking; X3 = harvested plant; X4 = sterilized plant; X5 = harvested ear; X6 = wet peeling; X7 = water content; X8 = dry shelling weight; X9 = weight of 1000 grain; X10 = plant height; X11 = ear height; X12 = ear length; X13 = ear diameter; and X14 = number of ear rows.

According to Singh and Chaudary (1979) the relation among parameters is based on the relation between the coefficient of correlation (r) and the path coefficient (p), as follows:

$$Rx1y = px1y + rx1x2px3y + rx1x4px4y + ... + rx1x13px13y$$

$$Rx2y = rx2x1px1y + px2y + rx2x3px3y + \ldots\ldots + rx2x13px13y$$

$$Rx3y = rx3x1px1y + rx3x2px3y + px3y + \ldots\ldots + rx3x13px13y$$

$$Rx13y = rx13x1px1y + rx13x2px13y + rx13x3px13y + \ldots + px13y$$

By the matrix model A = B.C and calculating the matrix inverse B, the path coefficient can be computed by $C = B^{-1}.A$.

Results and Discussion

The selection of 14 characters as independent variables to assess their effect on maize yield components under low N conditions was based on the understanding that the vegetative parts of a plant, particularly leaves, play an important role in different metabolic activities. Besides, it is known that both vegetative and generative characters are directly linked to yield components. (Miftahorachman *et al*. 1992).

Of these 14 characters, all but two did not have a significant direct or indirect effect on the yield components of MRSS-1 C0 under low N conditions. The two characters that were found to have a significant direct effect were wet peeling weight (X6) and plant height (X10) with a value of 0.978 and 0.541, respectively (Table 1).

The correlation coefficient of wet peeling weight (r 6.14 = 0.984) and its direct effect (p 6.14 = 0.987) made a significant contribution to grain yield. The direct effect calculated from the matrix of wet peeling weight and harvested plant (p 6.3 = 0.30), harvested ear (p 6.5 = 0.61), weight of 1000 grain (p 6.9 = 0.22), ear row number (p 6.14 = 0.53) was extremely significant to grain yield. The direct effect of the above two characters being high, they were used as selection parameters.

Although the direct effect of harvested plant, harvested ear, weight of 1000 grain, and number of ear rows was low, the total of their correlation, ie 0.30, 0.61, 0.22, and 0.53, respectively, was relatively high and significant. This was attributed to simple correlation of an irreversible relationship being measured without assessing its nature, while the path analysis was assessed both as a causing factor and its relative importance. (Dewey *et al*. 1595 *in* Pamin *et al*. 1993).

Table 1. Path value of yield components and agronomic character correlation with the yield of Synthetic Maros First Cycle population (MRSS-1 C0) under low-N conditions.

	Character (rij)													
PATH	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
X1		0.00338	0.00025	0.00077	0.00094	-0.01688	0.00001	0.00006	-0.00033	0.00032	0.00082	-0.0003	0.00006	0.00316
X2	-0.00367		0.00051	0.00068	0.00047	-0.03496	0.00001	0.00002	-0.00032	0.0002	0.00028	-0.00004	0.00009	-0.0032
X3	0.00014	-0.00026		-0.00068	0.00916	0.30232	0.00001	0.00003	0.0003	0.00062	-0.0004	-0.00004	-0.00001	0.00789
X4	-0.00036	0.00029	0.00057		-0.01319	-0.4171	0.00000	0.00003	-0.00036	-0.00079	0.0008	-0.00013	0.00006	0.0062
X5	-0.0002	0.00009	-0.00358	-0.00613		0.60805	0.00000	-0.00001	0.00054	0.00125	-0.00008	-0.00026	0.00001	-0.00093
X6	0.00008	-0.00015	-0.00015	-0.00411	0.01289		0.00000	0.00002	0.00212	0.00212	-0.00019	-0.00059	0.0001	-0.00261
X7	-0.00023	0.0003	0.0003	-0.00001	0.00008	-0.01786		0	-0.00004	-0.00046	0.00053	-0.00002	-0.00002	-0.00098
X8	0.00037	-0.00011	-0.00011	-0.00037	0.00027	-0.03119	0.00000		-0.0002	-0.00012	0.00055	-0.0001	-0.00001	-0.00094
X9	0.00027	-0.00024	-0.00024	-0.00064	0.00205	0.21592	0.00000	0.00002		0.00061	-0.00074	-0.00006	-0.00006	0.00064
X10	-0.00037	0.00021	0.00021	-0.00196	0.00665	0.53143	-0.00002	0.00002	0.00086		-0.00056	-0.0009	0.00014	0.00272
X11	0.00018	-0.00006	-0.00006	-0.00037	0.00008	0.00884	0.00000	0.00002	0.0002	0.00011		0.00535	-0.00116	0.03869
X12	-0.00023	0.00003	0.00003	0.00022	0.00092	0.09998	0.00000	-0.00001	0.00005	0.00061	0.01932		0.00112	-0.03698
X13	-0.00019	0.00024	0.00024	0.00038	0.00018	0.06504	0.00000	0	-0.00023	0.00036	0.01598	-0.0043		-0.01362
X14	-0.00009	-0.00008	-0.00008	0.00036	-0.00012	-0.01536	0.00000	0	0.00002	0.00006	-0.00481	0.00127	-0.00012	
	Direrct Effect (Pih)													
	-0.00448	0.00412	-0.00811	0.00963	0.02073	0.97797	0.00018	-0.00067	0.00549	0.54084	-0.02069	-0.00573	0.0015	0.16644
Total	-0.01223	-0.03581	0.31097	-0.41436	0.6195	0.98424	-0.0188	-0.03211	0.22282	0.54084	0.03101	0..07926	0.06539	0.1472

Conclusions

Entries being selected for tolerance to N deficiency have to be assessed under N deficiency conditions. Wet peeling weight and plant height can be used as selection traits for high-yielding capacity under N-deficiency conditions.

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Screening of Salt-Tolerant Grain Maize (*Zea mays* L.) Genotypes under Saline Conditions

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Abstract. This study was conducted to determine the response of maize genotypes under saline conditions at the Center of Agricultural Research and Natural Resources of Khorasan Razavi Province, Mashhad, Iran in 2007. Forty-two genotypes (20 inbred lines and 22 hybrids) were first tested in the greenhouse in four complete randomized designs under four irrigation treatments with different salinity levels [i electrical conductivity = 4 ds m^{-1}, 8 ds m^{-1}, 12 ds $m^{-1}$and 1 ds m^{-1} (control)]. The germination percentage, dried weight of plants and the vigor index were recorded separately. In the field experiment, 46 genotypes (23 inbred lines and 23 hybrids) were planted in two randomized complete block designs with 3 replications in two fields (the Mashhad and Neyshabour agricultural research stations with saturated-paste electrical conductivity of 5.9 ds m^{-1} and 8 ds m^{-1}, respectively). The evaluated traits were anthesis and silking time, anthesis–silking interval, plant height, ear height, physiological maturity, grain yield, ear length, kernel number, number of rows per ear, kernel number per row, kernel depth and 1000-kernel weight. The results showed no significant differences among the salt stress treatments for germination percentage, although the vigor index of plants was affected by higher levels of salt (8 ds m^{-1} and 12 ds m^{-1}). The results of the field experiment in Mashhad showed that saline conditions had nonfavorable effects on the maize genotypes, especially the inbred lines. The single-cross hybrids KE72012/1-12 × K2331, KSC500 and ZP434 recorded yields of 7.77 t ha^{-1}, 7.66 t ha^{-1} and 7.22 t ha^{-1} respectively. The inbred line KE72012/1-12 had the highest grain yield of 1.722 t ha^{-1}. The hybrid ETH-M24 and the inbred line OH43/1-42 recorded the lowest grain yields. In Neyshabour, many of the inbred lines and hybrids were removed due to high salinity; therefore, statistical analysis was not possible.

Key words: Salt stress, electrical conductivity, inbred line, hybrid, grain yield, maize *(Zea mays L.)*

Introduction

One of the most important limitations to maize production in arid and semiarid regions is soil and water salinity. Salinity affects about one billion ha of land in the world and 320 million ha in Asia (Khan and Weber 2006). In Iran, more than 18 million ha are affected by salinity (Koochaki 2007). Mass *et al.* (1983) reported that the relative salt tolerance of maize may increase at different stages of growth without a significant decrease in yield. Kostandi and Soliman (1992) found that despite grain yield decrease with salinity increase, using salty water especially during the late stages of growth is advisible. Jamaly *et al.* (2003) reported that maize is tolerant of salinity at the germination stage. Hoffman *et al.* (1993) showed that the threshold of tolerance to salinity in maize plants is 3.7 ds m^{-1}. Levay and Bauder (2002) evaluated 16 species of forage plants for salt tolerance in 2002 and found that the most tolerant species were tall fescue, slender wheatgrass, altai, wheatgrass, sorghum and maize, in that order. Cuevas (2003) evaluated the response of maize inbred lines to salinity and found that the inbred lines pi-21 and pi-31 were more tolerant due to the increased activation of the nitrate reductase enzyme**.** Zarandi *et al.* (2007) evaluated 27 hybrids of grain corn at electrical conductivity (EC) of 8.5 ds m^{-1}. The salt-tolerant hybrids were BC504, OSSK373, G-3337, G-54190, KSC301, KSC250 and NS540. El-lakany and Russel (1971) reported positive and significant correlation between inbred line traits and their hybrids' performances. Gama and Hallauer (1977) reported no significant correlation between inbred line traits and the yield of their test crosses, but there were indications that phenotypic selection for inbred line traits would be useful.

The objectives of our study were: (i) screening of maize genotypes (inbred lines and hybrids) for salt tolerance in the greenhouse and the field; and (ii) evaluation of salt stress effects on morphological traits, yield and yield components of different maize genotypes.

Materials and Method

The greenhouse and field study was conducted at the Center of Agricultural Research and Natural Resources of Khorasan Razavi Province in northeastern Iran (59æ%38'N, 36æ%16’E), a region which received 265 mm of rainfall in 2007. In the greenhouse, 42 genotypes (20 inbred lines and 22 hybrids) were evaluated in four complete randomized designs (CRD) under four irrigation treatments with different salinity levels [(EC_W = 4, 8, 12 ds m^{-1}, and 1ds m^{-1} (control)]. In each CRD design, 40 seeds of each genotype were planted in sand in 2 pots (as 2 replications). The germination percentage, dried weight of plants and the vigor index were recorded separately. The vigor index (VI) was calculated (Maguire 1962; Abdul-Baki and Anderson 1973) as

Vigor index (VI) = dry weight of plants (g) × germination percentage

In the field, 46 genotypes (23 inbred lines and 23 hybrids) were planted in two randomized complete block designs with 3 replications in two fields (at the agricultural research stations at Abbas-abad in Mashhad and at Feiz-abad in Neyshabour). The electrical conductivity of saturated paste (ECe) and water (EC_W) were 5.9 ds m^{-1} and 6 ds m^{-1} in Mashhad and 10.5 ds m^{-1} and 8 ds m^{-1} in Neyshabour, respectively. Each genotype was planted in 2 rows of 3 m length with 0.75 m distance between rows. Plant density for the early-maturity genotypes was 85 000 plants ha^{-1} and for mid- and late-maturity genotypes 75 000 plants ha^{-1}. The evaluated traits were anthesis and silking time, anthesis-silking interval (ASI), plant height, ear height, physiological maturity, grain yield, ear length, kernel number, number of rows per ear, kernel number per row, kernel depth and 1000-kernel weight. The salinity variation and chemical characteristics of the soil were determined before planting and after harvest. Analysis of variance (ANOVA) and Pearson’s simple correlations between yield and related traits were done by SAS (version 8.1). Means of the measured traits were compared by Duncan’s multiple range tests.

Results and Discussion

The results of the greenhouse experiments showed no significant differences among the salt stress treatments for the germination rate (Table 1; Fig. 1), while the vigor index of plants was affected by the higher rates (8 ds m^{-1} and 12 ds m^{-1}) of salinity (Table 1). In the first germination count (7th day after planting at 25æ%C), many of the genotypes were found to have a low germination rate at EC 8 ds m^{-1} and 12 ds m^{-1} due to high salinity, while in the second count (3 weeks after planting) the germination rate was higher and normal. Jamaly *et al.* (2003) had reported that maize is tolerant to salinity during the germination stage. Our findings conformed to this pattern.

Figure 1. Effects of salt stress on vegetative growth of maize var. KSC500.

Table 1. Results of ANOVA for seed germination, dry weight of plantlets and vigor index of maize genotypes.

SOV	Df	Means of square		
		Vigor index	Dry weight of plants (g)	Germination (%)
Salt stress (s)	3	243 389.270**	47.092**	6.503^{ns}
Salt/replication	4	9909.197	0.523	4.253
Genotypes (G)	41	261 716.442**	20.818**	165.673**
S × G	42	124 38.001**	1.024*	8.946**
Error	245	7612.365	0.767	3.704
Total	335	27.92	21.35	13.76

Despite the nonsignificance of salinity effects on germination percentage, salt stress had very significant effects on dried weight and vigor index (Table 1). Comparison of the means for the vigor index showed that the higher levels of salinity (8 ds m^{-1} and 12 ds m^{-1}) led to a severe decrease in the vigor index (Fig. 2). Results of ANOVA for the field experiments showed significant differences for all of the measured traits at the Abbas-abad, Mashhad research station (Tables 2 and 3). Salt stress had greater effect on inbred lines than hybrids. It led to a decrease in vegetative growth especially plant height and ear height, delay in flowering, increase in the ASI and decrease in grain yield and yield components. The single-cross hybrids KE72012/1-12 × K2331, KSC500 and ZP434 recorded grain yields of 7.77 t ha^{-1}, 7.66 t ha^{-1} and 7.22 t ha^{-1} respectively whereas inbred line KE72012/1-12 yielded 1.722 t ha^{-1}, ie, the highest. The hybrid ETH-M24 (2.894 t ha^{-1}) and the inbred line OH43/1-42 (279.8 $kgha^{-1}$) gave the lowest yields under saline conditions. KE72012/1-12 is one of the elite inbred lines for salt stress conditions due to its good performance alone and in combination with K2331. Also, the inbred lines K3640/5 and K19 were superior in terms of grain yield under salt

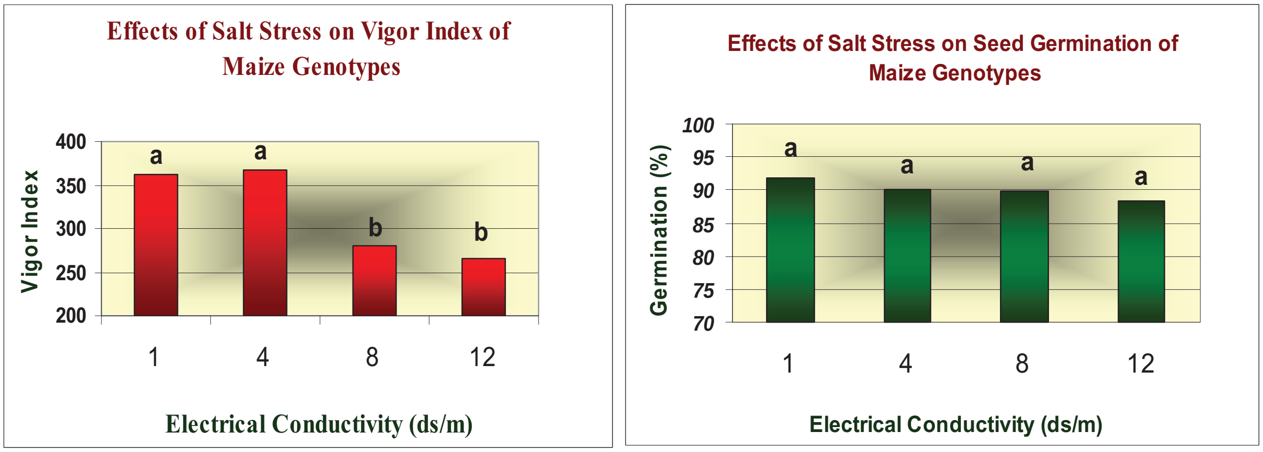

Figure 2. Effects of salt stress on seed germination and vigor index of maize genotypes, 2007.

Table 2. Analysis of variance of morphophysiological traits, yield and yield components of grain maize hybrids under salt stress conditions.

Ear length (cm)	Physiological maturity	Anthesis-silking interval	Silking time	Anthesis time	Ear height (cm)	Plant height (cm)	df	SOV
0.4.3	2.261	16.362	7.870	105.319	24.422	17.928	2	Replication
11.751**	118.401*	7.292*	91.953**	50.968**	431.45**	706.731**	22	Genotypes
2.030	53.367	3.696	9.324	10.198	30.068	49.145	44	Error
9.82	6.14	20.58	4.45	5.12	9.33	5		CV (%)

1000-seed weight (g)	No. of kernels	No. of rows	No. of kernels per row	Kernel depth (mm)	Grain yield (t ha^{-1})	df	SOV
2052.334	3099.838	0.025	7.523	6.744	4.236	2	Replication
2874.509**	24907.269**	6.877**	75.560**	4.212**	7.380**	22	Genotypes
997.682	5259.537	1.066	13.055	2.127	1.459	44	Error
14.35	14.78	6.65	11.47	16.28	17.12		CV (%)

stress conditions. At Neyshabour station many of the inbred lines and hybrids were removed due to high salinity; therefore, statistical analysis of variance was not possible. Salinity had undesired effects on the vegetative and generative growth stages of plants. The maximum (166.7 cm) and minimum (65.27 cm) of plant height belonged to the hybrid B73 × K19 and the inbred line K1263/1 respectively.

The hybrids L105 × K19 and L105 × K74/1 with 18.67 cm and 17.60 cm length respectively had the longest ears. The inbred line L105 (with 14.65 cm) was the best for ear length. The maximum (261.7 g) and minimum (86.67 g) 1000-kernel weight was recorded by SC704 (hybrid) and K18 (inbred line), respectively. Hoffman *et al*.s (1983) found that higher levels of salinity (more than 3.7 ds m^{-1}) led to a decrease in the 300-kernel weight and grain yield. Our results support that finding. The new early-maturing Iranian hybrid KSC400 had the most number of kernels per row (39.07), but the most number of rows per ear was found in the hybrid B73 × K74/1. The lowest number of rows (10) and number of kernels per row (10.33) were recorded by the inbred lines MO17 and OH43/1-42, respectively. The earliest and late-maturing inbred lines were S61 and K1264/1, taking 107 and 133 days from planting to physiological maturity, respectively. The highest anthesis-silking interval (ASI) was 12.62 days recorded by the K2816 inbred line. Najafi and Pooran (2000) reported that water salinity increased ASI by about 4-5 days. Simple coefficients of correlation between yield and agronomical traits like plant height (r = 0.826**), ear height (r = 0.771**), 1000-kernel weight (r = 0.684**), kernel depth (r =0.641**), number of kernels per row (r = 0.816**), number of of rows (r = 0.587) and ear length (r = 0.815**) were strongly positive but the correlation between ASI and other traits was negative and nonsignificant (r = -0.133) (Table 4).

Table 3. Analysis of variance of morphophysiological traits, yield and yield components of grain maize inbred lines under salt stress conditions.

Ear length (cm)	Physiological maturity	Anthesis-silking interval	Silking time	Anthesis time	Ear height (cm)	Plant height (cm)	df	SOV
2.184	19.580	8.739	43.884	37.841	188.552	930.466	2	Replication
18.704**	160.862**	27.808**	134.983**	178.026**	131.985**	361.503**	22	Genotypes
1.381	9.186	9.209	8.975	14.250	33.529	55.970	44	Error
13.75	2.50	20.02	3.88	5.38	17.67	8.32		CV (%)

1000-seed weight (g)	No. of kernels	No. of rows	No. of kernels per row	Kernel depth (mm)	Grain yield (t ha^{-1})	df	SOV
275.527	746.967	1.384	2.463	1.590	0.112	2	Replication
2707.047**	16958.105**	10.314**	74.274**	3.102**	0.461**	22	Genotypes
587.733	7299.130	3.648	16.830	1.085	0.040	44	Error
15.55	19.95	15.01	19.04	15.09	18.2		CV(%)

Table 4. Coefficients of correlation between grain yield and related traits in corn genotypes under saline conditions.

	Grain yield	ASI	Kernels no.	Ear lenght	Rows no.	Kernel no./row	1000- seeds weight	Kernel depth	Ear height
Plant height	0.826**	-0.093 ^{ns}	0.779**	0.793**	0.572**	0.765**	0.711**	0.579**	0.903**
Ear height	0.771**	-0.026 ^{ns}	0.739**	0.79**	0.511**	0.74**	0.715**	0.526**	
Kernel depth	0.641**	-0.152 ^{ns}	0.581**	0.444**	0.452**	0.536**	0.505**		
1000-seed weight	0.684**	0.027 ^{ns}	0.589**	0.657**	0.318**	0.62**			
Kernels no./row	0.816**	-0.202 ^{ns}	0.954**	0.83**	0.548**				
Rows no.	0.587**	-0.079 ^{ns}	0.754**	0.55**					
Ear length	0.815**	-0.149 ^{ns}	0.82**						
Kernels no.	0.836**	-0.17 *							
ASI	-0.133^{ns}								

Acknowledgment

This project was carried out at the Center of Agricultural Research and Natural Resources of Khorasan Razavi Province and supported by the Seed and Plant Improvement Institute (SPII), Karaj, Iran.

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Combining Ability of Drought-Tolerant Maize Inbred Lines

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Abstract. Ten tropical maize inbreds were crossed in a diallel mating system to determine the combining ability and compare the performance of the hybrids. Forty-five crosses were evaluated in separate trials for grain yield (GY), grain moisture content (MC) and kernel weight (KW) under water stress (WS) and no-water-stress (WW) conditions at Tak Fa, Nakhon Sawan Field Crops Research Center, Thailand during 2006-2007. A randomized complete block design was used with three replications. Water stress was managed by irrigation withdrawal, thereby achieving severe drought stress during the flowering stage. The no-water-stress condition received weekly irrigation. The Griffing (1956) method 4 showed that general combining ability (GCA) was significant for all the traits measured. Under the WS condition, GCA was more important than specific combining ability (SCA) for GY. Nei 452004 (0.603 t ha^{-1}), Nei 452026 (0.470 t ha^{-1}) and Nei 452008 (0.323 t ha^{-1}) had high GCA, which indicated that these inbreds would give high-yielding hybrids. High-yielding combinations including Nei 452008 × Nei 452019 (4.56 t ha^{-1}), Nei 452008 × Nei 452026 (4.06 t ha^{-1}) and Nei 452004 × Nei 412019 (4.00 t ha^{-1}) also had high SCA. Under the WW condition, GCA and SCA were significant. Nei 452017 (0.535 t ha^{-1}), Nei 452004 (0.478 t ha^{-1}), Nei 452026 (0.468 t ha^{-1}) and Nei 452008 (0.383 t ha^{-1}) had high GCA. Nei 452004 also had high GCA for KW under WS and WW conditions. Nei 452026 was a parent of the three highest combinations with high positive SCA, including Nei 452026 (9.46 t ha^{-1}), Nei 452017 (9.41 t ha^{-1}) and Nei 452029 (9.05 t ha^{-1}). Considering the GCA and SCA effects for GY, Nei 452026, Nei 452008 and Nei 452004 represented good inbred lines for developing drought-tolerant hybrids.

Key words: Drought, maize, stress, combining ability, general combining ability, specific combining ability

Introduction

Drought stress is the most widespread abiotic constraint to maize production in the tropics, where the crop is mostly grown under rainfed conditions in marginal production environments. Loss of maize grain production due to drought may reach up to 24 million t per year in the tropics, equivalent to 17% of production under well-watered conditions (Edmeades *et al*. 1999). In Thailand, drought is a priority constraint to maize production in many agroecological zones, occurring almost every year (Eskasingh *et al*. 2004). Maize can be affected by stress at any stage (Grant *et al*. 1989). Reduction of water availability down to permanent wilting point during preflowering, flowering and postflowering stages reduced grain yield by 25%, 50% and 21% respectively (Denmead and Shaw 1960). Therefore, breeders need to develop maize genotypes which cantolerate water stress (Betran *et al*. 1996). Genetic variations are the basis of genetic improvement in any crop. Crossing diverse inbred lines can provide sufficient genetic variability from which to make an effective selection for desirable traits. Identifying parents that will combine well and produce productive progenies mainly depends on gene action. Information on quantitative traits in terms of general and specific combining ability effects for a set of parents can be obtained from diallel analysis. The Nakhon Sawan Field Crops Research Center (NSFCRC) Maize Breeding Program has collaborated with CIMMYT on maize research and development for many years, and developed a number of inbreds through different breeding procedures (Grudloyma *et al*. 2005). In the present study, different sources of tropical late yellow inbreds were crossed in a diallel mating system to determine their combining ability and compare the performance of promising hybrids under water stress conditions.

Materials and Method

The study was conducted atNSFCRC in Tak Fa, Thailand during 2006-2007. A set of 10 tropical late yellowmaize inbreds were crossed in a diallel mating system with nonreciprocal cross (Table 1). The seed of 45 F_1s were sown in separate trials under no-water-stress (WW) conditions in the rainy and late rainy seasons of 2006 and under water stress (WS) in the summer season of 2007. A randomized complete block design was used with three replications. Each plot consisted of two rows 5.0 m long with an interrow spacing of 0.75 m and intrarow spacing of 0.20 m. Plant density was approximately 66 000 plants ha^{-1}. The WS experiment was subjected to irrigation withdrawal to achieve severe drought stress during flowering (irrigation was stopped from the 9th leaf stage to two weeks after mid-flowering). The WW condition received weekly irrigation. Soil analyses of testing sites indicated a pH of

7.3, organic matter content 2.87%, available P (Bray II) 61 ppm and exchangeable K 166 ppm. One hundred and fifty kg ha^{-1} of N fertilizer was applied to each experiment.

Data collection and statistical analysis

Days from planting to anthesis (AD) and silking (SD) were calculated as per the date on which 50% of the plants had begun shedding pollen or had silks emering from the husk. The anthesis-silking interval (ASI) was obtained by subtracting AD from SD. Grain yield (GY), expressed at 15.0% moisture content (MC), was obtained from shelled grain. The 100-kernel weight (KW) was obtained from 100 kernels weight. The drought index (DI) was the ratio of yield under stress to that under the no-stress condition, relative to the ratio of the mean yield of all the genotypes under stress conditions to that under no-stress conditions. Analyses of variance were performed using the MSTAT procedure. Combining ability studies for GY, KW and MC were conducted following the Griffing (1956) method 4 model 1.

Table 1. Pedigree and grain texture type of ten inbreds.

Name	Pedigree	Grain texture
Nei 412019	DK 888-14-1-2-1-BBBBB	Orange yellow, semiflint
Nei 452004	KS 23(S)C2-190-1-2-1-BBBBB	Orange yellow, semiflint
Nei 452008	Pio.3003-3-2-B-3-1-4-BBB	Orange yellow, flint
Nei 452010	Exp.9477-35-2-B-1-2-1-BBB	Orange yellow, semiflint
Nei 452017	(SW5(S)C3S2-76-2-4-1x (GHC2S2-18-1-1-1)-B-B-4-1-BBB	Orange yellow, flint
Nei 452019	C-5134064-17-3-B-2-1-1-BBB	Orange yellow, semiflint
Nei 452026	C-5124001-57-1-B-1-1-3-BBB	Orange yellow, semiflint
Nei 452029	LY-AL-TOL(S) C1-22-2-2-BBBBB	Orange yellow, flint
Nei 452030	NS-DR(S2)C1-60-1-4-BBBBB	Orange yellow, flint
Nei 452031	SW1(RRS)C2-458-1-3-1-2-BBBB	Orange yellow, flint

Results and Discussion

Analyses of variance indicated significantly different effects due to genotype for all of the traits measured. Due to error variances of WW experiments were having homogeneity, they were combined before their combining ability was estimated. In this case, the degree of freedom (df) of variance was 220. Under WS conditions, the general combining ability (GCA) effect was more important than the specific combining ability (SCA) effect for GY. Nei 452004 (0.603 t ha^{-1}), Nei 452026 (0.470 t ha^{-1}) and Nei 452008 (0.323 t ha^{-1}) were good combiners with positive GCA (Table 2). It indicated that they would give high-yielding drought-tolerant hybrids. In contrast, Nei 452017 (-0.718 t ha^{-1}), Nei 452030 (-0.365 t ha^{-1}) and Nei 412019 (-0.387 t ha^{-1}) were poor combiners with negative GCA. For grain moisture content, Nei 452019 (2.358%), Nei 452004 (1.109%) and Nei 452008 (2.703%) had positive GCA while Nei 452017 (-5.732%) and Nei 452029 (-0.834%) had negative GCA. Four inbreds, Nei 412019 (0.980 g), Nei 452004 (5.951 g), Nei 452010 (2.897g) and Nei 452019 (1.474 g), gave high positive GCA for KW. Under the WW treatment, GCA and SCA effects were both significant. Nei 452017 (0.535 t ha^{-1}), Nei 452004 (0.478 t ha^{-1}), Nei 452026 (0.468 t ha^{-1}) and Nei 452008

Table 2. Estimates of GCA effects for grain yield, moisture content and kernel weight of maize inbred lines under water stress and no-water-stress conditions, 2006-2007.

Inbred line	Water stress			No-water-stress		
	Grainyield	Moisturecontent	Kernel weight	Grainyield	Moisturecontent	Kernel weight
Nei 412019	-0.387**	2.358**	0.980**	-0.382**	0.750**	0.079
Nei 452004	0.603**	1.109*	5.951**	0.478**	0.067	5.524**
Nei 452008	0.323*	2.703**	-0.849*	0.383**	1.438**	-1.089**
Nei 452010	0.170	0.459	2.897**	-0.563**	-0.306*	0.822**
Nei 452017	-0.718**	-5.752**	-4.574**	0.535**	-2.119**	-0.015
Nei 452019	0.234	-0.687	1.474**	-0.486**	-0.864**	1.213**
Nei 452026	0.470**	0.381	-2.317**	0.468**	0.190	-2.893**
Nei 452029	-0.180	-0.834*	-1.522**	-0.105	0.580**	-1.020**
Nei 452030	-0.365**	0.229	-2.662**	-0.340**	0.630**	-3.803**
Nei 452031	-0.150	0.034	0.622	0.041	-0.368*	1.181**
se[g(i)]	0.136	0.434	0.364	0.069	0.141	0.269
se[g(i) - g(j)]	0.203	0.647	0.542	0.103	0.211	0.402

* Significant at 0.05 probability level. ** Significant at 0.01 probability level.

Table 3. Grain yield (upper diagonal, t ha^{-1}) and specific combining ability (parentheses) of inbreds under water stress conditions.

Male Female	Nei 452004	Nei 452008	Nei 452010	Nei 452017	Nei 452019	Nei 452026	Nei 452029	Nei 452030	Nei 452031
Nei 412019	4	1.53	2.72	2.34	2.73	3.22	3.04	2.43	1.71
	(0.804)*	(-1.379)**	(-0.039)	-0.462	(-0.096)	-0.154	-0.631	-0.2	(-0.736)*
Nei 452004		3.67	3.65	2.88	3.79	4	3.18	3.23	3.25
		(-0.239)	(-0.103)	-0.015	(-0.027)	(-0.050)	(-0.230)	-0.012	(-0.187)
Nei 452008			3.33	3.21	4.56	4.06	2.85	3.06	3.12
			(-0.142)	-0.629	(1.024)**	(0.887)*	(-0.273)	-0.126	(-0.033)
Nei 452010				2.63	2.91	3.47	3.08	3.18	3.2
				-0.198	(-0.470)	(-0.150)	-0.11	-0.392	-0.203
Nei 452017					1.88	2.16	2.06	1.84	2.08
					(-0.619)	(-0.569)	(-0.025)	(-0.057)	(-0.035)
Nei 452019						3.32	2.89	2.91	3.7
						(-0.360)	(-0.147)	-0.061	-0.633
Nei 452026							3.61	3.38	3.36
							-0.336	-0.291	-0.06
Nei 452029								1.67	3.01
								(-0.762)*	-0.356
Nei 452030									2.2
									(-0.262)

* Significant at 0.05 probability level. ** Significant at 0.01 probability level.
df = 88 se[s(i, j)] = 0.358
t (0.05) = 1.987 se[s(i, j) - s(i, k)] = 0.537
t (0.01) = 2.632 se[s(i, j) - s(k, l)] = 0.498

Table 4. Grain yield (upper diagonal, t ha^{-1}) and specific combining ability (parentheses) of inbreds under no–water-stress condition, 2006.

Male Female	Nei 452004	Nei 452008	Nei 452010	Nei 452017	Nei 452019	Nei 452026	Nei 452029	Nei 452030	Nei 452031
Nei 412019	8.19	7.96	7.08	8.27	7.39	8.32	7.29	7.4	7.69
	-0.051	(-0.109)	(-0.049)	-0.044	-0.19	-0.162	(-0.300)	-0.054	(-0.042)
Nei 452004		8.55	8.32	8.37	8.43	9.46	7.92	8.4	8.6
		(-0.357)*	(0.365)*	(-0.689)**	(0.399)*	(0.471)**	(-0.499)**	-0.224	-0.036
Nei 452008			8.18	9	8.37	8.98	8.23	7.79	8.66
			-0.29	-0.005	(0.400)*	-0.057	(-0.124)	(-0.324)	-0.163
Nei 452010				8.46	5.6	7.39	7.97	7.89	7.25
				(0.416)*	(-1.424)**	(-0.586)**	(0.570)**	(0.716)**	(-0.298)
Nei 452017					8.13	9.41	8.66	7.88	8.76
					-0.005	(0.357)*	-0.16	(-0.390)*	-0.112
Nei 452019						6.58	8.46	7.79	8.01
						(-1.475)**	(0.982)**	(0.542)**	(0.384)*
Nei 452026							9.05		
							(0.612)**	-0.167	-0.256
Nei 452029								6.74	7.5
								(-0.890)**	(-0.511)**
Nei 452030									7.67
									(-0.099)

* Significant at 0.05 probability level. ** Significant at 0.01 probability level.
df = 220 se[s(i, j)] = 0.182
t (0.05) = 1.960 se[s(i, j) - s(i, k)] = 0.273
t (0.01) = 2.756 se[s(i, j) - s(k, l)] = 0.252

Table 5. Grain yield (t ha^{-1}), yield loss (%) and drought index of selected hybrids over water stress and normal moisture conditions, 2006-2007.

Hybrid	Grain yield			Yield loss	Drought index
	Water stress	Normal moisture	Mean		
Nei 452004 × Nei 452026	4.00	9.46	7.64	58	1.16
Nei 452008 × Nei 452026	4.06	8.98	7.34	55	1.23
Nei 452026 × Nei 452029	3.61	9.05	7.23	60	1.09
Nei 452008 × Nei 452019	4.56	8.37	7.10	46	1.49
Nei 452008 × Nei 452017	3.21	9.00	7.07	64	0.98
Nei 452026 × Nei 452030	3.38	8.37	6.70	60	1.10
Nei 452017 × Nei 452026	2.16	9.41	6.99	77	0.64
Nei 452004 × Nei 452008	3.67	8.55	6.92	57	1.17
Nei 452004 × Nei 452019	3.79	8.43	6.88	55	1.23
Nei 452004 × Nei 452031	3.25	8.60	6.81	61	1.03

(0.383 t ha^{-1}) had high positive GCA. This indicated that these inbreds would give high-yielding hybrids under no-water-stress conditions. Nei 452004 was a good general combiner for KW under both conditions, indicating that it would give high-KW hybrids. The high-yielding combinations under WS conditions, including Nei 452008 × Nei 452019 (4.56 t ha^{-1}), Nei 452008 × Nei 452026 (4.06 t ha^{-1}) and Nei 452004 × Nei 412019 (4.00 t ha^{-1}) also had high positive SCA (Table 3). In the WW treatment, 12 hybrids had high positive SCA. Nei 452026 was a parent of three of the highest combinations with high positive SCA, including Nei 452026 with Nei 452004 (9.46 t ha^{-1}), Nei 452017 (9.41 t ha^{-1}) and Nei 452029 (9.05 t ha^{-1}), respectively (Table 4). Considering the GCA and SCA effects for GY, Nei 452026, Nei 452008 and Nei 452004 represented good inbred lines for developing drought-tolerant hybrids. The GY of the top ten selected hybrids ranged from 6.81 t ha^{-1} to 7.64 t ha^{-1} (Table 5). Nei 452004, Nei 452008 and Nei 452026 were parents of high-yielding hybrids with yield losses ranging from 46% to 64% over both conditions. While Nei 452017 gave high-yielding hybrid under no-water-stress condition.

Conclusion

Nei 452004, Nei 452008 and Nei 452026 were good combiners under water stress and no water stress conditions. It indicated that they would be good inbred lines for developing drought-tolerant hybrids. While Nei4 52017 was a good combiner under the no-water stress condition. Nei 452004 was also found to be a good general combiner for kernel weight under both conditions, indicating that it would give high kernel weight in a hybrid combination.

Acknowledgement

The authors wish to express their gratitude to Dr. Sansern Jumpatong, National Corn and Sorghum Research Center; Mr. Weerasak Doungjan, Bangkok Seeds Industry Co. Ltd., and Dr. Gary Atlin, CIMMYT, for their helpful advice.

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Screening of Corn Genotypes for Drought Tolerance Using the Sand Culture Technique

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Abstract. Drought is a major constraint to corn production in the Philippines. Production loss in 1990-93 was estimated at 478 000 t and valued at PhP 2.1 billion. It resulted in a shortage in the local supply and caused an increase in prices of livestock feeds and meat and poultry products. Development of drought-tolerant varieties is one way to increase grain production and minimize losses due to drought. The objective of this study was mass screening and evaluation of corn genotypes for drought tolerance under greenhouse conditions. Sand culture, a rapid screening method for corn genotypes against drought stress, was used in this experiment. The top-performing genotypes identified in terms of plant dry matter production were CML 453, CML 172, ARMP THAI 16 and CA 03116. These genotypes will be validated on-farm and recommended for consideration in generating populations with an emphasis on drought tolerance. Our study also showed that screening corn germplasm using the sand culture technique is effective. Evaluation could thus be done even during the rainy season, which would help hasten breeding work.

Key words: Corn genotypes, drought tolerance, sand culture technique

Introduction

Drought in 1990-1993 caused the most devastating damage to corn production in the past 26 years in the Philippines. Production loss was estimated at 478 000 t and valued at PhP 2.1 billion. It led to a shortage in the local corn supply and caused an increase in prices of livestock feeds and meat and poultry products. Then in 1997-1998 the occurrence of El Niño caused a significant decrease in corn production. Yellow corn yield from the dry-season crop declined by 37% while white corn yield fell by 27%, thereby decreasing production by 42% and 38%, respectively.

Development of drought-tolerant varieties is one way to increase grain production and minimize losses due to drought. However, field screening is difficult due to typhoons during the rainy season and sometimes during the dry season. A rapid screening method like the sand culture technique makes evaluation of corn genotypes possible even during wet/rainy season, therebyhastening breeding work.

Materials and Method

The greenhouse screening was conducted during the rainy season of 2006 at the Physiology Division of the Institute of Plant Breeding, University of the Philippines Los Baños, Laguna, Philippines. A total of 24 corn genotypes (Table 1) were considered for evaluation. The experiment was laid out in a split-split plot in a randomized complete block design (RCBD) with three replications. The main plot factor was the water level (W_1 = irrigated; W_2 = drought-imposed) while the subplot factor was the nitrogen level (N_1 = 0 kg N ha^{-1}, N_2 = 120 kg N ha^{-1}). A replicate comprised of 4 trays which contained 24 corn genotypes. These genotypes were designated as the sub-subplot factor and were randomized as per the tray.

The sand culture technique, which uses sand placed in styrofoam trays as the growth medium, was used to evaluate the performance of the 24 corn genotypes in terms of nitrogen-use efficiency and plant dry matter under irrigated and drought conditions (Fig. 1). The fertilizers used were urea (46-0-0) for nitrogen, solophos (0-18-0) for phosphorus and muriate of potash (0-0-60) for potassium. Urea was dissolved in such a way that each corn entry would receive 10mL of the assigned nutrient solution equivalent to the intended N rate. Solophos and muriate of potash were mixed with the sand before planting. The sand in all trays was irrigated to field capacity after planting. Drought stress was imposed one week after seedling establishment while the control plants received water continuously. Data on NUE and total plant weight, later oven dried, were taken after the imposition of the water treatment. The data gathered was analyzed using the analysis of variance for split-split plots in an RCBD.

Table 1. Means of plant dry matter (DM) across treatments under irrigated and drought conditions.

No.	Genotype	Across treatments DM (g)	Irrigated DM (g)	Drought DM (g)
1	IPB VAR. 7	2.36 Bcde[1]	2.43 bcde	2.28 bcde
2	ARMP THAI 41	2.10 De	2.12 e	2.08 e
3	ARMP 8	2.26 Cde	2.55 bcde	1.97 e
4	CA 34504	2.19 Cde	2.21 cde	2.17 de
5	CML 451	1.98 E	1.98 e	1.97 e
6	CML 487	2.39 Bcde	2.36 bcde	2.42 bcde
7	CML 453	4.53 A	5.35 a	3.71 b
8	CA 00314	2.25 Cde	2.24 cde	2.25 cde
9	IPB VAR 1	2.59 Bcde	2.51 bcde	2.66 bcde
10	CML 481	2.47 Bcde	2.53 bcde	2.42 bcde
11	CML 172	3.09 Bc	2.56 bcde	3.62 bcd
12	CA 03130 B	2.75 Bcde	3.08 bcde	2.41 bcde
13	ARMP THAI 49	2.48 Bcde	2.54 bcde	2.43 bcde
14	CML 21	2.46 Bcde	2.39 bcde	2.52 bcde
15	CML 452	2.30 Bcde	2.38 bcde	2.22 cde
16	ARMP THAI 16	4.83 A	3.63 bc	6.04 a
17	CML 427	2.30 Bcde	2.41 bcde	2.19 cde
18	CL 02836	2.94 Bcd	2.77 bcde	3.10bcde
19	ARMP THAI 20	2.15 Cde	2.18 cde	2.12 e
20	CL 02838	2.32 Bcde	2.68 bcde	1.97 e
21	ARMP THAI 36	2.34 Bcde	2.55 bcde	2.14 e
22	ARMP THAI 29	2.04 De	2.16 e	1.92 e
23	CL 00331	2.63 Bcde	3.09 bcde	2.18 cde
24	CA 03116	3.21 B	3.11 bcde	3.32 bcde

[1.] Means with the same letter are not significantly different.

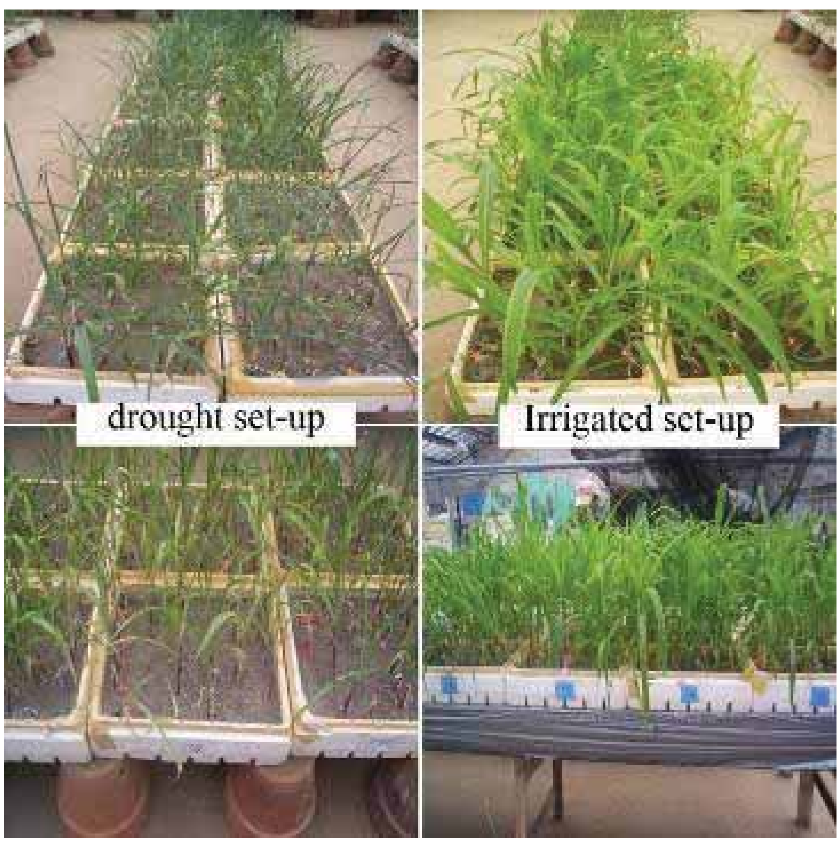

Figure 1. Sand culture, a rapid screening technique for maize germplasm against drought.

Results and Discussion

Analysis of variance showed that there was a highly significant interaction between the levels of water and genotypes in terms of plant dry matter (DM). Also, the main effects of nitrogen and genotype were found to be highly significant, indicating the existence of wide variability among the genotypes. However, there were no significant differences for the different treatments in terms of N-use efficiency (NUE). Regardless of nitrogen and water levels, the top ten genotypes in terms of DM production were ARMP THAI 16 (4.83 g), CML 453 (4.53 g), CA 03116 (3.21 g), CML 172 (3.09 g), CL 02836 (2.94 g), CA 03130 B (2.75 g), CL 00331 (2.63 g), IPB Var 1 (2.59 g), ARMP THAI 49 (2.48 g) and CML 481 (2.47 g). Genotype CML 451 hadthe lowest DM value of 1.98 g. Under the irrigated treatment, CML 453 (5.35 g), ARMP THAI 16 (3.63 g) and CA 03116 (3.11 g) were the top performers. Genotypes ARMP THAI 16, CML 453, CA 03116 and CML 172 were also the top performers under the drought set-up. In other words, these four genotypes were able to produce a high amount of dry matter even in the presence of drought stress.

The genotypes exhibiting high plant dry matter under drought conditions like ARMP THAI 16, CML 453, CA 03116 and CML 172 are therefore recommended for consideration in generating populations with emphasis on drought tolerance. It should be noted, however, that the top-performing genotypes identified in this study must be validated on-farm. Our study shows that screening of corn genotypes using the sand culture technique is effective and allows evaluation of genotypes even during the rainy season.

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Screening for Drought-Resistance Key Indices and Genetic Research in Silage Maize

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Abstract. By using six silage maize inbred lines with different drought-tolerance characteristics in diallel crossing as per the Griffing method 2, we determined that the key indexes for drought-resistance coefficients of kernel yield are the root cap ratio, leaf area, chlorophyll content and rate of photosynthesis, that have an established regression equation of the drought coefficients of kernel yield from path analysis. We also estimated the general combining ability (GCA), special combining ability (SCA) and other relevant genetic parameters of the material. The results show that GCA is obviously higher than SCA for the five characteristics and that the genetic accumulating effect is dominant. The h_B^2 (%) for the rate of photosynthesis was 93.07% at most, and the h_N^2 (%) for the grain yield drought index was 18.62% at most. The drought-resistance coefficients of kernel yield are more effective when selected in an earlier generation.

Key words: Silage maize, drought tolerance, key indices, genetic laws

Introduction

Silage maize is the most important source of livestock feed as it is rich in nutrition. It is cultivated over almost 3 million hm^2 in China. However, it is severely constrained by drought, which is the crop's most important abiotic stress factor. Over 70% of China's corn fields are affected by drought, suffering a production loss of over 15 million teach year. The hilly region of southwestern China is frequently and severely affected by seasonal drought. According to data pertaining to the period 1951-1995, the frequency of droughts in the spring, summer and middle summer was 58%, 76% and 67% respectively. In the hilly region of Sichuan, this was 89%, 92% and 62%, respectively. The year 2006 saw Sichuan being affected by the most serious drought since the foundation of the People's Republic of China. The direct economic loss from it reached RMB 12.57 billion, the loss to agriculture being RMB 10.8 billion. The corn crop is particularly seriously affected by drought in China.When drought occurs, farmers take recourse to silage maize in China. Therefore, there is a need to build a reliable quantitative and research-based indicator system to identify drought resistance in silage maize. A coefficient of drought tolerance is the key element in this effort.

Under drought conditions, solar energy utilization through photosynthesis decreases. Drought also plays an important role in the aging of plant organs. Aging first manifests itself in the leaves, which turn yellow (Yuan *et al.* 2002). The aging of the root system (including the stem) is a sign of the aging of the whole plant (Shen 2001). The key indices influencing drought tolerance in silage maize under conditions of drought stress in the hilly regions have not been worked out yet. Our study aims at identifying the key tolerance indices influencing yield of silage maize, and at providing a reliable theoretical basis for drought-resistance breeding in illy, arid and similar areas. Toward this objective, we carried out diallel crossing, as per the Griffing method 2, of six inbred lines of drought-resistant silage maize.

Materials and Method

The six silage maize inbred lines chosen for this study had different drought-resistance characteristics (Table 1). Match two groups of Griffing diallel crossing patters, in which orthogonal F_1 combination with number of a = p*(p+1)/2 = 6*96+1) = 21 and add parents. They were sown in an experimental field in Jianyang in Sichuan province where there is a Water Saving Key Lab. The experiment had three replications on plots in a random design. The experimental plots had four rows of 320 cm each with a spacing of 67 cm. Fifteen plants were planted within each row, giving a density of 4800 plants per hm^2.

The experiment consisted of two treatments: plots subjected to drought stress and plots supplied irrigation. The plots in the drought-stress treatment were rainfed and given one irrigation of 30 cubic meters. The plots in the irrigation treatment were rainfed too but given five irrigations at the seedling stage, elongating stage, early differentiating stage and milk-ripe stage. The total irrigation dosage per *mu* (a measurement of area) was 150 cubic metres, each irrigation being of 30 cubic metres. The water dosage was precisely controlled with a drip irrigation system. Recommended cultivation practices for silage maize were followed.

Sampling. We sampled 10 plants from each treated sampling area to carry out tests for the chosen characteristics

Sampling schedule. Recordings of the leaf area, chlorophyll content and photosynthetic rate were taken at the spinning stage; other indicators were measured at the harvest stage.

Measurement of chlorophyll content. We directly measured the chlorophyll content of each treated spike leaf with a Spad apparatus made in Japan.

Separating samples. The collected samples were cleaned and the root, stem, green leaves, yellow leaves and ears were separated. Their gross weight was recorded.

Fixing, drying and weighing. The samples (root, stem, green leaves, yellow leaves and ears) were fixed in water at 105°C for half an hour and then dried at 70~80°C for 1~2 days till their weight remained stable. The plant organs were weighed with an electronic balance of 0.0001 definition.

Indicator-measuring methods. The photosynthetic rate (Pn) was measured using a BAU photosynthetic system. Measurement of the leaf area was done as per the method of Zhang (1992). The drought index (DI) of yields was calculated as per (Fu *et al.* 2003)

(Ya/YM)/Ya [CK]/Ym [CK]

in which Ya and Ym represent the yields of the two treatments, drought-stress and irrigated conditions, respectively; and Ya[CK] and Ym[CK]] represent the yields of the two treatments involving *Chuandan 13* (a kind of seed) used as control.

Index-calculating methods. The following relations were used:

- root/top = root dry weight/soil-above dry weight
- ear/dry matter = fruit and are dry weight/whole plant dry weight
- stem/leaf = stem/whole leaf dry weight
- green leaf/yellow leaf = green leaf dry weight/yellow leaf dry weight
- green leaf/dry matter = green leaf dry weight/whole plant dry matter weight
- stem/dry matter = stem dry matter weight/whole plant dry matter weight
- root/dry matter = root dry matter weight/whole plant dry matter weight

Data processing. The data was analyzed on the DPS8.01 platform.

Statistical analysis

First, multiple regression analysis was done using the kernel drought-resistance coefficients as the target characters and comparing the importance of each character index to corn drought resistance. Then, a covariance analysis was performed on the screened key drought-resistance indices according to model I/model II designed in random groups. The significance of differences was recorded. Lastly, the combining abilities of model I/model II were determined by the Griffing method 4. Further, the genetic parameters of the group were estimated on this basis.

Results

Path analysis of drought-resistance key indicators

The efficiency of silage maize drought resistance depends on several characters acting together. But overall, it should be measured in terms of the drought-resistance coefficients of kernel yield. After we did the stepwise regression analysis on leaf area ($X_{1)}$, ear/dry matter ratio (X_2), root cap ratio (X_3), stem/leaf ratio (X_4), stem/whole plant dry matter ratio (X_5), chlorophyll content (X_6), rate of photosynthesis (X_7), green leaf/yellow leaf ratio (X_8), green leaf/whole plant dry matter ratio (X_9), root/whole plant dry matter ratio (X_{10}) and the drought-resistance coefficients of drought-treated kernel yield (Y), we foundthat (Fig. 1) if we estimate Y with a linear regression equation, the linear relation is clear (R = 0.9316), and its value can reach 86.79%. This shows that leaf area, root cap ratio, chlorophyll content and photosynthetic rate are the main component factors of drought-resistance coefficients of kernel yield. The regression equation was as follows:

$$Y = 1.376 + 0.000036X_1 + 5.872X_3 - 0.0079X_6 - 0.0320X_7$$

The analysis shows that both X_1 and X_3 are positive direct actions in which the root cap ratio ($P_{0.3} = 0.850$) is the first positive factor influencing the drought-resistance coefficients of kernel yield, with the dependent coefficient ($d_{0.2}$ = 0.7237) in it being the most important. The contribution of R^2 (the degree of the estimate of the regression equation) is 52.230%. The second important factor is leaf area ($p_{0.1}$ = 0.288), with the dependent coefficient ($d_{0.2}$) being 0.0829. X6 and X_7 are negative direct actions of which the photosynthetic rate ($P_{0.7}$ = –0.730) is the most influential, with the dependent coefficient ($d_{0.7}$) being 0.533 and the the contribution of R^2 being 55.90%. The second important negative factor is chlorophyll content ($p_{0.6}$ = –0.429), with the dependent coefficient ($d_{0.6}$) being 0.184. Seen from an indirect action perspective, in which the chlorophyll content contributes positive action through the indirect action of the leaf area and the root cap ratio, the product of these actions is 0.620, the highest value. The positive indirect action of the photosynthetic rate through the leaf area is second highest, with a value of 0.025. The indirect actions among indicators are negative values. The facts above indicate that for the drought-resistance coefficients of silage maize kernel yield, the positive influence of the root cap ratio is primarily direct action, the negative indirect actions being small. The positive direct action and the negative indirect action of the leaf area are quite equal. The positive indirect action of chlorophyll content is bigger than its negative direct action.

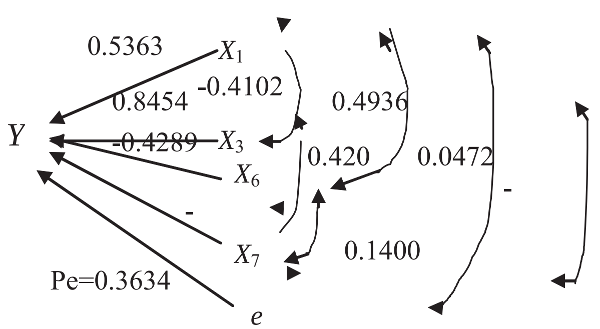

Figure 1. Path analysis graph of different factors affecting the corn yield drought index.

and the photosynthetic rate is primarily a negative direct action and an indirect one.

Combining abilities of key drought-resistance indicators and genetic parameter analysis

The results of the path analysis indicate that leaf area, root cap ratio, chlorophyll content and photosynthetic rate are key indices of the drought-resistance coefficients of kernel yield. Through a covariance analysis of the five indices, we found that differences among the character combinations were very evident, which may be arising from genetic factors. This needed an analysis of their combining abilities.

The results showed that covariance of the GCA and SCA of the five indices was quite evident, which further indicates that the addictive and nonaddictive genetic effects in the test materials are very important to the performance of each character. The covariance of GCA of the five indices was particularly higher than the SCA, which indicates that the genes which control these characters are mainly cumulative in effect but different in degree.

Effects of combining abilities

By estimating the general combining abilities of the parents, we found that (Table 2) the GCA of the five characteristics of all of the parent inbred lines have two kinds of effect values, ie, positive and negative, which indicates that the effect of one inbred line on each characteristic in F_1 combination is different, as is the effect of different inbred lines on the same characteristic in F_1 combination. Inbred line 2018 can increase the root cap ratio, chlorophyll content, PN and DI value and bring down the leaf area in F_1, which makes it an ideal drought-resistant inbred line. DIKM can increase the leaf area and DI value, and C 161 can improve the leaf area, chlorophyll content and DI value in $F_{1.}$ Both of them are ideal inbred lines. Q

Table 1. Drought-related traits of six inbred lines of silage maize.

Inbred line	Leaf area (cm^2)		Ratio of root cap		Chlorophyll content ($mg.g^{-1}.FW$)		Photosynthetic rate ($\mu mol\ CO_2/m^2.s$)		Drought index of corn yield	
	X	CV%	X	CV%	X	CV%	X	CV%	X	CV%
2018	2874.49	41.87	0.08	4.06	53.87	2.58	14.60	1.05	1.13	13.10
Q 205	5108.73	27.64	0.06	25.79	39.07	5.14	19.07	2.02	0.75	12.79
DIKM	4367.78	35.94	0.05	7.14	38.23	22.53	7.03	1.25	1.31	16.96
C 161	5262.20	9.18	0.03	9.26	44.30	3.51	14.40	1.84	0.92	9.03
1437	2547.62	43.51	0.05	21.93	48.97	4.68	14.77	2.15	0.98	3.47
SH 21	3387.45	11.57	0.06	20.19	43.83	16.74	13.97	2.35	0.83	8.16

Table 2. GCA effects of inbred lines.

Inbred line	Leaf area (cm^2)	Ratio of root cap	Chlorophyll content ($mg.g^{-1}.FW$)	Photosynthetic rate ($\mu mol\ CO_2/m^2.s$)	Drought index of corn yield
2018	-413.415	0.011**	1.444**	0.451**	0.039**
Q 205	162.969*	-0.002	-4.235	1.018**	-0.0843
DIKM	197.120*	-0.005	-2.756	-2.094	0.0799**
C 161	1141.041**	-0.007	1.140**	-0.069**	0.0094*
1437	14.145	0.001	3.186**	0.372**	-0.0093
SH 21	-1101.861	0.001*	1.219**	0.322**	-0.0347

Significant at 0.05 probability level.
** Significant at 0.01 probability level.

Table 3. SCA effects of crosses.

Cross	Leaf area (cm^2)	Ratio of root cap	Chlorophyll content ($mg.g^{-1}.FW$)	Photosynthetic rate ($\mu mol\ CO_2/m^2.s$)	Drought index of corn yield
2018 × Q 205	147.373	-0.0174	-2.902	-3.211	0.0432
2018 × DIKM	1436.32	-0.0137	-3.548	2.002	-0.1076
2018 × C 161	794.352	0.00120	1.923	0.177	0.0261
2018 × 1437	905.972	-0.0125	1.111	2.702	-0.0651
2018 × SH 21	539.778	-0.0177	2.711	0.019	-0.043
Q 205 × DIKM	-291.471	-0.017	-2.935	3.235	-0.1243
Q 205 × C 161	257.917	-0.003	5.636	-1.890	0.0595
Q 205 × 1437	933.938	0.001	6.923	-1.565	0.0349
Q 205 × SH 21	613.111	-0.0131	-0.544	-1.548	0.1203
DIKM × C 161	2401.542	-0.0032	3.290	-0.544	-0.0314
DIKM × 1437	625.862	-0.0045	4.077	-1.219	-0.0793
DIKM × SH 21	-892.881	-0.0023	12.877	3.164	0.0061
C 161 × 1437	2875.167	0.0083	3.448	1.289	0.0411
C 161 × SH 21	-1062.760	-0.0065	2.915	0.973	0.0532
1437 × SH 21	846.851	-0.0043	0.502	-0.169	0.0286

205,01437 and SH 21 all can decrease the DI value in F_1, which is not ideal.

We can see in Table 3 that the special combining ability of the adrought-resistance index vary with the cross, as do the SCA effects of different indices of the same cross. In C161 × 1437, the effective values of special combining ability for leaf area and root cap ratio are the highest and the DI positive value is big, which indicates a cross with good comprehensive drought resistance. From the different crosses in which the special combining ability has a positive value, it can be seen that in the inbred lines combination of drought-resistant character, there always has combination appear whose special combining ability of drought-resistant character is positive value, which indicates drought-resistant character can appear combination in which special combining ability is positive value from in some genetic background for inbred lines whose general combining ability is negative effect.

In order to further understand the genetic basis of the drought-resistance key indices of each test material, we estimated the related genetic parameters according to the results of the covariance analysis (Table 4). We see that the addictive covariance of DI is obviously bigger than dominant covariance, which indicates that the expression of the character in F_1 is controlled mainly by genetic addictive action. The expression of the dominant covariance of the other four indices in F_1 is bigger than addictive covariance, which explains that the expression of the leaf area, root cap ratio, chlorophyll content and photosynthetic rate in F_1 is controlled mainly by dominant action. Seen from the perspective of broad-sense heritability h_N^2, PN heritability reaches 93.07%, which is high; for the root cap ratio it is 50.00% and the other values are smaller. Seen from narrow-sense heritability h_N^2, the value of DI can be 18.62% at most, which suggests that the DI value can be better in early-generation selection. The genetic coefficient indicates that the genetic variation coefficients of leaf area and root cap ratio are bigger, which indicates that these characters have a wide range of differences among the inbred lines tested. We can further reorganize, innovate and breed inbred lines which conform

Table 4. Genetic rules of drought-resistantce key indices.

Genetic parameter	Leaf area (cm^2)	Ratio of root cap	Chlorophyll content ($mg.g^{-1}.FW$)	Photosynthetic rate ($\mu mol\ CO_2/m^2.s$)	Drought index of corn yield
X	5613.2219	0.0385	50.6254	14.5413	0.9821
	337 097.1307	0.00001	5.9439	1.1056	0.0046
	1 835 273.5126	0.0002	26.7602	4.8290	0.0018
	2 172 370.6432	0.0002	32.7042	5.9346	0.0063
	3 661 490.7056	0.0002	43.0368	0.4416	0.0184
	5 833 861.3488	0.0004	75.7410	6.3762	0.0247
$\hat{h}_B^2$ (%)	37.2373	50.0000	43.1790	93.0742	25.5061
$\hat{h}_N^2$ (%)	5.7783	2.7100	7.8477	17.3395	18.6235
GCV(%)	26.2578	36.7328	11.2962	16.7530	8.0819

to drought-resistance target characters by integrating options from other lines.

Results and Discussion

Screening for drought-resistance key indices

In the process of core drought-resistance breeding, the appraising method and the index of drought-resistant genotypes are the key factors in the success and efficiency of breeding. At present, the drought-resistance mechanism of corn is not very clear, and there is no unified drought-resistant appraising index. Researchers such as Zhang Baoshi (1996) consider that drought resistance of corn is a complicated physiological trait that cannot be appraised in the form of one or two simple indices. Researchers such as Li Wanzhen (2001) consider that the rate of emergence of core drought-resistance coefficients of biological yield and their product are reliable indices for appraisal of drought resistance at the corn seedling stage.

We consider that among the many agricultural and physiological characters of silage maize, four key indices can be helpful in selecting for drought resistance, and they are evidently reliable. Path analysis shows that the root cap ratio should be high during the maturity period and materials have to be chosen whose root system is strong and the canopy part is sizable. Similarly, bigger leaf area must be chosen at the spinning stage which can improve the light energy intercept area. Leaves should have high chlorophyll content. The photosynthetic rate should be maintained at a proper level so as to keep the fluency of fluid. Higher root cap ratio is good for the drought resistance of plants (Passioura 1983). This provides reliable indices for appraisal of drought resistance in silage maize in hilly regions.

Compared with previous studies (Zhang 1996; Li 2001; Passioura 1983; He *et al.* 1995; Shan and Luo 2001), this assay not only screened key indices for appraisal of drought-resistance in silage maize from agricultural and physiological indicators but also studied their genetic basis. Researchers such as He *et al.* (1995) consider that characters related to drought resistance are influenced by additive genetic effects to a great extent. Shang and Luo (2001) consider that the drought indexes are mainly controlled by additive effects. Wang and Guo (1998)(consider that root length, root number and root cap ratio in the corn root system have dominant and additive effects with narrow sense heritability being moderate.

We believe that additive covariance of the drought-resistance coefficients of silage maize is greater than dominant covariance, which is consistent with the said combining abilities. The indices are mainly controlled by genetic additive action and is the highest of the narrow sense heritability in this assay, but with moderate narrow sense heritability value. Therefore, through selection and refining of the parents, the drought-resistance coefficients of corn kernel yield can be effectively refined. But this index reacts well with environment, so the selection of authenticated environment should be emphasized, which is consistent with earlier research. The dominant covariance of the four indices, – leaf area, root cap ratio, chlorophyll content and photosynthetic rate – is bigger than their additive covariance. These indices are mainly controlled by dominant actions. Therefore, we should utilize them through crossing, which is inconsistent earlier research. It is the result of selecting different crosses or different ecological conditions, or it is the result of a deviation in experimental result due to different drought intensity.

Our study indicates that in order to obtain better kernel yield of silage maize under drought stress, we must fully consider the reaction among genes. We also need to pay attention to the selection of the parents. What needs to be emphasized is that the same cross has different genetic effects for different indices. Therefore, quantitative heredity of the core drought resistance needs further detailed research, o as to determine its regularity and provide guidance for drought-resistance breeding or drought-resistance genetic improvement.

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Study of Morpho-Phenotypic Traits of Grain White Anoman-1 under Drought Conditions

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Abstract. Drought in Indonesia is a universal abiotic stress condition. Anoman-1 (Tuxpeno sequia-C7), a white-grain, open-pollinated variety (OPV), was selected for its drought tolerance during the critical flowering stage and successfully released in Indonesia in 2006. Anoman-1 was improved one cycle from a CIMMYT population. Some morpho-phenotypic traits of this variety such as means (µ) correlation between all variables (r), deviation of the means (ó$_x$,) and coefficient heritability (h^2) of Anoman-1 were studied under normal and drought stress conditions. Two sets of experiments were conducted: (1) under normal water availability conditions at the Bajeng and Muneng experimental farms;‘ and (2) under water stress conditions at south Sulawesi (Jeneponto district). The results show that Anoman-1 yielded a maximum of 152.0% more under normal water condictions than drought conditions. The problem of rolling leaves under drought conditions was scored 3.52. There were significant differences relating to the anthesis-silking interval (ASI): it was six days under drought conditions, and three days under normal water conditions. As regards reaction to major diseases, the two locations had the similar scores for maydis leaf blight and rust. The yield of Anoman-1 had a positive correlation to ear height, tasseling and silking under drought conditions.

Key words: Drought, correlation, Anoman-1

Introduction

Drylands account for 20.27% (14.9 million ha) of total farm land in Indonesia (BPS 2006). The eastern part of Indonesia has the largest area affected by drought, including East and West Nusa Tenggara (NTT, NTB) and Southeast Maluku where rainfall is low. Total rainfall during the rainy season in these regions varies from 500 mm to 1100 mm. Erratic rainfall and high variability in soil fertility cause wide yield variability across years and locations. The grain yield loss suffered due to drought during the flowering and seed-filling stages is about 25%. Further, there is variability of yield losses among genotypes within a crop (Dahlan *et al*. 1996). In NTT for instance the maximum yield of maize (opv. Arjuna) under dry conditions was 2.0 t ha^{-1} (Yasin *et al.* 1990). Edmeades *et al.* (1992) reported that in developing countries yield losses per year under drought conditions could go up to 60%.

Anoman-1, a white-grain variety from a CIMMYT population (Tuxpeno Sequia cycle 6) was released in 2005 in Indonesia after improvement by one cycle to C7. S_1 families were generated at ICERI, Maros and screening was done at two sites to select families with drought tolerance. Some morpho-phenotypic traits of this variet were observed during experiments conducted for the release of the variety. Trials of the selected families were carried out in target areas, particularly in the eastern part of Indonesia like NTT, NTB and Southest Maluku. In this area, farmers commonly use white maize as their staple food. The objective of this paper was to study some morho-phenotypic traits like means of variables, deviation of means, heritability and simple correlation of the traits.

Morpho-phenotypic Traits

The experiment was conducted in a randomized complete block design with three replications for evt, and a simple lattice design with two replications for selected superior families in South Sulawesi (Bajeng, Jeneponto) and East Java (Muneng-Probolinggo). The climate of the experimental farm was of the type E3, ie, 3-5 wet months withrainfall >100 mm per month (Oldeman dan Darmiyati 1977). Germplasm was selected from the population Tuxpeno Sequia C6 to improve families by S_1 to recombine after screening under water stress conditions. The drought treatment involved stoppage of water from the tasseling stage (50 days) until harvesting time. The normal water treatment had normal supply of water until harvest. The selected families' tolerance for drought was calculated as per the drought index formula of Edmeades *et al.* (1992). The means (µ,) standard deviation (ó$_x$), coefficient of varitaion (cv) and heritability (h^2) of morpho-phenotypic traits were recorded and their simple coefficient of correlation (r) was computed by

$$r = [\text{Ó}x_iy_i-(\text{Ó}x_i)(\text{Ó}y_i)/n]/\text{''}[\text{Ó}x_i^2-(\text{Ó}x_i)^2/n-(\text{Ó}y_i^2)(\text{Ó}y_i)^2/n].$$

The test of significance used degrees freedom (df) = n-2 (Gomez and Gomez 1984).

The results of some morpho-phenotypic traits under normal and drought conditions are shown in Table 1. The maximum difference in yield between the drought and normal water treatments at the two locations was 152.0%. Leaves of Anoman-1 under drought were scored 3.52. The anthesis-silking interval (ASI) was found to be six days under drought conditions, and three days under normal water. Yield was low under drought because there is no synchronization between flowering andr high value of ASI (Beck *et al.* 1996 Blum 1986; Bolanos and Edmeades 1993). Reaction to major diseases maydis leaf blight and rust, was similar (score of 1.0).

Correlation of Morpho-Phenotypic Traits

Simple correlation of coefficients at the two locations is shown in Table 2. The highest correlation of yield in the normal water treatment was founded to be with plant height and water content of seeds at harvest time. A negative correlation of yield was found with ear height, tasseling and silking stage, and visually observed aspects (plant, ear and husk aspects). Under drought conditions, yield was found to have a positive correlation with ear height, tasseling, and silking. Correlation of yield under normal water treatment was significantly different with plant height, silking period and plant aspects. Under drought conditions, correlation of yield was significant with plant aspects only.

Conclusion

The yield of Anoman-1 was 152% higher under normal conditions. The morpho-phenotypic trait of rolling leaves under drought conditions was scored 3.52. There were significant differences as regards the anthesis-sinlking interval (ASI): it was six days under drought conditions, and three days under normal water availability conditions. Maydis leaf blight and rust had similar scores under both treatments. The yield of Anoman-1 had positive correlation to ear height, tasseling and silking under drought conditions.

Table 1. Morpho-phenotypic traits[1] of Anoman-1 under normal and drought conditions.

Normal conditions	μ	$ó_x$	cv	h^2
1. Yield (t ha^{-1})	6.320	0.806	15.470	63.850
2. Tasseling (days)	48.657	3.396	2.815	40.362
3. Flowering (days)	51.167	2.339	2.235	77.751
4. Plant height (cm)	174.500	9.552	9.305	40.347
5. Ear height (cm)	51.167	2.339	13.900	77.755
6. Plant aspect (score)	1.667	0.745	42.510	45.062
7. Husk cover (score)	1.687	0.745	35.455	36.287
8. Ear aspect (score)	1.500	0.500	30.322	40.974
9. Water content of seeds harvest ed (%)	28.817	3.145	6.250	78.253
10. Maydis leaf blight score *(Helminthosporium maydis)*	1.417	0.449	30.485	74.520
11. Maize rust score (*Puccinia sarghi*)	1.833	0.236	21.32	36.792
12. Rolling leaves score	1.000	0.225	15.300	98.500
Drought conditions				
1. Yield (t ha^{-1})	2.5000	0.483	19.025	63.662
2. Tasseling (days)	55.758	2.403	1.710	63.654
3. Flowering (days)	59.035	1.572	1.690	77.785
4. Plant height (cm)	140.000	87.000	9.665	43.673
5. Ear height (cm)	87.000	15.695	14.275	43.662
6. Plant aspect score	1.530	0.764	37.980	45.050
7. Husk cover score	2.167	0.373	40.450	65.214
8. Ear aspect score	3.000	1.291	38.800	40.922
9. Water content of seed harvest ed (%)	34.483	4.129	5.518	78.235
10. Maydis leaf blight score *(Helminthosporium maydis)*	1.335	0.471	28.458	74.661
11. Maize rust score (*Puccinia sarghi)*	1.566	0.746	30.255	98.795
12. Rolling leaves score	3.520	1.225	17.553	65.500

1. μ = means; $ó_{x\,=}$ deviation of the means; cv = coefficient of variation (%.); h^2 = heritability.

Table 2. Simple coefficient of correlation between variables* under normal water and drought conditions, Bajeng-Muneng, Jeneponto, Indonesia, 2004.

	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Under normal water conditions											
(a)	1.000	0.816	-0.178	-0.830	-0.842	-0.813	-0.521	-0.645	-0.748	-0.579	0.811
(b)	-	1.000	-0.304	-0.790	-0.652	-0.608	-0.374	-0.401	-0.592	-0.851	0.686
(c)	-	-	1.000	0.594	0.601	0.293	0.843	0.573	0.623	0.289	-0.596
(d)	-	-	-	1.000	0.908	0.745	0.745	0.764	0.801	0.554	-0.936
(e)	-	-	-	-	1.000	0.892	0.892	0.926	0.965	0.352	-0.979
(f)	-	-	-	-	-	1.000	0.700	0.894	0.913	0.158	-0.907
(g)	-	-	-	-	-	-	1.000	0.894	0.913	0.158	-0.858
(h)	-	-	-	-	-	-	-	1.000	0.928	0.00	-0.938
(i)	-	-	-	-	-	-	-	-	1.000	0.262	-0.944
(j)	-	-	-	-	-	-	-	-	-	1.000	-0.333
(k)											1.000
Under drought conditions											
(a)	1.00	-0.279	0.398	0.319	0.419	-0.844	-0.638	0.309	-0.749	-0.597	0.811
(b)	-	1.000	0.526	-0.709	-0.951	0.310	0.138	0.495	-0.592	-0.851	0.685
(c)	-	-	1.000	-0.397	-0.411	-0.125	-0.199	0.361	0.623	0.289	-0.596
(d)	-	-	-	1.000	0.734	-0.660	-0.699	-0.263	0.801	0.554	-0.936
(e)	-	-	-	-	1.000	-0.486	-0.237	-0.574	0.965	0.352	-0.979
(f)	-	-	-	-	-	1.000	0.878	-0.169	0.913	0.158	-0.907
(g)	-	-	-	-	-	-	1.000	-0.346	0.913	0.158	-0.858
(h)	-	-	-	-	-	-	-	1.000	0.928	0.00	-0.938
(i)	-	-	-	-	-	-	-	-	1.000	0.262	-0.938
(j)	-	-	-	-	-	-	-	-	-	1.000	-0.944
(k)											1.000

* (a) Yield (t ha^{-1}); (b) plant height (cm); (c) ear height (cm); (d) male tasseling (days); (e) female silking (days); (f) plant aspect; (g) ear aspect; (h) husk cover; (i) maydis leaf blight; (j) rusk; (k) water content of seeds (%);
Statistical test 5%: 0.811.

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Maize Pathology in Asia: Opportunities and Challenges for Breeding Disease-Resistant Maize

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Abstract. More than 12% of maize productivity is lost to diseases annually in Asia. Sources of resistance to several important diseases have been identified in advanced CIMMYT germplasm but the genetics of resistance is poorly understood. Disease resistance in maize is reported to be conditioned by both major (qualitative) genes and minor (quantitative) genes or quantitative trait loci (QTL). To date, 437 quantitative disease resistance loci or dQTL, 17 major resistance genes and 25 resistance gene analogs (RGAs) associated with resistance to 11 maize diseases have been described in a few of these sources. QTL mapping studies conducted in maize thus far have provided information on the genetic architecture of disease resistance including the number, location and action of chromosomal segments conditioning the trait. The availability of the maize genome sequence offers new opportunities for a detailed understanding of the organization and architecture of disease resistance, fine mapping of QTL, validating candidate genes and identifying effective molecular markers to improve disease resistance. However, full exploitation of genomic approaches and information to develop and release maize cultivars more resilient to diseases will only be possible through (i) a deeper integration of genomic approaches with conventional breeding methodologies; (ii) a capacity to reliably and accurately phenotype diseases on a large scale; and (iii) a sound multidisciplinary knowledge of the biochemical and physiological processes determining crop yield and its stability under different disease stress regimes. A major emphasis for the Asian region will have to include the collaborative evaluation of promising sources of resistance for specific traits using standardized protocols that would allow harmonization of disease phenotypic data for use in identifying the most promising resistance genes, validating molecular markers liked to effective dQTL and major resistance genes and incorporation of the most promising sources in improvement activities.

Introduction

The importance of maize to sustainable development cannot be overstated. With a production of 0.78 billion t in 2007, and providing approximately 12% of the calories consumed worldwide, maize is the most important staple food crop in the world (FAO 2007; http://faostat.fao.org/). Maize is utilized as food for human consumption, as feed for livestock and as a raw material in industry (FAO 1992). Recent projections indicate that by 2020, global maize demand will increase by 50% (IFPRI 2008; Pingali and Pandey 2001). The increase will be acute in Asia where it is expected to rise by 87% from 162 million t in 1995 to 303 million t in 2020 (IFPRI 2000). Rising incomes, population growth, urbanization and expanding energy demand are the fators responsible for much of this increase. Rapid economic growth and increased consumer purchasing power are shifting food demand away from traditional staples and toward higher-value foods like meat and dairy products. This dietary shift is leading to increased demand for grains used to feed livestock (Wada *et al.* 2008).

The increasing diversion of maize toward biofuel production (especially in the USA, currently the largest maize exporter, where 30% of maize production will go toward ethanol in 2008) rather than into world food and feed markets (IFPRI 2008) means that Asia cannot rely on imports. The growing demand for maize in Asia will have to be met through accelerated production at home, either through an augmentation of crop area or increased adoption and use of high-yielding varieties. As land is limited in most countries, increased production will have to come from adoption of new technologies. Efforts to reduce maize losses due to disease through adoption of resistant crop varieties, pesticides and other cultural practices offer tremendous opportunities for increasing and stabilizing maize productivity. This paper aims at highlighting opportunities and challenges of using host resistance to manage maize diseases and identifies some researchable areas to alleviate losses due to diseases.

Economic Importance of Diseases in Maize Production

Diseases are important constraints to maize production in Asia. Losses from disease vary according to disease, occurrence, environmental conditions, host genotype and time (growth stage of the crop) of infection. Loss estimates in Asia have been put at around 12% (Table 1) but actual losses can be as high as 80%. For example, in 1987, an epidemic of downy mildew in Nepal caused 50% yield loss (Gerpacio and Pingali 2007). During the 1996 cropping season in Central Lampung, Java, Indonesia, downy mildew (*Peronosclerospora maydis)* affected a total of 7665 ha, of which 2880 ha (about 38%) were totally damaged (Dalmacio 2000). Maize losses due to disease result from reduced yields, increased production costs (fungicide purchases and application), lost market opportunities due to poor grain quality and contamination by kernel and ear molds. Furthermore, kernel and ear rot pathogens, especially *Aspergillus* and *Fusarium* species, produce aflatoxin and fumonisin mycotoxins that are toxic to humans and animals. Contaminated grain pose an important health risk and an economic burden on livestock and poultry producers. These mycotoxins have been shown to affect uptake of nutrients, vitamins and micronutrients resulting in stunting in children; aggravate the effects of HIV/AIDS; increase the incidence of neural tube defects leading to abortion and, when high concentrations are consumed, fatalities (Williams *et al.* 2004).

Table 1. Estimated yield losses from different biotic constraints.

Region	Loss (%)			
	Attainable yield ($t \times 10^6$)[1]	Diseases	Animals/ insects	Weeds
Africa	74	16	20	18
Central and South America	98	12	17	19
Asia	203	12	18	12
North America	258	9	11	11
Europe	68	7	9	9

[1.] Based on 1988-1990 data summarized in Oerke 1994.

Important Maize Diseases in Asia

Many diseases threaten maize production in Asia (Dalmcio 2000). The important diseases are similar across maize production environments in the continent, differing only in intensity from country to country, location to location or season to season within a country, under the influence of cultural and cropping practices (Table 2). Of the reported maize diseases, the ones considered of economic importance are maize dwarf mosaic (including sugar cane mosaic); the downy mildews; Diplodia, northern, southern and banded leaf and sheath blights (BLSB); southern and common rusts; Diplodia, Fusarium, charcoal and bacterial root and stalk rots; Rhizoctonia, Diplodia, Fusarium and Aspergillus kernel and ear rots; and common and head smuts. Disease occurrence is dynamic and will remain so, while some of the currently important diseases may become less important as resistant cultivars are developed, and others presently considered not important may become more prevalent with changes in climate, cropping practices and introduction of new, high-yielding cultivars. Climate change will modify the distribution and adaptation range of many diseases, and research is needed to document the effects of climate change on pathogen dynamics in Asia.

Table 2. Important diseases of maize in Asia.

	A	B	C	D	E	F	G	H	I	J	K	L	M
Highlands	4.0[1]	2.0	5.0	3.5	5.0	3.0	5.0	4.5	1.5	1.8	4.8	1.0	5.0
Upper wet MA	2.5	1.5	4.0	3.0	5.0	3.0	5.0	3.5	1.5	2.0	4.0	1.0	4.5
Lower wet MA	2.0	1.5	1.5	2.0	2.0	3.0	2.5	3.0	1.5	2.0	3.5	1.5	3.0
Dry mid-altitudes	3.5	3.0	2.0	4.0	3.0	4.0	2.5	2.0	4.0	2.5	2.5	1.5	3.0
Wet lowlands	1.5	4.5	1.0	3.8	1.3	4.0	1.5	2.0	3.5	1.5	2.0	1.0	2.5
Dry lowlands	3.5	5.0	2.0	5.0	1.8	5.0	1.5	2.0	4.8	2.0	2.0	1.0	3.5

A = Banded leaf and sheath blight
B = Common rust
C = Downy mildews
D = Gray leaf spot
E = Maydis leaf blight
F = Phaeosphaeria leaf spot
G = Polysora rust
H = SCMV/MDMV
I = Turcicum leaf blight
J = Ear rots
K = Asperigillus ear rots
L = Fusarium verticillioides ear and stem rot
M = Stenocarpella ear rot (Diploida)
[1.] Classification based on scores on a 1-5 rating scale, where 1 = economically very important; and 5 = not economically important.

Increased adoption of zero tillage and conservation agriculture will change disease dynamics as has been observed for gray leaf spot (*Cercospora zeae-maydis)* in Nepal, Vietnam, India and China, where recent epidemics have been experienced following adoption of conservation agriculture technologies. The rapid shift to rice-maize cropping systems by Asian farmers over the past two decades in response to reduced availability of water, increased costs associated with irrigation and increased demand of maize for feed, will likely increase the incidence and prevalence of BLSB. The disease has already become a major constraint to maize production in regions where rice-maize systems have long been established (eg, China, Vietnam, Indonesia, India and the Philippines). Research is needed to develop maize varieties and hybrids with broad resilience to a range of diseases that are suited to these types of cropping systems. Broad-spectrum resistance at the genotypic level and sustainability at the cropping system level are therefore complementary approaches in managing maize diseases.

Strategies for Managing Maize Diseases

Numerous options have been recommended for the control and management of maize diseases (Pratt *et al.* 2003). The availability, feasibility and cost-effectiveness of each method will differ among production regions and settings. Recommended practices include conventional tillage that buries crop residues, crop rotation and fungicide application, and the use of resistant varieties. Host plant resistance will remain the most economical way of controlling maize diseases. It is especially attractive to smallholder farmers because once the technology is developed, it is packed and disseminated as seed, which makes it practical, cost-effective and environmentally friendly. Resistance is available to almost all the major diseases of maize. The big challenge to breeders is to incorporate these genes into elite but susceptible cultivars. This requires tools and methodologies for reliable identification of superior disease-resistance genes for use in breeding programs and protocols for identifying plants carrying the resistance gene during the breeding process. The variable nature inherent in both the incidence and severity of natural disease epiphytotics complicates breeding for disease resistance. The complexity arising from a host organism, a pathogenic microorganism, the interaction between them, and their interaction with the environment can make expedient and efficient selection difficult. Testing across locations or seasons and/or artificial inoculation systems are usually necessary to accurately characterize host response. Development of highly efficient inoculation techniques or identification of sites with consistent, severe disease pressure may be used to overcome fluctuations in disease pressure. All these efforts will benefit from availability of standardized disease screening protocols for use in breeding programs. In addition, management of resistance genes to prolong their effectiveness or slow down their breakdown would require integration of other strategies, including the use of chemicals, biocontrol agents and cultural practices.

Finding the right genes – the key to durable disease management

A broad range of germplasm with adequate resistance to many pathogens has been identified by different scientists in different organizations (Gerpacio and Pingali 2007; Lal *et al*. 2000; Welz and Geiger 2000). In addition, CIMMYT has germplasm with resistance to several important maize diseases in Asia (Jeffers *et al.* 2000). As the population structure of many maize pathogens has not been sufficiently elucidated, there is a need to widely test potential sources of resistance in order to expose them to as wide a spectrum of the pathogen as possible, thereby enabling the identification of the most suitable and effective resistance genes for use in breeding programs. Research at a regional level is needed to test potential sources of resistance and identify superior resistance genes or gene combinations for use in breeding programs. This would benefit from the establishment of a regional disease nursery and cooperative testing for resistance to major diseases in each country. Such efforts have already resulted in the rapid development and deployment of downy mildew-resistant varieties (Gerpacio and Pingali 2007; Raymundo 2000). These efforts should be revived and extended to other pathogens. For such efforts to bear fruit there is a need for the development of standardized disease screening techniques to reliably distinguish resistant plants from escapes.

Standardized disease screening protocols

The rate of progress toward development of stable and durable disease resistance in maize will depend on availability of methodologies and protocols for reliable differentiation of resistant and susceptible genotypes. Establishing adequate and uniform disease pressure is crucial for progress. Low disease pressure will result in too many escapes and nonreliable results, and thus slow down the rate of genetic gain toward development of stable resistance. Conversely, too high a disease pressure will eliminate low levels of resistance inherent in adapted germplasm, drastically narrowing the germplasm base. Therefore, establishing adequate levels of disease pressure is critical for reliable differentiation of test entries. Although methodologies and protocols for generating adequate disease pressure have been developed and reported (Jeffers *et al.* 2000), they have not been standardized. It is not uncommon for different scientists within the same or different institutions and countries to use different disease screening techniques and protocols. Such exercises limit the usefulness of the information generated, and make it difficult to exchange, compare and harmonize data from different organizations. A concerted regional effort to identify and monitor disease resistance genes will accelerate progress toward development of maize varieties and hybrids with durable resistance. This will greatly benefit from the use of a common set of standardized protocols and methodologies. The methodological issues that have been raised include the lack of standardized protocols (including different scoring systems) within and between countries and the lack of common checks, use of which will facilitate the exchange of data between institutions and countries. All these issues limit efforts to harmonize data and information from different organizations.

Disease rating keys and scales

Perhaps this is the most abused system that is often poorly harmonized even within institutions. Disease rating scales of 1 or 0 to 3, 1 or 0 to 5, 1 or 0 to 7, and 1 or 0 to 9 are the most commonly used. The interpretation of these scales is as variable as the scales themselves, and in some instances, a rating of 1 represents a highly resistant genotype while in other institutions a rating of 1 represents a highly susceptible entry. There is no clear definition of rating scales, which have been modified to suit different conditions by many scientists. Therefore, a rating of 3 might represent different disease pressures and intensities to different people. To avoid this bias and work toward harmonizing disease rating keys in maize, there is a need to develop pictorial type disease rating scales to use as reference points and narrow down errors that might be generated through lack of experience. Furthermore, diseases rarely occur alone in nature and mixed infections have been observed. To an inexperienced eye, symptoms from different diseases might be difficult to differentiate which might further compound difficulties in disease rating. Disease guides with typical and atypical symptoms on genotypes with different levels of resistance might help minimize these errors. Once this is implemented, it will allow us to harmonize data from different institutions and work toward a concerted effort with regional relevance. In addition, it is important that the amount of data and information to be collected relates to the objectives of the study.

Development of standardized disease screening protocols and methodologies is crucial for progress toward development of stable disease resistance in maize. Most of these methodologies have been developed and optimized by various scientists, but they have not been sufficiently documented for the benefit of other scientists in the region. CIMMYT in conjunction with IITA and scientists from national agricultural research systems (NARS) are working to develop standardized protocols and methodologies for reliable phenotyping of diseases.

Genetics of Resistance to Maize Diseases

Several studies have elucidated the genetics of disease resistance in maize (Pratt and Gordon 2006; Wisser *et al.* 2006). Disease resistance in maize is conditioned by both qualitative and quantitative genes. Qualitative resistance is based on single dominant or recessive genes, while quantitative resistance is typically partial and race-nonspecific in phenotype, oligogenic or polygenic in inheritance and is conditioned by additive or partially dominant genes. Although it is easier to work with qualitative resistance in breeding, quantitative resistance is often the more useful in an agronomic context, due to its generally higher durability and broader specificity. However, very little is known about the molecular or physiological basis of quantitative resistance. In maize, the majority of disease resistance deployed in elite varieties in the field is quantitative in nature. Although numerous sources of resistance have been described, the genetics of resistance has not been sufficiently documented. This information is crucial for breeders to identify genes and gene combinations that when stacked together may result in stable resistance. Although considerable progress has been made, much more remains to be done to fully take advantage of the plethora of disease resistance genes that exist in maize. The establishment of a disease resistance nursery for wider evaluation will likely result in the identification of superior disease resistance genes for use in breeding programs.

Architecture of disease resistance genes

Wisser *et al.* (2006) analyzed the locations of 437 quantitative trait loci (QTL) for disease (dQTL), 17 resistance genes (R-genes), and 25 resistance gene analogs (RGAs) that have been reported in the literature, in an attempt to harmonize disease resistance gene mapping information into formats useful for breeding purposes. They found that disease resistance genes are organized into clusters on all 10 maize chromosomes. The revelation that disease resistance genes are organized into 'hot spots' opens new doors and opportunities for marker-aided breeding, as breeding programs will target the introgression of chromosomal segment 'hot spots' associated with resistance genes for different diseases rather than focus only on introgression of single-disease resistance genes. This is most likely going to make marker-assisted breeding (MAB) more efficient and feasible, but there is a need to test the feasibility of this notion. Since then more studies to identify and map dQTL have been conducted, and there is a need to harmonize this information and make sense of the architecture of dQTL to make practical use of molecular markers in maize breeding. These efforts will benefit from the availability of good quality phenotypic data, preferably from multiple locations. The regional disease nursery is timely as it will identify superior diseases resistance genes and generate much-needed multilocation phenotypic data that will be used to validate candidate-disease resistance markers and their subsequent use in MAB.The availability of the full sequence of the maize genome will provide information useful for marker-trait association to identify and develop molecular markers for use in MAB.

Molecular marker development and marker-assisted selection (MAS)

Considerable research has been undertaken in genetic mapping of QTL to assist the development of varieties with improved host resistance (Wisser *et al.* 2006). Resistance QTL associated with NLB, downy mildew, SLB, rust, GLS and many other diseases have been mapped in maize (Wisser *et al.* 2006), making molecular MAS a potentially viable strategy to improve resistance to these diseases. However, few molecular markers are being used in breeding programs largely because few efforts have been put into molecular marker validation outside the mapping population. This step would require collection of quality phenotypic data, which will depend on accurate and reproducible disease induction and quantification. The nature of minor genes and QTL is still elusive and will require considerable effort to understand. Genomic approaches may be of considerable value in helping us reveal the role of minor genes, but again, proof of impact on the phenotype will depend on good and reliable phenotypic data. Improved methods are being developed for gene mining (Dong *et al.* 2003) using genomic and proteomic approaches. Increases in our understanding of pathogen variation will also enable determination of specific interactions between host and pathogen and enable optimization of MAS strategies.

Directions for Future Research

A concerted regional effort to identify superior disease resistance genes will accelerate progress toward development of maize varieties and hybrids with durable resistance. Only through cooperative research can we enhance our ability to improve host resistance of germplasm in a gainful manner. Two major objectives for the future would be: (1) to develop standardized protocols and methodologies for disease establishment and rating. As most methodologies have been developed and optimized by various scientists, a major effort should be the documentation of these protocols for the benefit of maize scientists in the region; and (2) to establish a regional disease resistance nursery and subsequent testing by all member countries. Such a nursery offers several advantages including the ability to (i) identify superior sources of disease resistance; (ii) ascertain the quality and stability of identified disease resistance genes; (iii) monitor the emergence of new pathogen strains or diseases; and (iv) generate information to make scientists better prepared for disease challenges that might emerge in response to climate change. Through the regional disease nursery, good phenotypic data will be generated for many diseases on the same set of materials, thus affording the consortium to take advantage of the availability of the maize genome sequence for mining candidate genes for disease. Furthermore, such a set will prove to be an excellent experimental material for validating molecular markers and subsequent application in MAB.

Conclusions

Disease occurrence will remain dynamic and its incidence and economic impact will vary from one country to another. Therefore, breeding for disease resistance will remain the most economical way of controlling maize diseases. Resistance is available to almost all major diseases of maize, and the big challenge to breeders is to incorporate these genes into elite but susceptible varieties. Significant gains toward development of durable disease resistance in maize will only be feasible when available sources of resistance are tested in as many environments as possible, and using as wide a spectrum of genetic variation in the pathogen as possible to identify superior sources of disease resistance. A paradigm shift from thinking locally to regionally is necessary for cooperative evaluation of potential sources of resistance. This can only be feasible through the use of standardized disease screening protocols and methodologies, including common checks, to allow consolidation and harmonization of data. Recent developments in biotechnology offer an opportunity to harness molecular markers to speed the introgression of resistance genes into elite materials. However, this requires quality phenotypic data for marker trait validation. Setting up a regional nursery offers the opportunity to collect such data and to facilitate validation of potential molecular markers and their subsequent use in MAB. Shared responsibility in the face of limited resources will allow us to generate more information that will bring about significant gains in our quest to use host resistance to durably manage maize diseases than single institutions would achieve alone.

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Identification of Maize Germplasm for Resistance to Maize Insect Pests in Thailand

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Abstract. Insect pests are major constraints to maize production in Thailand. During 2006/2007, 10 elite maize inbreds and 45 single-cross hybrids, including 5 commercial hybrids, were evaluated separately for resistance to Asian corn borer (ACB) (Ostrinia furnacalis Guenee) and maize weevil (MW) (Sitophilus zeamais Motsch.). Evaluation for resistance to ACB was conducted under artificial infestation in the greenhouse. Each plant was infested with 30 second instar larvae which were deposited in the whorl of 20-day old plants. Plant damage rating was done on a 1-9 scale five days after infestation. Significant differences were not found among the inbreds and hybrids. However, 12 hybrids and 7 inbreds were identified as having intermediate resistance. All hybrids that had Nei 412019 as one of the parents had significantly less leaf-feeding damage rating than others. The evaluation of resistance to maize weevil was conducted in no-choice tests in the laboratory. Thirty gram grain samples of each inbred and hybrid were infested with 25 adult weevils for 7 days. The weevils were removed and incubated for a further 90 days before loss of grain weight was recorded. No significant differences were found among the inbreds and hybrids. However, 15 hybrids and 11 inbreds were identified as resistant. Hybrids which had the parent Nei 452008 had smaller percentages of grain weight loss than others. These superior maize genotypes will be considered for further use in a maize breeding project for insect resistance.

Key words: Maize, Asian corn borer (Ostrinia furnacalis Guenee), maize weevil (Sitophilus zeamais Motsch.), screening for insect resistance

Introduction

Maize is one of the most important sources of animal feed in Thailand. Asian corn borer (ACB) is the crop's most serious insect pest, which can attack the maize plant in the whorl stage, postflowering and ear-formation stages. Serious damage by ACB can easily be seen in late rainy-season maize (Kongkanjana and Chunhawong 1991). Maize weevil (*Sitophilus zeamais* Motsch.) is the most serious storage insect pest of maize, causing storage losses of up to 90%, particulary when grain is stored without chemical protection (Sukprakran 1982). Several cultural and chemical practices are undertaken to control these pests, but the focus now is on integrated pest management whose main goal is the development of insect-resistant varieties. The objectives of this study were to identify maize germplasm with resistance to Asian corn borer and maize weevil.

Materials and Methods

Ten elite maize inbred lines and 45 single-cross hybrids developed from these lines, including 5 commercial hybrids obtained from the breeding project of the Nakhon Sawan Field Crops Research Center, Thailand, were evaluated separately for resistance to Asian corn borer and maize weevil.

Screening for resistance to Asian corn borer

The maize germplasm were sown in the greenhouse. The experiments were arranged in a randomized complete-block design with 5 replications. Asian corn borer larvae were obtained from a culture maintained on a diet of mung bean (Ketnuti 1982) in the laboratory ($27\pm1^{o}C$; 75 ± 5 % rh) at the Nakhon Sawan Field Crops Research Center in order to produce 2nd instar larvae in sufficient supply for the experiment. Each maize plant was manually infested with thirty 2nd instar larvae which were introduced to the spirally rolled leaves in the whorl of plants 20 days after emergence. The infestations were made in 2 applications of fifteen larvae spaced 1 day apart. One week after infestation, leaf-feeding damage was visually rated using a 1-9 rating system (1 = no damage; 9 = extreme damage). Resistant leves were identified by the method developed by Guthrie *et al.* (1960).

Screening for maize weevil (S. zeamais Motsch.) resistance

The experimental hybrids and inbreds were separately screened in no-choice tests using the assessment methods proposed by Bergvinson *et al*. (2002). Maize germplasm were planted in the field. All ears from each genotype were harvested and shelled. Grain were cleaned and dried in the sun to bring the grain moisture content down to 13-14%. Stock cultures of maize weevils (MW) were established in the laboratory (27±1ºC; 75±5% rh) to produce a sufficient number of adults. The experiment was arranged in a randomized complete-block design with 3 replications. Thirty grams of grain of each genotype were put in a glasss jar with a ventilated lid and stored at -20ºC for two weeks to kill field infestation. The experimental jars were then incubated in the laboratory for two weeks prior to infestation. Twenty-five unsexed and equal-age (1-7 days old) adult weevils were randomly introduced into each jar. After seven days the parent weevils were removed and the experimental jars were kept in the laboratory for 90 days. After incubation, samples were passed through meshed screens to separate kernels, adult weevils and flour fractions. The parameters used to evaluate the damage to the maize genotypes were percentage of grain damage and grain weight loss. Data for these two parameters were arcsine transformed prior to analysis.

Table 1. Response of maize inbred lines to infestation with Asian corn borer under greenhouse conditions at Nakhon Sawan Field Crops Reseach Center, Thailand.

Inbred line	Damage rating[1]	Level of resistance[2]
Nei 412019	7.00	S
Nei 452010	7.00	S
Nei 452029	7.25	S
Nei 452004	6.00	IR
Nei 452026	6.00	IR
Nei 452031	6.25	IR
Nei 452017	6.50	IR
Nei 452019	6.50	IR
Nei 452030	6.50	IR
Nei 452008	6.75	IR
Mean	6.57	
F-test	ns[3]	
CV (%)	15.20	

1. Leaf-feeding damage scored on a 1-9 damage rating scale (1 = no damage, 9 = extreme damage).
2. Resistance classification based on method developed by Guthrie *et al.* (1960). IR = Intermediate resistance; S = Susceptible.
3. ns = nonsignificant.

Results and Discussion

Screening for Asian corn borer resistance

No significant differences were found among inbred lines (Table 1) and hybrids (Table 2) for leaf-feeding damage due to ACB infestation. The average leaf-feeding damage scores for the inbreds and hybrids were 6.57 and 7.07,

Table 2. Leaf-feeding damage rating and level of resistance of maize hybrids to ACB under greenhouse conditions at the Nakhon Sawan Field Crops Reseach Center, Thailand.

Single-cross hybrids	Leaf-feeding damage rating[1]	Level of resistance[2]
Nei 412019 × Nei 452008, Nei 412019 × Nei 452017, Nei 412019 × Nei 452017, Nei 452004 × Nei 452026, Nei 452004 × Nei 452031, Nei 452008 × Nei 452031, Nei 452010 × Nei 452031, Nei 452017 × Nei 452031, Nei 452019 × Nei 452026, Nei 452026 × Nei 452031	6.00-6.75	IR
Nei 412019 × Nei 452004, Nei 412019 × Nei 452010, Nei 412019 × Nei 452019, Nei 412019 × Nei 452026, Nei 412019 × Nei 452029, Nei 412019 × Nei 452030, Nei 412019 × Nei 452031, Nei 452004 × Nei 452008, Nei 452004 × Nei 452010, Nei 452004 × Nei 452017, Nei 452004 × Nei 452019, Nei 452004 × Nei 452030, Nei 452008 × Nei 452010, Nei 452008 × Nei 452017, Nei 452008 × Nei 452019, Nei 452008 × Nei 452026, Nei 452008 × Nei 452029, Nei 452008 × Nei 452030, Nei 452010 × Nei 452017, Nei 452010 × Nei 452019, Nei 452010 × Nei 452026, Nei 452010 × Nei 452029, Nei 452010 × Nei 452030, Nei 452017 × Nei 452019, Nei 452017 × Nei 452026, Nei 452017 × Nei 452029, Nei 452017 × Nei 452030, Nei 452019 × Nei 452029, Nei 452019 × Nei 452030, Nei 452019 × Nei 452031, Nei 452026 × Nei 452029, Nei 452026 × Nei 452030, Nei 452029 × Nei 452030, Nei 452029 × Nei 452031, Nei 452030 × Nei 452031	7.00-7.50	S
CP-DK 888	7.25	S
Big 919	6.75	IR
NK 48	6.75	IR
NS 2	7.5	S
NS 72	7.25	S
Mean	7.07	
F-test	ns[3]	
CV (%)	8.86	

1. Leaf-feeding damage scored on a 1-9 damage rating scale (1 = no damage; 9 = extreme damage).
2. Resistance classification based on method developed by Guthrie *et al.* (1960). IR = Intermediate resistance; S = Susceptible.
3. ns = Nonsignificant.

respectively. However, by the ACB resistance evaluation method developed by Guthrie *et al*. (1960), seven inbred lines were identified as having an intermediate level of resistance to ACB while the rest were susceptible. Similarly, 10 promising hybrids and 2 commercial hybrids were identified as having intermediate resistance while the rest were susceptible. All of the hybrids that had an intermediate response to ACB had a common parent, Nei 412019 (6.36), which seems to imply that this parent was the source of resistance (Table 3).

Screening for maize weevil resistance

There were no statistically significant differences among inbreds (Table 4) and hybrids (Table 5) for the percentage of grain damage and grain weight loss. The average percentage of grain damage among inbreds and hybrids was 40.53% and 62.52% respectively. The average percentage of grain weight loss was 6.28% and 12.86% for inbreds and hybrids respectively (Horber1987). As no maize weevil-resistant variety was used in the experiment, resistance was identified on the basis of levels of resistance reported in Bergvinson *et al*. (2002) and Bergvinson (2003). Using these indices, nine inbred lines were identified as resistant and one as susceptible. Sixteen promising hybrids and one commercial hybrid were identified as resistant to maize weevil, 16 promising hybrids and 4 commercial hybrids as moderately resistant, while 13 promising hybrids were susceptible. A group of promising hybrids which had the parent Nei 452008 in common had less damage and percentage of grain weight loss than others (Table 6).

Table 3. Leaf-feeding damage rating on a group of common-parent hybrids caused by ACB under greenhouse conditions at the Nakhon Sawan Field Crops Reseach Center, Thailand.

Single-cross hybrids \	Average leaf-feeding damage rating[1]
Nei 412019 × 9 inbreds	6.36
Nei 452004 × 9 inbreds	6.92
Nei 452031 × 9 inbreds	6.92
Nei 452008 × 9 inbreds	7.0
Nei 452026 × 9 inbreds	7.0
Nei 452017 × 9 inbreds	7.03
Nei 452019 × 9 inbreds	7.08
Nei 452029 × 9 inbreds	7.14
Nei 452010 × 9 inbreds	7.22
Nei 452030 × 9 inbreds	7.22

1. Leaf-feeding damage scored on a 1-9 damage rating scale (1 = no damage; 9 = extreme damage).

Conclusions

The results of the two experiments identified seven inbred lines and 12 hybrids that had moderate but promising resistance to ACB. Similarly, 9 inbreds and 17 hybrids were identified as having promising resistance to MW. The inbred lines Nei 412019 and Nei 452008 have the greaest potential of being good inbred lines for developing insect-resistant hybrids. These superior maize genotypes will be considered for further use in maize breeding projects for insect resistance.

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Table 4. Percentage of grain damage and weight loss caused by maize weevil on inbred lines after 90 days of storage at the Nakhon Sawan Field Crops Research Center, Thailand.

Inbred line	% grain damage[1]	% grain weight loss	Level of resistance[2]
Nei 452031	20.47	2.65	R
Nei 412019	22.02	2.66	R
Nei 452010	33.79	4.25	R
Nei 452008	37.44	5.13	R
Nei 452004	37.11	5.28	R
Nei 452029	35.28	5.33	R
Nei 452026	38.85	5.56	R
Nei 452017	48.94	7.82	R
Nei 452030	57.11	8.05	R
Nei 452019	74.34	16.08	S
Mean	40.53	6.28	
F-test	ns[3]	ns	
CV (%)	38.23	88.50	

1. Data were analyzed by transformation to arc sine and shown in the table as back-transformed data. CV (%) was calculated by transformed data.
2. Level of resistance based on percentage of grain weight loss (R = Resistant; MR = Moderately resistant; S = Susceptible) as per Bergvinson (2003).
3. ns = Nonsignificant.

Table 5. Percentage of grain damage and grain weight loss caused by maize weevil on hybrids after 90 days of storage, Nakhon Sawan Field Crops Research Center, Thailand.

Inbred line	% grain damage[1]	% grain weight loss	Level of resistance[2]
Nei 452008 × Nei 452031, Nei 452008 × Nei 452010, Nei 452026 × Nei 452029, Nei 452004 × Nei 452019, Nei 452030 × Nei 452031, Nei 452008 × Nei 452030, Nei 412019 × Nei 452008, Nei 452008 × Nei 452029, Nei 412019 × Nei 452029, Nei 412019 × Nei 452017, Nei 452004 × Nei 452030, Nei 452008 × Nei 452017, Nei 452004 × Nei 452026, Nei 412019 × Nei 452026, Nei 412019 × Nei 452019, Nei 452004 × Nei 452008	27.12-73.92	4.04-9.67	R
Nei 452004 × Nei 452008, Nei 452019 × Nei 452030, Nei 452026 × Nei 452031, Nei 452017 × Nei 452031, Nei 452004 × Nei 452010, Nei 452010 × Nei 452030, Nei 452004 × Nei 452031, Nei 412019 × Nei 452031, Nei 452019 × Nei 452026, Nei 452008 × Nei 452019, Nei 452029 × Nei 452030, Nei 452008 × Nei 452026, Nei 452017 × Nei 452030, Nei 452017 × Nei 452029, Nei 452019 × Nei 452029, Nei 452010 × Nei 452017, Nei 412019 × Nei 452004	47.21-83.21	10.48-14.20	MR
Nei 412019 × Nei 452010, Nei 452017 × Nei 452019, Nei 452004 × Nei 452029, Nei 452010 × Nei 452029, Nei 452004 × Nei 452017, Nei 452010 × Nei 452026, Nei 412019 × Nei 452030, Nei 452019 × Nei 452031, Nei 452026 × Nei 452030, Nei 452017 × Nei 452026, Nei 452029 × Nei 452031, Nei 452010 × Nei 452031, Nei 452010 × Nei 452019	52.78- 86.90	17.33-27.84	S
CP-DK 888	64.51	10.71	MR
Big 919	59.11	12.00	MR
NK 48	50.61	6.63	R
NS 2	56.85	11.48	MR
NS 72	76.56	15.93	MR
Mean	62.52	12.86	
F-test	ns[3]	ns	
CV (%)	29.42	33.88	

1. Data were analyzed by transformation to arc sine and shown in the table as back transformed data. CV (%) was calculated by transformed data.
2. Level of resistance based on percentage of grain weight loss (R = Resistant; MR = Moderately resistant; S = Susceptible) as per Bergvinson (2003).
3. ns = Nonsignificant.

Table 6. Range and means of percentage of grain damage and grain weight loss caused by maize weevil on group of hybrids with one common parent and evaluated after 90 days of storage.

Hybrids	% grain damage		% grain weight loss	
	Range	Mean	Range	Mean
Nei 452008 × (9 inbreds)	27.12 – 62.96	47.05	4.04 – 12.29	8.49
Nei 412019 × (9 inbreds)	35.04 – 83.21	57.29	8.11 – 20.37	11.93
Nei 452004 × (9 inbreds)	52.48 – 83.21	64.85	7.20 – 18.88	12.05
Nei 452030 × (9 inbreds)	40.27 – 83.84	60.57	7.97 – 20.77	12.43
Nei 452029 × (9 inbreds)	35.04 – 81.95	65.80	7.04 – 22.17	13.40
Nei 452026 × (9 inbreds)	48.29 – 83.84	64.32	7.04 – 20.79	13.53
Nei 452031 × (9 inbreds)	27.12 – 86.90	65.24	4.04 – 22.87	13.66
Nei 452017 × (9 inbreds)	52.50 – 81.95	67.03	8.37 – 20.79	13.83
Nei 452019 × (9 inbreds)	47.21 – 81.90	63.26	7.20 – 27.84	14.51
Nei 452010 × (9 inbreds)	31.58 – 86.90	70.92	5.25 – 27.84	16.45

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Reduction of *Chilo partellus* Egg Load through Intercroping in Maize

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Abstract. Studies on the ovipositional behavior of Chilo partellus (Swinhoe) in maize agroecosystems were conducted during the kharif (rainy season) of 2006 in the entomology unit of the Directorate of Maize Research, Indian Agricultural Research Institute (IARI), New Delhi, India. In order to determine the influence of intercropping on oviposition, five crops – groundnut, cowpea, soybean, green gram and black gram – were intercropped with maize hybrid Bio-9681. The number of eggs laid per maize plant was lowest when maize was intercropped with cowpea, and highest when intercropped with soybean followed by black gram, groundnut and green gram. Conversely, the average number of eggs per plant on cowpea was highest while soybean plants did not attract females for oviposition. Likewise, the least number of egg patches was observed on maize plants in maize-cowpea intercropping and the most in maize-soybean intercropping. On the other hand, the maximum number of egg patches per plant were observed on cowpea intercropped with maize followed by groundnut, green gram and black gram. The plant height of the intercrop influenced the oviposition of C. partellus moths: there were significant differences in the number of eggs laid on maize at different heights. The tallest plants of cowpea attracted C. partellus moths to lay the maximum number of eggs per plant but no such relation was observed in the other crops. Chilo partellus egg load was considerably reduced on maize when it was intercropped with cowpea.

Key words: Maize, Chilo partellus, oviposition, intercrops

Introduction

Maize (*Zea mays* L.) is attacked by a number of insect pests in India. Mathur (1987) observed that over 250 insect species are associated with maize in field and storage conditions. Of these, 74 have only appeared recently and about a dozen are of potential economic importance (Mathur 1992). Lepidopteran pests, particularly stem borers, are major constraints to productivity and are of economic importance in most maize-growing countries around the world. Of these, the maize stem borer, *Chilo partellus* (Swinhoe), is an important pest. Yield losses of 24.3-36.3% have been estimated due to it (Chatterji *et al*. 1969). It has been shown that the feeding, growth and development of *C. partellus* larvae depend on both the physical and chemical characteristics of the host plant (Khan *et al.* 2006; van den Berg 2006) as is reflected by their performance on resistant cultivars of maize compared to susceptible ones (Dass and Aggarwal 1993). Different control measures have been recommended against this pest. Cultural practices like intercropping, balanced fertilization, appropriate plant population density and use of least susceptible varieties have been effective in its management. Insects have selective preferences of the host plant, plant age, site and time of oviposition to give their progeny a better chance of survival. Eggs and neonate larvae remain exposed to natural enemies and hence are vulnerable during these stages. It is, therefore, easy to manage the population of this pest at this stage by use of biological and chemical pesticides. Thus information on the ovipositional preferences of *C. partellus* can be used in the management of this pest in the egg stage.

Materials and Methods

Chilo partellus larvae were collected by splitting infested maize stalks obtained from the field. They were reared on cut pieces of maize stems (7.5 cm long) till pupation. Pupae thus collected were kept in glass rearing jars (10 cm × 15 cm). Upon emergence, the moths (1 Male:1 Female) were transferred to oviposition jars kept at $27^{o}\pm2^{o}$C as per the method described by Siddiqui *et al. (*1977). Egg-laying was examined on alternate days and portions of butter paper containing the egg mass were cut and incubated at $27^{o}\pm2^{o}$C. Live moths were again transferred to fresh egg-laying jars and kept in the BOD for further egg-laying. The eggs thus obtained served as a nucleus for the mass rearing of *C. partellus* on an artificial diet in the laboratory. To study the ovipositional behavior of C. *partellus* on maize and its intercrops, maize hybrid Bio-9681 along with five intercrops (groundnut, cowpea, soybean, green gram and black gram) were sown in a net house covered with a nylon mesh during the *kharif* (rainy season) of 2006. Fifteen days after sowing (DAS), 21 pairs of laboratory-reared, one-day-old *C. partellus* moths were released into the net house in the morning (0900 h) and allowed to oviposit for 5 days. At the end of the 5th day, each plant in the plot was examined very carefully for the presence of egg patches. The number of egg patches was recorded and the eggs in each patch were counted. The average number of egg patches and eggs per plant in each treatment was recorded. In each treatment five plants from the intercrops were randomly selected and their height was measured and the mean height was calculated for each treatment. The ovipositional preference of *C. partellus* was compared among the five treatments.

The data were averaged for each treatment and subjected to one-way analysis of variance (ANOVA) using AGRES software. Data pertaining to egg patches and number of eggs per plant were subjected to square-root transformation after adding 0.5 to each figure (LeClerg *et al.* 1962). Such data were then subjected to analysis of variance.

Results and Discussion

Data were recorded for the number of egg patches and eggs laid per plant on the maize sole crop and on the intercrops, which were taken as treatments. Data on the number of eggs laid by *C. partellus* per maize plant in the intercrop ecosystem are presented in Table 1. It is evident from the data that the intercrops had a significant influence on the ovipositional preference of *C. partellus* on maize plants. The number of eggs laid per maize plant was least (0.54) when maize was intercropped with cowpea and highest (3.56) when intercropped with soybean, followed by black gram (3.55), groundnut (3.14) and green gram (2.27). The interactions of different intercrops with maize cultivar Bio-9681 were significant. Maize intercropping with cowpea or green gram was found significantly superior to the other three intercropping systems. The results presented in Figure 1 clearly bring out the egg-laying behavior on maize plants when intercropped with different crops. The least number of eggs per plant was recorded when maize was intercropped with cowpea followed by green gram. There was no significant advantage when maize was intercropped with soybean, black gram or groundnut.

Data on the number of eggs per plant on different intercrops presented in Table 2 show the effect of different intercrops on the ovipositional preference of *C. partellus*. Cowpea was the most preferred crop for oviposition among

Table 1. Number of *C. partellus* eggs laid per plant on maize (hybrid Bio-9681) in different intercropping systems.

Maize intercrop	R[1] I	R II	R III	R IV	Tr. mean[2]
Groundnut	3.61	1.71	4.41	2.82	3.14
	(2.03)[3]	(1.49)	(2.22)	(1.82)	(1.91)
Cowpea	2.15	0.00	0.00	0.00	0.54
	(1.63)	(0.71)	(0.71)	(0.71)	(1.02)
Soybean	5.00	3.53	2.64	3.07	3.56
	(2.35)	(2.01)	(1.77)	(1.89)	(2.01)
Green gram	3.80	2.26	3.00	0.00	2.27
	(2.07)	(1.66)	(1.87)	(0.71)	(1.66)
Black gram	3.43	6.53	1.00	3.22	3.55
	(1.98)	(2.65)	(1.22)	(1.93)	(2.01)
F-test					Sig.
SEM±					0.18
CD at 5%					0.66
CD at 1%					0.93

1. R = Replications
2. Tr. mean = Treatment mean.
3. Figures in parentheses are square root-transformed values after adding 0.5 to each value.

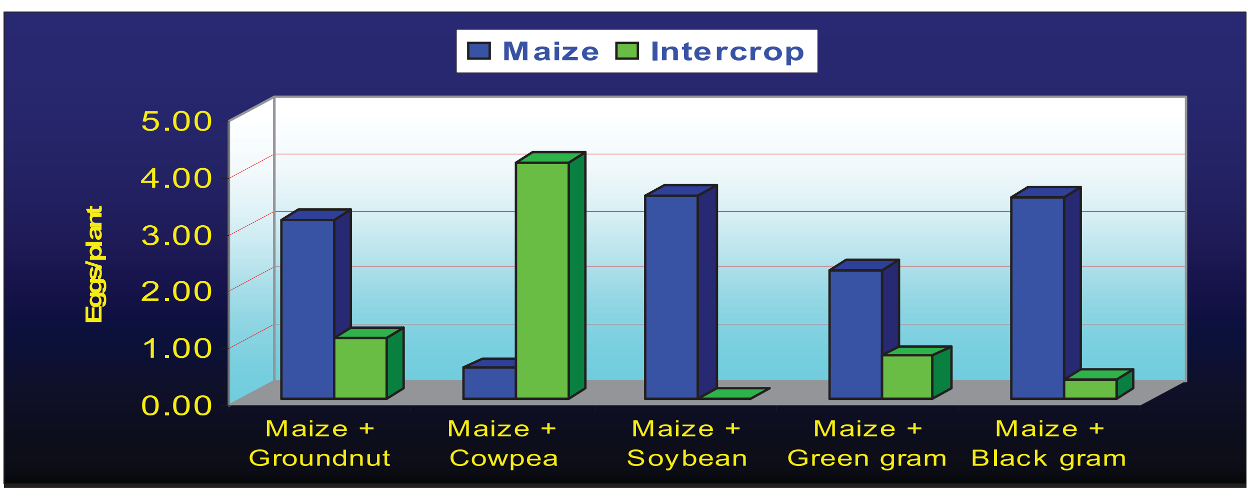

Figure 1. Number of eggs laid per plant by *Chilo partellus* on maize (hybrid Bio-9681) and on intercrops.

the five intercrops. The average number of eggs per plant on cowpea was 4.15 while in contrast soybean plants did not attract *C. partellus* females for oviposition. The other experimental intercrops, ie, groundnut, green gram and black gram, received 1.06, 0.74 and 0.33 eggs per plant respectively.

Data presented in Table 3 reveal a similar trend in the case of the number of egg patches per plant laid on maize when intercropped with different crops. The least number of egg patches per maize plant (0.02) was observed in the maize-cowpea intercrop and the most (0.17) in the maize-soybean intercrop. The number of egg patches per maize plant in both maize-groundnut and maize-black gram intercropping were 0.13. The maize-green gram intercropping had relatively fewer egg patches per plant, ie, 0.08. Maize-cowpea intercropping showed a positive effect in reducing the number of egg patches laid by *C. partellus* on maize and differed significantly from all the other treatments (Fig. 2).

Data on the number of egg patches per plant laid on the crops that were intercropped with maize showed the ovipositional preference of *C. partellus* (Table 4). The maximum number of egg patches per plant were observed on cowpea (0.16) followed by groundnut (0.06), green gram (0.03) and black gram (0.01). Soybean plants were not preferred for oviposition by *C. partellus.* The number

Table 2. Number of eggs laid per plant by *Chilo partellus* when maize is intercropped with different crops.

Treatment	R[1] I	R II	R III	R IV	Tr. mean[2]
Groundnut	2.37	0.00	0.00	1.89	1.06
	(1.69)[3]	(0.71)	(0.71)	(1.55)	(1.25)
Cowpea	4.75	2.78	5.40	3.68	4.15
	(2.29)	(1.81)	(2.43)	(2.04)	(2.16)
Soybean	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
Green gram	0.00	1.13	0.00	1.85	0.74
	(0.71)	(1.27)	(0.71)	(1.53)	(1.12)
Black gram	1.31	0.00	0.00	0.00	0.33
	(1.35)	(0.71)	(0.71)	(0.71)	(0.91)
F test					Sig.
SEM±					0.13
CD at 5%					0.54
CD at 1%					0.76

1. R = Replication.
2. Tr. mean = Treatment mean.
3. Figures in parentheses are square root transformed values after adding 0.5 to each value.

Table 3. Number of egg patches per plant laid on maize by *C. partellus* when intercropped with different crops.

Treatment	R[1] I	R II	R III	R IV	Tr. mean[2]
Groundnut	0.15	0.07	0.18	0.12	0.13
	(0.81)[3]	(0.76)	(0.82)	(0.79)	(0.79)
Cowpea	0.08	0.00	0.00	0.00	0.02
	(0.76)	(0.71)	(0.71)	(0.71)	(0.72)
Soybean	0.20	0.20	0.14	0.13	0.17
	(0.84)	(0.84)	(0.80)	(0.80)	(0.82)
Green gram	0.13	0.07	0.13	0.00	0.08
	(0.80)	(0.75)	(0.80)	(0.71)	(0.76)
Black gram	0.13	0.23	0.08	0.09	0.13
	(0.79)	(0.85)	(0.76)	(0.77)	(0.80)
F-test					Sig
SEM±					0.01
CD at 5%					0.04
CD at 1%					0.06

1. R = Replication.
2. Tr. mean = Treatment mean.
3. Figures in parentheses are square-root transformed values after adding 0.5 to each value.

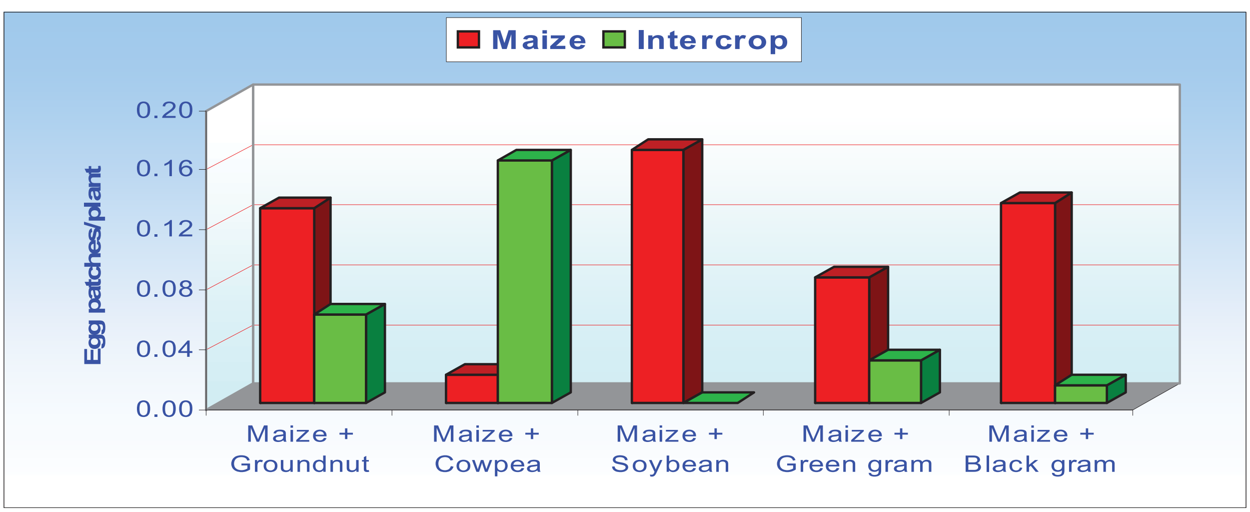

Figure 2. Number of egg patches laid per plant by *C. partellus* on maize and on intercrops.

varied significantly with the intercrop, ranging from 0.00 per plant in soybean to 0.16 in cowpea.

Among the five crops intercropped with maize, their heights at 15 DAS significantly differed from each other except soybean and black gram. The maximum height of 33.50 cm was for cowpea followed by 30.90 cm, 28.38 cm, 28.08 cm, 19.40 cm for green gram, black gram, soybean and groundnut respectively (Table 5; Fig. 3). Plant heights of the intercrops had a direct influence on the oviposition of *C. partellus* moths in terms of significant differences in the number of eggs laid on intercrops at different heights (Fig. 3). The tallest crop, cowpea, received the highest number of eggs per plant (Table 2) but no correlation was noticed when observed in other intercrops. Though taller crops attracted more moths for oviposition, this was not the case for soybean.

Table 4. Number of egg patches laid per plant by *C. partellus* on different crops when intercropped with maize.

Treatment	R[1] I	R II	R III	R IV	Tr. mean[2]
Groundnut	0.13	0.00	0.00	0.11	0.06
	(0.79)[3]	(0.71)	(0.71)	(0.78)	(0.75)
Cowpea	0.25	0.07	0.20	0.13	0.16
	(0.87)	(0.76)	(0.84)	(0.79)	(0.81)
Soybean	0.00	0.00	0.00	0.00	0.00
	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
Green gram	0.00	0.06	0.00	0.05	0.03
	(0.71)	(0.75)	(0.71)	(0.74)	(0.73)
Black gram	0.05	0.00	0.00	0.00	0.01
	(0.74)	(0.71)	(0.71)	(0.71)	(0.72)
F-test					Sig.
SEM±					0.01
CD at 5%					0.04
CD at 1%					0.06

1. R = Replication.
2. Tr. Mean = Treatment mean.
3. Figures in parentheses are square-root transformed values after adding 0.5 to each value.

Our study was aimed at identifying suitable crops that could be intercropped with maize to reduce the *C. partellus* egg load on the maize crop. Intercrops influence the oviposition in many ways: they compete for oviposition with the main crop; they mask the main crop, thus limiting the access of females. The search for a suitable host also might require excessive energy and time, which might affect the rate of reproduction. Furthermore, expenditure of energy may increase mortality among the population, thus reducing the adult numbers that reach the intercropped host plant compared with host plants grown in monoculture.

The importance of intercropping as a method for controling stem borers in sorghum and maize has been reported (Amoako-Atta and Omolo 1983; Ampong-Nyarko *et al.* 1994; Skovgaard *et al.* (1996); Ayisi *et al.* 2001;

Table 5. Mean plant height (cm) of different intercrops when sown with maize.

Treatment	R[1] I	R II	R III	R IV	Tr. mean[2]
Groundnut	18.80	19.70	19.20	19.90	19.40
Cowpea	34.80	32.30	33.40	33.50	33.50
Soybean	28.00	28.10	29.00	27.20	28.08
Green gram	31.60	31.20	30.10	30.70	30.90
Black gram	28.70	27.70	28.50	28.60	28.38
F-test					Sig.
SEM±					0.36
CD at 5%					1.13
CD at 1%					1.59

1. R = Replication.
2. Tr. mean = Treatment mean.

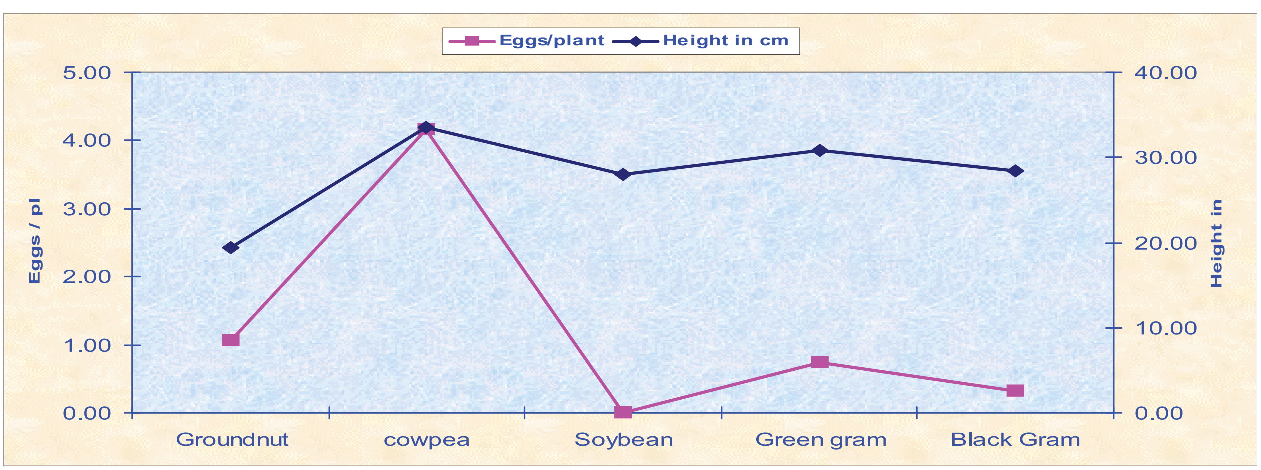

Figure 3. Effect of the height of intercrops on the oviposition preference of *C. partellus*.

Ndemah *et al.* 2003). Among the five crops intercropped with maize for this study, cowpea was preferred most by *C. partellus* for oviposition, as was evident from the number of eggs laid per cowpea plant (4.15). The females exhausted their eggs on cowpea, and, consequently, the egg load on maize plants was reduced substantially (0.54 eggs per plant). This finding is in variance with Paets *et al.* (1997), who studied the influence of intercropping on the abundance, distribution and parasitism of *C. partellus* and *C. orichalcociliellus* in maize. They did not observe a significant difference in the number of egg batches per plant between the treatments, and batches were randomly distributed on both cowpea and maize plants. Maluleke *et al.* (2005) studied *C. partellus* incidence in sole-culture maize and in maize/lablab intercropping. They observed that stem borer infestation was more severe in sole-culture maize than maize in the maize/lablab intercrop. Kwesi *et al.* (1994) also recorded a reduction in *C. partellus* incidence in sorghum when intercropped with cowpea. Jiang e*t al.* (2006), while studying the effect of mixed-cropping systems containing maize, sorghum, millet and beans on infestation of stem borers, found that systems containing the nonhost bean were more efficient in reducing pest densities than those with millet or sorghum only. In our study, intercropping reduced the oviposition load of *C. partellus* to varying degrees on maize.

Heavy egg-laying on cowpea may be primarily because of the preference of *C. partellus* for cowpea, but the height of cowpea plants may also be responsible for masking the maize plant, thus compelling the females to unload their eggs on cowpea. The pest also laid some eggs on groundnut (1.06 eggs per plant). Soybean, green gram and black gram may not be suitable crops to be intercropped with maize.

Cowpea can be gainfully intercropped with maize as the heavy egg-laying on it, ie, a nonhost crop, reduces the chances of neonate larvae emerging from egg patches to reach the maize plant. These research findings may be useful to both farmers and scientists as a way of reducing damage from *C. partellus*.

Acknowledgement

We thank Dr. Meenu Agarwal for rendering technical help for this study.

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Insect Resistance Management: the Philippines Experience

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Abstract. Bt corn is cultivated over about 300 000 ha in the Philippines, which ranks 13th among countries cultivating genetically modified (GM) crops over 50 000 ha or more. The Philippines was the first country in Asia to formulate biosafety rules and regulations on GM crops. Insect Resistance Management (IRM) is a major component of the measures prescribed by the Philippines government governing the cultivation of GM crops such as Bt corn. In this paper, we present an outline of the cooperative endeavors relating to Bt corn taken up by technology developers (Monsanto, Pioneer, Syngenta and academic institutions), regulators, farmers and scientists. So far, there has been no observed development of resistance by from Asian corn borer to Bt corn. The country's IRM strategy utilizes the services of the IRM Technical Advisory Team of the Department of Agriculture of the Government of the Philippines. This strategy has been incorporated into the requirements stipulated for the continued commercialization of Bt corn (MON 810) by Monsanto. Soon, RR soybean, Bt cotton, PRSV-resistant papaya, vitamin A-fortified rice and Bt eggplant will be commercially available in the country.

Key words: Bt corn, Insect Resistance Management, Philippines, Asian corn borer

AO: Administrative Order, DA: Department of Agriculture, DENR: Department of Environment and Natural Resources, DOH: Department of Health, DOST: Department of Science and Technology, EO: Executive Order, Memorandum Circular, SO: Special Order

Introduction

The Philippines is ranked 13th among countries cultivating GM crops over 50 000 ha or more (James 2007). Bt corn is grown over more than 300 000 ha in the country. The Philippines was the first country in Asia to formulate biosafety rules and regulations for GM crops in which Insect Resistance Management is a major component. Insect Resistance Management (IRM) refers to the deployment of a combination of strategies designed to reduce the risk of the target insect (in this case Asian corn borer) developing resistance to the product (Bt corn). A refuge-plus-high-dosage strategy is recommended for IRM of most varieties of transgenic insecticide corn (Andow *et al.* 2000; Andow and Hutchinson 1998; Alstad and Andow 1996). This paper is aimed at documenting and sharing the Philippines experience in IRM.

Biosafety Regulations on GMOs

In 1987, an ad hoc committee on biosafety was set up drawing participation from the University of the Philippines Los Baños, the International Rice Research Institute (IRRI) and the Department of Agriculture. The committee recommended:

- creation of a National Committee on Biosafety
- formulation of national policies and guidelines on biosafety

The National Committee on Biosafety of the Philippines was created in 1990 by an executive order (430 S of 1990). It is an interagency body (with the participation of the Department of Health, the Department of Science and Technology, the Department of Agriculture and the Department of Environment and Natural Resources) with four scientists and two community representatives. The committee is replicated at various institutions such as the University of the Philippines Los Baños through Institutional Biosafety Committees. This committee

- issues guidelines (for experimental use);
- conducts risk assessments with the Scientific and Technical Review Committee (STRP); and
- evaluates risks related to Potentially Harmful Exotic Species (PHES).

In 2002, the Department of Agriculture by way of an administrative order (8 S of 2002) laid down guidelines for the import and use (except contained use) of plants and plant products derived from the use of modern biotechnology. Since 2003, the GM corn program under Dr. Artemio M. Salazar has actively advocated Insect Resistance Management (IRM) of Bt corn in the Philippines. Under this stimulus, the Department of Agriculture (DA), through a memorandum circular (MC 17 S of 2003) adopted additional requirements for Insect Resistance Management (IRM) for Bt corn and set compliance standards for the industry. The DA's Bureau of Plant Industry (BPI) monitors industry's compliance with IRM norms and reviews and revalidates the existing IRM system to ensure that it addresses the needs of resistance management. The DA (memorandum circular O8 S of 2005) further strengthened the science-based IRM norms for Bt corn and amended Sec. III a (ii) of MC 17 S 2003 to create an IRM Technical Advisory Team. Further, the DA's special order (7 S of 2006) set up an Insect Resistance Management Advisory Team (IRMAT) that will provide scientific guidance and advice related to IRM in the Philippines.

Data collection

Facts and figures related to IRM for this paper have been taken from the actual experiences of the authors regarding the conceptualization and implementation of IRM in the Philippines. Data on various statutes related to IRM were taken from the DA's archives and/or records. Field data were gathered by the authors and staff of the Crop Protection Division, the Bureau of Plant Industry and the Biotech Core Team.

Adoption of Bt corn

In 2003, Bt corn adoption in the Philippines was a mere 10 776 ha. This grew to 58 293 ha in 2005, 96 710 ha in 2006 and 122 608 ha in 2007. The island of Luzon has the highest adoption rate of Bt corn followed by Mindanao and Visayas. The acreage dipped in 2008 as the result of a lack of available feeds and due to an increase in the planting of rice arising from fears of a rice shortage that the country had experienced. In 2006, two more traits were introduced (Bt and Roundup Ready). On the whole, adoption of GM corn has been increasing in the Philippines.

IRM strategy

1. The Philippines' IRM strategy for Bt corn stipulates a high dosage to ensure 99% or more mortality of the Asian corn borer and the use of refuge. It also requires monitoring of resistance development in coordination with the DA's IRM Program Management Team. This is done by establishing permanent sites for ACB monitoring in five corn-producing regions with a high or increasing rate of adoption of Bt corn, areas with regular occurrence of ACB and contiguous areas planted to corn for five years starting 2006. (Fig.1)

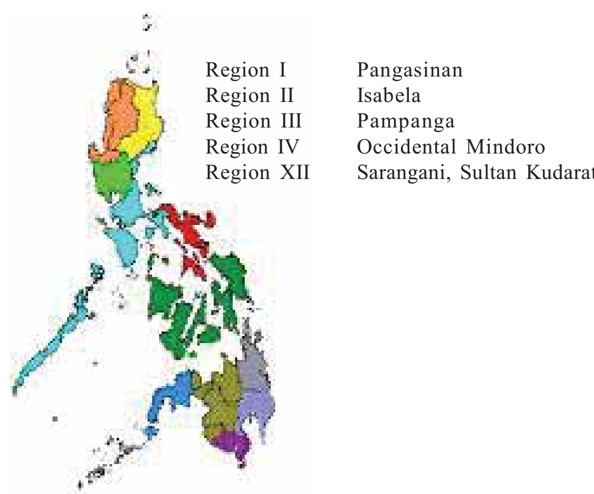

Figure 1. Monitoring of Asian corn borer resistance to Bt corn in the Philippines.

Table 1. Acreage (ha) of genetically modified corn planted in the Philippines*.

	TRAITS										
			Bt				Stacked (Bt + RR)			RR	
Island	2004	2005	2006	2007	2008	2006	2007	2008	2006	2007	2008
Luzon	48,516	43,735	85,612	103,438	18,473	3,879	59,346	13,180	11,685	54,509	1,909
Visayas	534	445	405	2,546	79	232	2,472	0	4,424	8,925	0
Mindanao	10,706	10,693	10,693	16,624	26,499	469	9,461	1,653	10,384	56,589	167,589
Grand Total	59,756	5,829	96,710	122,608	45,051	4,580	71,279	14,833	26,493	120,023	18,667

*2003: 10 776 ha Bt corn.

2. The IRM strategy also monitors industry compliance with biosafety norms and provides support to related research studies and capability-building efforts by the participating agencies. Compliance is ensured by submission of Bt adoption reports at the municipal level every two months; submission of reports relating to IRM awareness training programs, and submission of reports on industry's implementation of the enhanced IRM plan.
3. Another part of the IRM strategy is to educate all stakeholders in collaboration with the DA, the University of the Philippines Los Baños, the DA's Biotechnology Advisory Team, BPI, NGOs (Biotech Coalition of the Philippines) and the technology developers (Monsanto, Syngenta, Pioneer). This involves organizing IRM awareness training programs for farmers and seminars on IRM for DA/RCPC personnel involved in IRM
4. The strategy also includes mitigating measures such as planting 80% Bt corn and 20% non-Bt corn and its isolines over 200 ha of contiguous area and placing a cap on sales in areas with 95% adoption rate.
5. Another part of the IRM strategy is to hold regular reviews of the IRM strategy by IRMAT and other stakeholders.

Industry's enhanced IRM plan

1. Monitoring adoption by determining the physical areas in each municipality
2. Focused ACB resistance monitoring activities in Bt corn-growing areas which may possibly reach the contiguous 200 ha zone due to continuous corn cropping.
3. Increasing the frequency of reporting Bt adoption from seasonally to bimonthly.
4. Deploying other Bt genes with a different mode of action
5. Mitigating measure such as deployment of nonBt corn for about 1 month following Bt corn adoption of 80% for two consecutive seasons
6. Educating distributors, financiers, dealers and farmers on IRM:
    - Establishment of linkages with agricultural training institutes and local government units to disseminate information on IRM to farmers
    - Updating IRM modules as new information becomes available

Research and development activities taken up

Crop life/Technology developers

1. Baseline population susceptibility
2. Alternate-host and biology of ACB and its alternate-host preferences
3. IPM relating to ACB, predator population and ACB damage on Bt and isohybrids
4. Insect biodiversity for flying insects
5. Developmental period, mating period and ovipositional preference of ACB in some yellow corn varieties

PBS-BBI-USAID-supported projects

1. Investigation of secondary ecological effects of Bt corn in the Philippines. Alcantara *et al.* (2004-2009), Institute of Biotechnology, University of the Philippines Los Baños.
2. Post-commercialization monitoring of Asian corn borer (*Ostrinia furnacalis* Guenee) resistance to Bt corn in the Philippines and the impact of pollen dispersal on nontarget Lepidoptera. Cayabyab *et al.* (2006-2009) NCPC-CPC, University of the Philippines Los Baños.
3. Ecosystems approach in the assessment of the impacts of herbicide-tolerant corn on wild biodiversity in selected areas in Luzon, the Philippines. C.I.Villamor (2005-2009).

The concerns that need to be addressed are: (1) farmers' use of F_2 seeds of RR and stacked corn; (2) need for an accurate method of estimating adoption rates; (3) farmers' nonacceptance of 80-20 'bag-in-a-bag' innovation; (4) continuing IRM education of farmers; and (5) need for sensitive methods of detecting resistance.

Conclusion

Commercial use of Bt corn in the Philippines is increasing. An Insect Resistance Management (IRM) strategy is needed to prolong the benefits of this technology. Current concerns related to IRM need immediate attention. The cooperation of all stakeholders is a prerequisite for successful IRM in the Philippines.

Acknowledgement

We acknowledge the sponsorship of the Department of Agriculture, the Indonesian Agency for Agricultural Research and Development (IAARD), the Government of South Sulawesi, the International Maize and Wheat Improvement Center (CIMMYT) and the Indonesian Cereals Research Institute (ICERI). The support of the University of the Philippines Los Baños and PBS-BBI-USAID is also gratefully acknowledged. We are also thankful to Ms. Alice G. Aquino for the layout and preparation of this paper.

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Effect of Phenolic Acid of QPM Maize Lines on Resistance to Maize Weevil

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Abstract. The objective of this research was to measure the effect of the phenolic acid content of nine Quality Protein Maize (QPM) lines on resistance to the maize weevil (Sitophilus zeamais). The QPM lines tested were S99TL YQ-AB, S99TL YQ GH-AB, S99TL YQ-A, Tomeguin 8565, S99TL WQ-A, S99TL WQ-AB, S99TL WQ (F/D), ACROSS 8762 and POZARICA 8563. The varieties Sukmaraga and Bisma were included as resistant and susceptible controls, respectively. Chemical analysis was done on the QPM lines to measure their phenolic acid content. Grain of each QPM line were infested with the maize weevil. The results indicated that four lines [ACROSS 8762, POZARICA, S99TL WQ-AB and S99TL WQ (F/D)] which had high phenolic acid content had high levels of resistance to maize weevil.

Key words: Phenolic acid, quality protein maize, Sitophilus zeamais

Introduction

Maize production in Indonesia has grown 1.78% per year, and is projected to reach 12.1 million t in 2009 (BPS 2003). One of the strategies for maize development in Indonesia is to increase grain quality, which is dependent on both yield and postharvest handling and storage. The maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is the main storage pest of maize (Dobie *et al.* 1984). Adult weevils mate and deposit eggs in the grain and the larvae emerge to feed on the grain.

Yield losses in storage vary from country to country. In Mexico, 30% yield loss after six months of storage has been reported (Bergvinson 2002). Yield losses caused by maize weevil in Indonesia are significant. Grain damage was as high as 85% and weight loss about 17% after storage for six months in Maros, southern Indonesia (Tandiabang 1998). Such heavy losses make insect control strategies imperative. Use of resistant varieties is one such strategy. The discovery of QPM maize types with the Opaque-2 mutant gene, which changes the protein composition and increases the lysine and tryptophan content, has opened a new perspective for use of maize in human nutrition. However, the soft endosperm of QPM maize may result in huge damage and loss due to the maize weevil.

It has been reported that the chemical content and physical characteristics of maize grain influence the oviposition behavior and development of the maize weevil (Jorge 1981). Phenolic acid as a biochemical component of maize grain has been correlated with resistance to maize weevil (Serratos *et al.* 1987). The purpose of our study was to measure the phenolic acid content in nine QPM lines and relate it to maize weevil resistance.

Materials and Methods

Our study was conducted at the entomology and phytophatology laboratory of the Bogor Agriculture Institute, Indonesia during April-Nov 2003.

Mass rearing of maize weevils

Before mass rearing, the weevils have to be separated by gender. Species identification was done on the basis of the *aedeagus* and the *'Y'* shape (Fig. 1), and sex identification was based on rostral characterictics (Dobie *et al.*1984; Hidayat *et al.* 1996; Reddy *et al.* 1951) (Fig. 2).

About 250 adult maize weevils were put in a jar which contained 2 litters of maize, and kept for one week for the insects to deposit their eggs. The adults were then removed leaving the maize seeds in place for the eggs to develop into adults. Emerging adults of uniform age were used in the study.

Maize grain preparation

The grain used in this study came from 11 varieties/ lines: S99TL YQ-AB, S99TL YQ GH-AB, S99TL YQ-A, Tomegium 8565, S99TL WQ-A, S99TL WQ-AB, S00TL WQ-F/D, ACROSS 8762, POZARICA 8563, Sukmaraga and Bisma, the latter two being controls. The grain were put in

Figure 1. Species identification of *S. zeamais* by the *aedeagus (male)* and the '*Y' shape (female*).

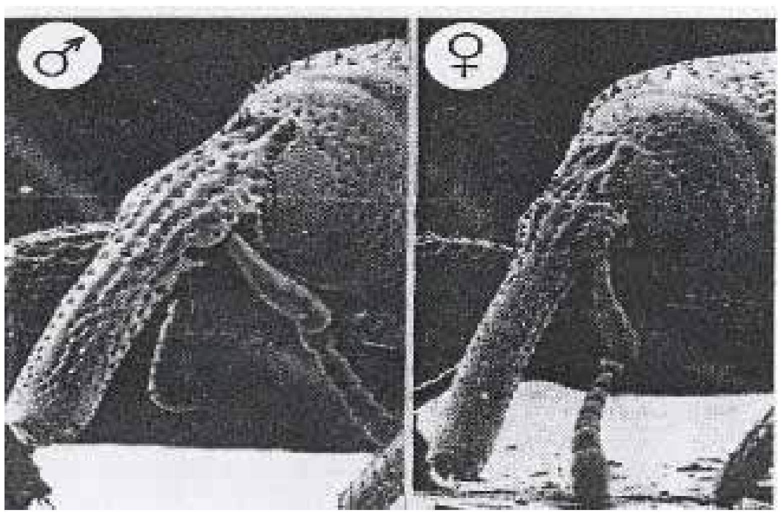

Figure 2. Rostral characteristics of male and female *S. zeamais*.

a refrigerator at -10^{o}C for 7 days to kill any weevils from the field.

Susceptibility index

Three hundred grain of each variety/line were divided into three replications, each consisting of 100 g of seed. The seeds were placed in jars. The grain in each jar were infected with 7-day-old maize weevils in the proportion of 20 females for every 5 males. The jars were put in an incubator at 30^{o}C and RH 70%. The maize weevils were allowed to feed and oviposit for 7 days, after which they were removed. The jars containing the grain were kept in the incubator at 30^{o}C and RH 70%. Emerged adults of *S. zeamais* were observed every 2 days, and removed to avoid oviposition. Observation continued until no more adults emerged. The susceptibility index (SIS) was determined according to Dobie (1977):

$$SI = \frac{\text{Ln } F \times 100}{DME}$$

where
SI : Susceptibility index
Ln : Logaritma
F : Number of F_1 adult insects emerging daily
DME : Median development period (Gudrups *et al*. 2001).

Grain damage

After all the adult weevils emerged, the grain damage was calculated as per Bergvinson (2002).

$$\text{Grain damage} = \frac{\text{Grain damaged}}{\text{Total grain (damaged + nondamaged grain)}} \times 100\%$$

Phenolic acid analysis

The phenolic acid content of the grain was analyzed by the hydrolysis method according to the method of Sosulski *et al.* (1982).

Statistical Analysis

A complete randomized design was used for this study, and data were analyzed using the IRRISTAT software. The difference between treatments was analyzed by the smallest real difference test at the 5% level.

Results and Discussion

Grain damage

The results showed that four lines, ACROSS 8762, POZARICA, S99TL WQ-AB and S99TL WQ (F/D), had low grain damage (Fig.3)

The grain damage to the QPM lines was corrected to the number of F_1 progeny and the chemical composition of the grain.

F_1 progeny

The number of F_1 progeny of the weevils recovered from each tested line varied from 6.33 recovered from ACROSS 8762 to 30.67 from S99TL WQ-A. The number of emerging F_1 weevil progeny recovered from ACROSS 8762, POZARICA, S99TL WQ-AB and S99TL WQ (F/D) was low, but was not significant for the varieties Bisma and Sukmaraga. The number of emerging F_1 weevils recovered from the lines S99TL YQ-AB, S99TL YQ-GH-AB was significantly higher than the varieties Sukmaraga, Bisma, ACROSS 8762, POZARICA, S99TL WQ-AB and S99TL WQ (F/D) (Fig. 4).

Phenolic acid

The phenolic acid content of all the varieties/lines tested (Fig. 5) ranged from 0.140 mg 100 g^{-1} for S99TL YQ-AB to 0.910 mg 100 g^{-1} for ACROSS 8762.

Susceptibility Index

Figure 6 presents the susceptibility index of the experimental varieties/lines to *S. zeamais*. The index was low for ACROSS 8762 (2.84%), S99TL WQ-F/D (3.77%), S99TL WQ AB (4.45%), POZARICA (4.40%) and high for S99TL WQ-A (5.72%), S99TL YQ-AB (5.63%), S99TL YQ-GH-AB (5.50%).

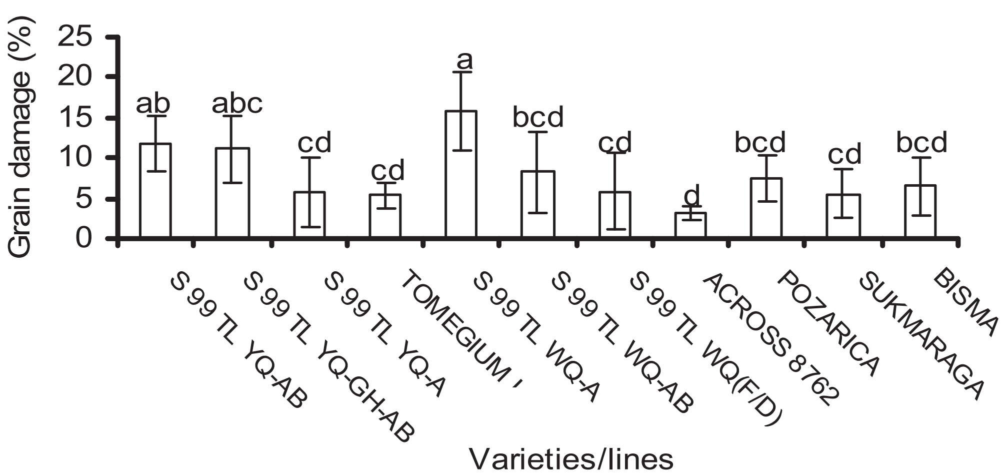

Figure 3. Average grain damage (%) suffered by 9 varieties/lines and 2 control varieties due to *Sitophilus zeamais.* Bar with the same letter was not statistically different as per the Duncan Test at the 5% level.

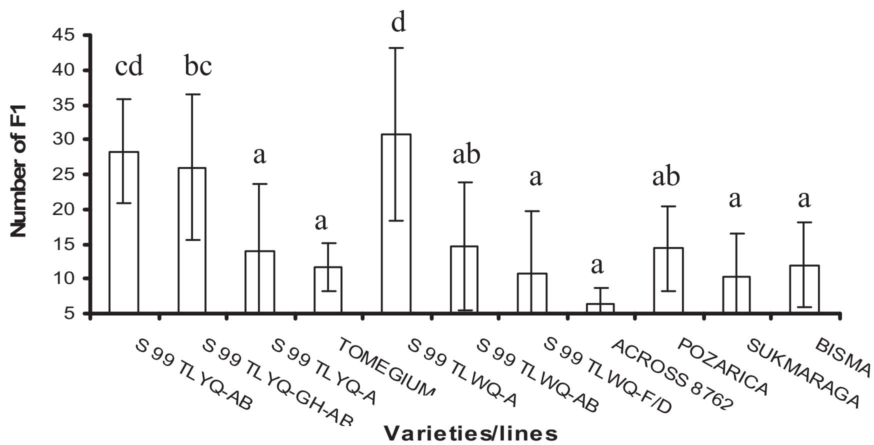

Figure 4. The average number of F_1 weevil progeny emerging from 9 QPM maize varieties/lines and 2 control varieties. Bars with similar letters were not significant as per the Duncan Test at the 5% level.

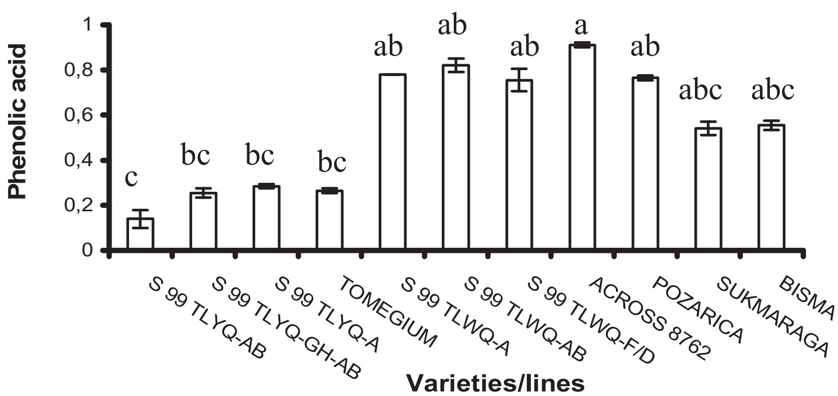

Figure 5. Average phenolic acid content of 9 varieties/lines of QPM maize and 2 control varieties. Bars with similar letters were not different absolutely based on Duncan Test at the 5% level.

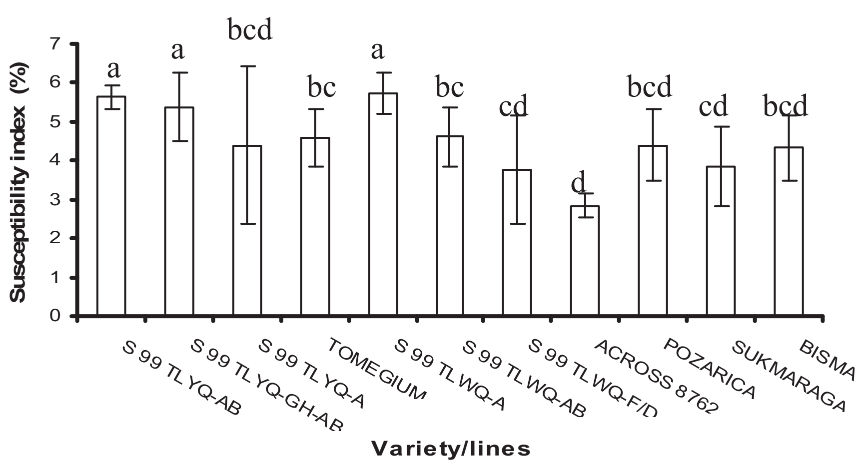

Figure 6. Susceptibility index of 9 variety/lines of QPM maize and 2 varieties of control to the maize weevil, *S. zeamais.* Bars with the same letters are not really different as per the Duncan Test at 5% level.

Discussion

The results reveal that the QPM lines had low grain damage, fewer F_1 weevil progenies, high phenolic acid content and a low susceptibility index. Grain hardness is the main factor in the beginning of maize weevil infestation (Seshagiri 1953). Hardness of the maize grain is influenced by the phenolic acid content in the seed: the higher the phenolic acid content, the harder the grain. So oviposition by female weevils was low in lines with higher phenolic acid content (Classen 1990). The harder the grain, the more difficult it is for the female weevil to penetrate the grain to oviposit eggs. Hard grain lead to lower numbers of weevil progeny and, therefore, less grain damage. The susceptibility index is a factor of the number of F_1 progeny emerging and the developing period of the maize weevil. Lines with low SI have lower numbers of emerging progeny with a longer developing period. Arnason *et al.* (1994) reported that maize genotypes resistant to maize weevil have a high concentration of diferulic acid compared to susceptible maize genotypes.

The phenolic acid compound found in the aleurm layer of the cell wall of the maize grain probably acts as an antibiotic factor which retards development and causes high mortality in the larvae (Panda and Gundev 1955; Cuthbert *et al.* 1974; Arnason *et al.* 1977).

Conclusion

Four of the nine varieties/lines of QPM maize tested showed high levels of resistance to the maize weevil. These included ACROSS 8762, POZARICA, S99TL WQ-AB and S99TL WQ (F/D). The susceptibility index of these lines was significantly different from that of the susceptible lines. The susceptibility index of the four QPM lines was in the range of 2.84-4.59%, while the susceptibility index of the susceptible lines reached 5.72%. The internal factor possessed by QPM lines which contributes to the lower susceptibility to *S. zeamais* is phenolic acid. The higher the content of phenolic acid, the more resistant the lines were to *S. zeamais* attack.

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Resistance of Corn Cultivars/Lines from Indonesia and the Philippines to Stenocarpella Leaf Blight *(Stenocarpella macrospora)*

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Abstract. Stenocarpella macrospora on corn can cause leaf blight, ear rot and stalk rot. These diseases are distributed worldwide and are important in the Philippines and Indonesia. In the Philippines, it was noted that S. macrospora associated with leaf blight and ear rot on corn could be a threat to corn production because incidence of these two diseases had caused substantial damage in a major corn-growing area in Mindanao. Stenocarpella leaf blight symptoms are similar to blights caused by Helminthosporium turcicum (northern corn leaf blight) and some researchers have difficulty in differentiating one from the other. We studied the resistance of 37 corn cultivars/lines from Indonesia and the Philippines to Stenocarpella leaf blight. An isolate having a high degree of virulence selected from major corn-growing areas in the Philippines was used for inoculation. The fungus was cultured on oatmeal agar for pycnidial production. The resulting suspension was adjusted to a concentration of 2×10^5 conidia mL^{-1} and inoculated with a hand-held sprayer. After inoculation, the plants were covered with plastic bags for 20 hours. The trial was arranged in a completely randomized design with three replications. The observed disease severity percentage and infection rate showed that the cultivar Tiniguib-Bukidnon Visca 8762-N was highly resistant to the disease as it had the lowest disease severity percentage and the lowest apparent infection rate ($r = 0.0388$). The other resistant lines included Bisma (S1)C1, Bisma (S1)C0, Gumarang, Pioneer 3023, Red Batuan Visca 8866, Senorita, Bisma (S2)C1, Bukidnon-Mixed and Wisanggeni.

Key words: corn cultivars/lines, leaf blight, Stenocarpella macrospora

Introduction

Stenocarpella (= *Diplodia*) fungi on corn can cause stalk rot, ear rot and leaf blight diseases. These diseases are important in the Philippines (Stevens and Celino 1931; Dalmacio and Lozano 1987) and Indonesia (Semangun 1991), and are widely distributed (Shurtleff 1980; Semangun 1991; Morant *et al.* 1993).

One of the important *Stenocarpella* species is *S. macrospora.* This fungus attacks the leaf blades and leaf sheaths of corn. The infected areas appear water-soaked to yellowish at first and later become brown when the tissue is killed by the fungus (Eddins 1930; Llano and Schieber 1980). The disease may appear on any part of the blade and sheath, although the latter is most often infected at the base. Lesions on the blade are irregular and elongate in shape, and may coalesce and form dead spots two inches in length and half an inch in width. The spots usually bear a few scattered pycnidia (Eddins 1930). Leaf blight with pycnidia is a diagnostic sign of leaf blight caused by *S. macrospora* (Dalmacio and Lozano 1987a). *S. macrospora* also attacks the ears and stalks of corn (Eddins 1930). The disease is most severe on leaves approaching maturity and is very destructive on ears (Llano and Schieber 1980). It was reported in 1976 that *S. macrospora* leaf disease of corn could be serious and was a threat to extensive corn plantings in the United States (Latterell *et al.* 1976). This disease is occasionally found in the mountain areas of the southern states of the United States (White 1999). Leaf blight and ear rot of corn caused by *S. macrospora* have also become major biotic constraints to corn cultivation in the mid-altitude ecologies of Nigeria (Olatinwo *et al.* 1999).

In the Philippines, it was noted that *S. macrospora* associated with leaf blight and ear rot on corn could be a threat to corn production because the incidence of these two diseases was commonly observed and caused substantial damage in a major corn-growing area in Mindanao. The fungus also caused ear rot reaching as high as 20% in some farmers' fields in South Cotabato (Dalmacio and Lozano 1987a; 1987b). It is a big problem in seed-production fields in the cooler areas of Mindanao where losses as high as 40% have been reported. Much of the available literature on *Stenocarpella* documents methodologies for screening stalk and ear rots, especially for *S. maydis* which does not incite leaf blight. On the other hand, literature regarding *S. macrospora*, the cause of leaf blight on corn, is still limited, even though it has been mentioned that stalk and ear infection by *S.*

macrospora appears to be highly dependent upon leaf infection, and that adequate leaf blight phase resistance should greatly minimize damage by the disease (Dalmacio and Lozano 1987a). However, no study has focused on resistance to *S. macrospora* leaf blight. This study, therefore, was undertaken to identify resistance to Stenocarpella leaf blight in corn germplasm from Indonesia and the Philippines.

Materials and Method

Collection and planting of test plants

Thirty-seven corn populations – 23 cultivars from Indonesia, 12 from the Philippines and 2 hybrid lines (Pioneer 3023 and Pioneer 3012) from the Pioneer Overseas Corporation identified as resistant and susceptible controls – were used in this study. The trial was conducted in a screen house at the Department of Plant Pathology, University of the Philippines Los Baños. Three seeds of each population were sown in a pot of size 6 containing sterilized soil, and 6 g of complete fertilizer was applied, half at sowing and a half at 3 weeks after sowing. Before inoculation, stands were thinned to 2 plants in each pot which served as a replication. Three weeks after sowing, the plants were sprayed with a conidial suspension of *S. macrospora* at a concentration of 2×10^5 conidia mL^{-1}.

Source of culture

Isolate Sm-14 isolated from a leaf sample taken from Poblacion, Dangcagan, Bukidnon province in the Philippines and which showed the highest degree of virulence compared to 18 other isolates, was used in the study.

Preparation and inoculation of leaf blight

To produce conidia, a 5-mm diameter plug of mycelium from a 5-to-6-day-old culture was placed at the center of an agar plate containing 15 mL oatmeal agar with 2 drops of 25% lactic acid (Alovera 2001). The plates were incubated at room temperature and under continuous light for 30 days to allow abundant production of pycnidia. Cultures with pycnidia were macerated using a Waring blender and filtered through two layers of muslin cloth to remove the agar medium. The resulting suspension was adjusted to a concentration of 2×10^5 conidia per mL^{-1}. A wetting agent, Tween-20, was added to the suspension at the rate of 1 drop per 200 mL. The plants were inoculated with a hand-held sprayer delivering a fine mist of the inoculum. After inoculation, the plants were covered with plastic bags for 20 hours to provide favorable conditions for disease development. The plants were watered daily and the surrounding area sprayed with water using a fine nozzle three to four times a day to provide adequate humidity. The experiment was arranged in a completely randomized design with three replications.

Disease severity

Disease severity was initially assessed one week after inoculation and at one-week intervals thereafter for five weeks. Disease severity was recorded at the seventh leaf as good symptoms appeared only then. Rating was done by establishing lesion-size categories and counting the number of lesions in each category, starting from the first week up to the fifth week after inoculation at one week-intervals. The lesion-size categories that frequently appeared were 0.5 × 1 mm, 1 × 2.5 mm, 2 ×5 mm, 3 × 10 mm, 4 × 15 mm and 5 × 20 mm. The data obtained were converted to percentage diseased leaf area by considering the leaf area of the assessed leaf after it was fully extended. The disease severity percentage for each leaf sample was calculated using the following equation.

Percent disease severity =

where
X_i = lesion-size categories
Y_i = number of lesions in each lesion-size category
La = area of the seventh leaf

The apparent infection rate (r) was calculated using Vander Plank's equation (Vander Plank 1963):

$$r = \left(\frac{1}{t_2 - t_1}\right)\left(\log_e \frac{x_2}{1 - x_2} - \log_e \frac{x_1}{1 - x_1}\right)$$

where
r = apparent rate of infection
t_1 = initial time of disease observation
t_2 = final time of disease observation
$\log_e$ = natural logarithm
x_1 = percent disease severity at t_1
x_2 = percent disease severity at t_2

Results and Discussion

The leaf blight symptoms started as small yellowish specks gradually enlarging into oval spots with gray centers. The time from inoculation to first symptom appearance varied among the cultivars/lines. This incubation period ranged from 48.9 hours to 72 hours or 2-3 days after inoculation. Using the whorl inoculation method with infected leaves or sorghum grain inocula, the first symptom appeared as early as 5 days (Dalmacio and Lozano 1987a).

Percent disease severity

The percent disease severity varied significantly among the 37 cultivars/lines used in our study, ranging from 4.79% to 30% at the 35th day after inoculation (Table 1). The data showed that the optimum time to observe disease severity was 30-35 days after inoculation. The lowest percent disease severity was recorded for Tiniguib-Bukidnon Visca 8762-N with a mean of 4.79% and an apparent infection rate of 0.0388 per unit per day. It was not significantly different from cultivars/lines such as

Table 1. Percent disease severity and apparent infection rate (r) of corn cultivars/lines from Indonesia and the Philippines at different time intervals following incolation of *Stenocarpella macrospora* isolate Sm-14.

Cultivar/ line	Origin[1]	Percent disease severity[2]					r value3
		Days after inoculation					
		7	14	21	28	35[4]	
Tiniguib-Mindanao	P	4.13	6.92	11.33	22.00	30.00 a	0.0821
Tiniguib-Pioneer	P	3.75	4.92	8.08	19.00	25.67 ab	0.0779
Pioneer 3012	Pi	3.92	4.92	7.75	16.67	24.96 abc	0.0806
Phil DMR1	P	4.08	5.96	9.33	17.00	23.50 bcd	0.0706
Maros Sintetis1(S1)C1	I	2.33	3.08	6.00	15.50	22.67 b-e	0.0896
SATP-2(S2) C6	I	2.71	4.25	6.67	15.83	22.17 b-f	0.0830
Pool-2(S1) C8	I	2.33	3.58	5.75	15.17	21.17 b-g	0.0865
UPCA var 2	P	3.00	4.08	6.25	14.83	20.83 b-h	0.0765
BK(HS)C1	I	2.71	3.84	5.67	14.17	19.33 c-i	0.0769
Lokal Madura	I	2.42	4.33	5.75	12.00	18.67 d-i	0.0795
Cebu White Flint	P	1.83	2.50	4.50	11.67	18.17 d-j	0.0884
AMATL(HS)C2	I	3.00	4.00	5.50	11.83	17.83 d-j	0.0696
Lokal Banjarbaru	I	3.00	3.92	4.83	10.33	17.50 d-j	0.0688
UPCA var 1	P	2.92	4.00	5.08	11.67	17.00 e-k	0.0685
Bayu	I	4.00	5.42	7.50	13.33	16.58 f-l	0.0558
Lokal Koasa	I	3.58	4.83	7.42	13.33	16.50 f-l	0.0597
Aroman White Flint	P	2.63	3.25	4.25	11.00	16.00 g-l	0.0698
Pulut Takalar	I	2.25	3.29	4.58	9.67	15.50 g-l	0.0741
Kresna	I	3.13	3.96	5.75	10.67	15.17 g-l	0.0611
BK(S1)C1	I	2.50	3.09	4.67	11.00	15.00 h-l	0.0689
Bisma	I	3.00	3.67	5.25	10.50	14.83 h-l	0.0617
Lokal Gorontalo	I	3.67	5.17	7.08	11.00	14.50 i-l	0.0533
AMATL(S1)C3	I	2.58	3.88	5.54	9.00	13.67 i-l	0.0639
Davao 1	P	3.00	4.00	4.92	8.83	13.50 i-l	0.0578
SATP-1(S2)C6	I	2.58	3.17	4.75	8.50	12.33 j-m	0.0596
Lamuru	I	2.59	3.17	4.67	8.67	11.42 k-n	0.0564
Lagaligo	I	2.50	3.13	3.88	8.00	11.33 k-o	0.0574
Wisanggeni	I	2.58	3.13	4.33	8.83	10.83 l-p	0.0544
Bukidnon-Mixed	P	1.92	2.34	2.75	5.67	7.42 m-q	0.0503
Bisma (S2)C1	I	2.25	2.71	3.50	5.67	6.58 m-q	0.0399
Senorita	P	1.96	2.38	2.67	5.17	6.42 m-q	0.0440
Red Batuan Visca 8866	P	2.33	2.67	3.08	5.33	6.17 n-q	0.0362
Pioneer 3023	Pi	1.83	2.25	2.92	4.83	6.08 n-q	0.0445
Gumarang	I	1.96	2.34	2.75	4.75	5.33 opq	0.0370
Bisma(S1)C0	I	2.00	2.33	2.88	4.92	5.17 pq	0.0351
Bisma(S1)C1	I	2.00	2.29	3.00	4.17	4.92 pq	0.0332
Tiniguib-Bukidnon Visca 8762-N	P	1.67	2.09	2.38	3.83	4.79 q	0.0388

1. P: Philippines; I: Indonesia; Pi: Pioneer (control).
2. Average of three replications, two plants for replication.
3. Apparent infection rate (r) calculated as described in text.
4. Means with the same letter in the same column are not significantly different at 5% level based on LSD.

Bisma (S1)C1, Bisma(S1)C0, Gumarang, Red Batuan Visca 8866, Senorita, Bisma (S2)C1, Bukidnon Mixed and Pioneer 3023. Most of those were not significantly different from Wisanggeni. The resistant control Pioneer 3023 had a percent disease severity mean of 6.08% and a corresponding apparent infection rate of 0.0445 per unit per day. From the least significant difference analysis of percent disease severities, the populations named above could be categorized as a resistant group whose percent disease severity at the 35th day was less than or around 10%. With respect to percent disease severities in the initial and final observations, the apparent infection rates of resistant cultivars/lines were generally lower than those of susceptible cultivars/lines. Ringer and Grybauskas (1995) also noted that the apparent infection rates of gray leaf spot on resistant hybrids were lower than those on susceptible hybrids.

The highest percent disease severity was recorded for Tiniguib-Mindanao with a mean of 30% and an apparent infection rate of 0.0821 per unit per day (Table 1). It was not significantly different from Tiniguib-Pioneer and Pioneer 3012 with percent disease severity means of 25.67% and 24.96%, and apparent infection rates of 0.0779 and 0.0806 per unit per day, respectively. The percent disease severities of these two varieties were not significantly different from those of Phil DMR 1, Maros Sintetis 1 (S1) C1, SATP-2 (S2)C6, Pool-2 (S1)C8 and UPCA Var 2. These populations could thus be categorized as a susceptible group whose percent disease severity at the 35th day was more than 20%.

Table 2. **Grouping of corn populations in terms of resistance to Stenocarpella leaf blight.**

Category	Cultivars/lines
Resistant	Tiniguib-Bukidnon Visca 8762-N, Bisma (S1)C1, Bisma (S1)C0, Gumarang, Pioneer 3023, Red Batuan Visca 8866, Senirita, Bisma (S2)C1, Bukidnon-Mixed, Wisanggeni
Moderately resistant	Lagaligo, Lamuru, SATP-1(S2)C6, Davao 1, AMATL(S1)C3, Lokal Gorontalo, Bisma, BK(S1)C1, Kresna, Pulut Takalar, Aroman White Flint, Lokal Koasa, Bayu, UPCA Var 1
Moderately susceptible	Lokal Banjarbaru, AMATL(HS)C2, Cebu White Flint, Lokal Madura, BK(HS)C1
Susceptible	UPCA Var 2, Pool-2(S1)C8, SATP-2(S2)C6, Maros Sintetis1 (S1)C1, Phil DMR 1, Pioneer 3012, Tiniguib-Pioneer, Tiniguib-Mindanao

Other cultivars/lines had percent disease severities between 10% and 20%. By using group differences, these populations could be categorized into a moderate group. These moderate populations could be classified into two groups, ie, moderately resistant and moderately susceptible. The moderately resistant populations consisted of Lagaligo, Lamuru, SATP-1 (S2)C6, Davao 1, AMATL (S1)C3, Lokal Gorontalo, Bisma, BK (S1)C1, Kresna, Pulut Takalar, Aroman White Flint, Lokal Koasa, Bayu and UPCA Var 1, with percent disease severities between 11% and 17%. The moderately susceptible populations consisted of Lokal Banjarbaru, AMATL (HS)C2, Cebu White Flint, Lokal Madura and BK(HS)C1 with the percent disease severities between 17% and 20%.

In sum, on the basis of differences in percent disease severity, the populations tested could be classified into four groups of resistances: resistant, moderately resistant, moderately susceptible and susceptible. This grouping of resistances of corn population can be seen in Table 2.

Conclusions

1. Corn cultivars/lines which were resistant to Stenocarpella leaf blight include Tiniguib-Bukidnon Visca 8762-N, Bisma (S1)C1, Bisma (S1)C0, Gumarang, Pioneer 3023, Red Batuan Visca 8866, Senorita, Bisma (S2)C1, Bukidnon-Mixed and Wisanggeni.
2. The susceptible corn cultivars/lines included Tiniguib-Mindanao, Tiniguib-Pioneer, Pioneer 3012, Phil DMR 1, Maros Sintetis (S1)C1, SATP-2(S2)C6, Pool-2(S1)C8, UPCA Var 2.
3. The rest of the cultivars were categorized as moderately resistant or moderately susceptible to Stenocarpella leaf blight.

Acknowledgment

The author would like to express his gratitude to Dr. Avelino D. Raymundo of the University of the Philippines Los Baños for his suggestions; Dr. Samuel C. Dalmacio of Pioneer Hi-Bred Philippines; Dr. Marsum M. Dahlan and Mr. Oman Suherman of the Indonesian Cereal Research Institute (ICERI) as well as Mr. Nestor C. Altoveros from National Plant Genetic Resources, IPB, the Philippines for supplying corn germplasm.

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Reaction of Sweet Corn Inbred Lines to Downy Mildew

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Abstract. Downy mildew caused by Peronosclerospora sorghi is a major disease of sweet corn in Thailand. Our study investigated the reactions of 20 sweet corn inbred lines and 2 commercial varieties, Hibrix-3 and ATS-5, to downy mildew infection at the Chai Nat Field Crops Research Center during the early rainy season of 2007. Of the experimental lines, CNSH 1819 and Hibrix3/CNSSW59(S)3-1-B-1-B/Inb# No.40 suffered infection percentage of 22.5% and 25.0% respectively, compared to 35.85% for Hibrix-3 and 100% for ATS-5. The overall results indicate that the former two lines are resistant to downy mildew.

Key words: Downy mildew, Peronosclerospora sorghi, sweet corn inbred lines

Introduction

Downy mildew caused by *Peronosclerospora sorghi* (Westeon & Uppal) C.G. Shaw is a major disease of corn in Thailand. It was observed in Phayuhakeeree and Thatago districts in Nakhonsawan province in 1968, and has since spread to other provinces such as Lopburi, Tak, Sukhothai, Pissanulok, Petchaboon and Nakhonratchasima. The disease seriously damages corn in every corn production area, especially Khanjanaburi and Uthaitanee where corn is grown continuously throughout the year. The fungicide Metalaxly has been used unsuccessfully in these areas to contain the disease, which can result in 100% yield reduction. *P. sorghi* can damage corn from the seeding until the flowering stage and grows well at temperatures of 12 ^{o}C to 32^{o}C (Kimigafukuro 1988). The disease cultured from single conidia on corn can produce spores in many shapes and sizes of around 24-26 × 12-20 microns (Pupipat 1976). The spread of the disease is serious in the rainy season (May-Sep) when relative humidity is high. It has been reported that Metalaxly inoculated on corn seeds at the rate of 7 g 1 kg^{-1} seed can control the disease effectively, but only in some areas. Therefore, a more widely effective method of control needs to be found. Our study of the reaction of sweet corn inbred lines to downy mildew is an effort in that direction.

Materials and Method

The experiment was conducted in the rainy season of 2007 at the Chai Nat Field Crops Research Center. Twenty sweet corn inbred lines and 2 commercial varieties, Hibrix-3 and ATS-5, were used in the study. A susceptible variety, Sukhothai 1, was used as a disease spreader. The spreader rows were sprayed with a spore suspension with a concentration of 5×10^4 spores mL^{-1} (prepared from a sample of sweet corn leaf infected by *P. sorghi*) for two consecutive nights 7 days after planting. The test varieties were sown when corn in the spread rows had developed disease symptoms of over 70% (3 weeks). Sprinkler and furrow irrigation was used to increase humidity in the canopy and thus enhance fungal infection and disease establishment (Figure 1). Data on percent inflection and rating of diseases severity were recorded 50 days after planting (Figure 2). Disease incidence and severity was recorded as per a modified method reported by Craig *et al.* (1977):

Figure 1. Sweet corn plants 25 days after emergence (foreground) and spreader rows (back).

Figure 2. Symptoms of downy mildew disease caused by *Peronosclerospora sorghi*.

0% infection (no symptom) = Highly Resistant (HR)
1-10% infection = Resistant (R)
11-25% infection = Moderately resistant (MR)
26-50% infection = Moderately susceptible (MS)
51-75% infection = Susceptible (S)
76-100% infection = Highly susceptible (HS)

Results and Discussion

The sweet corn lines CNSH 1819 and Hibrix3/CNSSW59(S)3-1-B-1-B/Inb#No.40 showed lower infection percentages of 22.5% and 25.0%, respectively, compared to 35.85% and 100% for the standard varieties, Hibrix-3 and ATS-5, respectively. Hibrix3/CNSSW59(S)3-2-1-B/Inb# No.40, Hibrix4(S)9-1-B-B-B/Inb# No.19, Fx 3(S)-3/Inb# No.40, Fx 3-1/Inb# No.19, CNSH 1840, Fx 3(S)-2/Inb# No.40, Hibrix3/CNSSW59(S)11-1-B/Inb# No.40 and Fx 3(S)-8/Inb# No.40 were moderately susceptible with disease severities ranging from 32.5% to 50.0%. Hibrix3/CNSSW59(S)26-1-B/Inb# No.40, Agsh2(S)-B/Inb# No.40, SSH001(S)11-1-B-B-3/Inb# No.19, SSH001(S)11-1-1-B-B/Inb# No.19, Hibrix4(S)9-1-B-B-B/Inb# No.40, Hibrix3/CNSSW59(S)4-B-B-1/Inb# No.40 and MIxSh2(S)14-1-B-2-B-1/Inb# No.40 had disease severities between 55.0% and 75.0% and were considered susceptible. SSH001(S)11-1-B-B-3/Inb# No.40, CNSH 440 and Insee1(S)11-1-1-B-B-B/Inb# No.40 with disease infections between 82.5% and 84.5% were considered highly susceptible varieties (Table 1).

Table 1. Percent infection and reaction of 22 sweet corn lines/varieties to downy mildew disease, Chai Nat Field Crops Research Center, Thailand, 2007.

Line/variety	% infection	Disease reaction[1]
CNSH 1840	42.50	MS
Hibrix3/CNSSW59(S)3-1- B-1-B/Inb# No.40	25.00	MR
Hibrix3/CNSSW59(S)4- B-B-1/Inb# No.40	70.00	S
Hibrix3/CNSSW59(S)11- 1-B/Inb# No.40	45.00	MS
Hibrix4(S)9-1- B-B-B/Inb# No.40	62.50	S
Hibrix3/CNSSW59(S)3- 2-1-B/Inb# No.40	32.50	MS
Fx 3(S)-2/Inb# No.40	42.50	MS
MIxSh2(S)14-1-B-2-B-1/Inb# No.40	75.00	S
SSH001(S)11-1-B-B-3/Inb# No.40	82.50	HS
Fx 3(S)-3/Inb# No.40	37.50	MS
Insee1(S)11-1-1-B-B-B/Inb# No.40	84.50	HS
Agsh2(S)-B/Inb# No.40	56.05	S
Hibrix3/CNSSW59(S)26-1-B/Inb# No.40	55.00	S
Fx 3(S)-8/Inb# No.40	50.00	MS
CNSH 1819	22.50	MR
Fx 3-1/Inb# No.19	37.50	MS
SSH001(S)11-1-1-B-B/Inb# No.19	57.50	S
SSH001(S)11-1-B-B-3/Inb# No.19	57.35	S
Hibrix4(S)9-1- B-B-B/Inb# No.19	32.50	MS
CNSH 440	82.50	HS
Hibrix-3	35.85	MS
ATS-5	100.00	HS

[1.] 0% infection (No symptom) = Highly Resistant (HR); 1-10% infection = Resistant (R); 11-25% infection = Moderately resistant (MR); 26-50% infection = Moderately susceptible (MS); 51-75% infection = Susceptible (S); and 76-100% infection = Highly susceptible (HS).

The overall results indicate that CNSH 1819 and Hibrix3/CNSSW59(S)3-1-B-1-B/Inb# No. 40 were resistant to *P. sorghi*. Earlier results may be different from the results reported here because Shurtleff (1980) and Cordwell *et al.* (1977) reported that disease incidence and prevalence depend on the prevailing weather conditions, particularly relative humidity and temperature. They suggested that a suitable temperature for disease infection is about 65-85°F accompanied by high humidity and foggy conditions. Kimigafukuro (1988) also reported that sporulation depends on day and night temperatures and high relative humidity.

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Major Corn Diseases in Palu Valley of Indonesia and Their Control

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Abstract. Diseases cause loss of corn production in Palu Valley in Central Sulawesi, Indonesia. They reduce the value and quality of the grain and may increase the harvesting costs when the affected plants lodge. A survey was conducted during the crop season of 2007 to identify the major diseases affecting corn in Palu Valley, especially in Donggala district. Based on these observations, seven major diseases and two pathogens infecting ear/seeds of corn were identified. These were downy mildew (Peronosclerospora maydis), maydis leaf blight (Bipolaris maydis), banded leaf and sheath blight (Rhizoctonia solani), polysora rust (Puccinia polysora), curvularia leaf spot (Curvularia lunata), bacterial stalk rot (Erwinia chrysantemi pv. Zeae), common smut (Ustilago maydis), aspergillus ear rot (Aspergillus plavus) and gray ear rot (Physalospora zeae). Among them, the most important corn diseases in Palu Valley are downy mildew, maydis leaf blight, polysora rust, banded leaf and sheath blight and aspergillus ear rot.

Key words: Palu Valley, corn diseases

Introduction

Corn (*Zea mays* L.) is one of the most important food crops grown throughout ythe world in temperate, subtropical and tropical zones wherever rainfall or irrigation is adequate (Shurtleff 1980). Most of the area sown to corn (58%) is in developing countries; of this, approximately 50 million ha are in the tropics, mainly at low elevations. However, about two-thirds of the world's corn is produced in developed countries where climates are almost entirely temperate. There are large differences in yield between developed and developing countries.

In some Asian countries, including Indonesia and the Philippines, corn is the second most important cereal crop after rice. Although grown principally for animal feed, it is also a staple food crop for human consumption, particularly in Mexico and other Central and South American countries, Africa and Asia (Shurtleff 1980).

In Indonesia, the demand for corn as food and feed is steadily increasing (Swastika *et al.* 2001). While more than 70% of the corn produced in the country is consumed as food (CBS 1999), development of the crop has in recent years taken an orientation toward agribusiness. This has resulted in high-frequency cultivation of corn, especially in the newer areas. Given the availability of host plants, this in turn has led to the emergence of insect pests and pathogens that formerly had not been dominant.

Corn is one of the most important food crops in Central Sulawesi Province, including the two districts of Palu Valley, Donggala and Palu. Of the two, Donggala has the more extensive corn sown area. A district of 10 471.71 km^2, Donggala lies between 0.3^{o}N and 2.2 ^{o}S latitudes and 119.45^{o}E and 121.45^{o}E longitudes. The temperature ranges from 20^{o}C to 25^{o}C at night and 34^{o}C and 37^{o}C at noon. The average relative humidity ranges from 64% to 85% with yearly rainfall of 1,500-3,000 mm except in Palu Valley which only receives 600-800 mm. Palu Valley is known as an off-season area due to the climatic conditions. Nevertheless, many crops can be sown all the year round because of the availability of irrigation.

All parts of the corn plant are susceptible to a wide variety of diseases which can reduce the yield and quality of its products. So far there has been no completely effective measure available for control of corn diseases. However, losses can be minimized by following certain recommended practices. It is imperative therefore for farmers to be aware of corn diseasess and the factors influencing their severity. Downy mildew is endemic throughout Indonesia and is the most important production constraint of corn. The severity of damage varies, depending on varietal tolerance and agroclimatic conditions (ICERI 2008).

The aim of this study was to determine the major corn diseases affecting corn in Palu Valley and identify methods for their control.

Materials and Method

The survey was conducted during the cropping season of 2007. Descriptitive analytical methods were used with nonprobability sampling (Sumaryanto 2004) in order to determine the sample area. Samples were collected randomly in corn planted areas and disease incidence was observed directly. Observations were recoded in three subdistricts of Donggala: Biromaru (two villages, Jono Oge and Sidondo), Palolo (three villages, Bahagia, Bobo and Petimbe) and Labuan (two villages, Labuan Toposo and Labuan Panimba). Samples were collected on the basis of the observed symptoms and taken to the laboratoty of AIAT Central Sulawesi for microscopic study.

Result and Discussion

As per secondary data, planted area, productivity and production of corn in Donggala district have fluctuated in the last five years (Table 1), the lowest production (27 863 t) occurring in 2004 and the highest (52 243 t) in 2007. These production figures are strongly influenced by the corn area: the crop was sown over 10 389 ha in 2004, and 14 112 ha in 2007.

Nine major diseases of corn were observed in Palu Valley, two of which were found on ear/seeds. Disease severities varied depending on the pathogen and the variety planted in each location (Table 2). The diseases were downy mildew *(Peronosclerospora maydis)*, maydis leaf blight (*Bipolaris maydis*), banded leaf and sheath blight (*Rhizoctonia solani*), polysora rust (*Puccinia polysora*), curvularia leaf spot (*Curvularia lunata*), bacterial stalk rot (*Erwinia chrysantemi* pv. *zeae*), common smut (*Ustilago maydis*), aspergillus ear rot (*Aspergillus flavus*) and gray ear rot (*Physalospora zeae*). Among them, the most important were found to be downy mildew, maydis leaf blight, polysora rust, banded leaf and sheath blight and aspergillus ear rot. Downy mildew was the most destructive, causing 100% yield losses in some parts of the valley.

Table 1. Corn area, yield and production in Donggala district, Indonesia 2003-2007*.

Year	Planted area (ha)	Yield (quintal ha^{-1})	Production (t)
2003	11 313	25.43	28 769
2004	10 389	26.82	27 863
2005	10 847	27.52	29 853
2006	10 245	28.87	29 574
2007	14 112	37.02	52 243

*Source: CBS (2003-2007).

The high incidence of downy mildew in all locations indicated that this disease was endemic in Palu Valley where the warm and humid climate were suitable for its development. According to Bonde *et al*. (1992), *P. maydis* produces large numbers of conidia at 18-23°C (the optimum temperature for sporulation) in the presence of dew for 5-6 h. Sporulation requires darkness. *P. maydis* has a broad tange of optimum temperature for germination (10-30°C) and germ tube growth (18-30°C).

Infected corn plants grown during the dry season are the primary source of inoculum. The fungus may survive as mycelium in kernels, making it a source of inoculum. Infection by conidia occurs through the stomata of young plants and local lesions elongate toward the meristem, inducing systemic infection.

The farmers' paucity of knowledge of how to prevent the disease has resulted in the pathogen spreading rapidly. It seems that most farmers in the affected areas do not treat seed with the fungicide metalaxyl before planting, while in other locations where farmers applied metalaxyl, downy mildew incidence was negligible or nonexistent. Seed treatment with metalaxyl provides excellent protection against *P. maydis* infection for 42 days (CPC 2004).

Polysora rust is the second major corn disease in Palu Valley. The disease is common in hot and humid lowland tropical and subtropical corn-growing areas of the world (CIMMYT 2004; CPC 2004). In our study, we found the highest disease incidence in Kayu Malue Ngapa village in Palu Valley on Sukmaraga and Lamuru varieties. Most disease incidence occurred during the generative stage.

Lack of awareness among farmers regarding this disease is a problem impeding effective disease management. We found that none of the farmers in this

Table 2. Disease severity of major corn diseases in several villages in Palu Valley, Central Sulawesi, Indonesia.

Corn disease	Disease severity[1]
Downy mildew (*Peronosclerospora maydis*)	+++
Maydis leaf blight (*Bipolaris maydis* syn. *Helminthosporium maydis*)	+++
Banded leaf and sheath blight (*Rhizoctonia solani*)	+++
Polysora rust (*Puccinia polysora*)	+++
Curvularia leaf spot (*Curvularia lunata*)	++
Bacterial stalk rot (*Erwinia chrysanthemi* pv. Zeae)	+
Common smut (*Ustilago maydis*)	+
Aspergillus ear rot (*Aspergillus flavus*)	+++
Gray ear rot (*Physalospora zeae*)	++

[1.] +++: heavy; ++: moderate; +: light.

area understood the strategies for managing the disease. So far, there no varieties have been identified with resistance to polysora rust in Palu Valley. According to CPC (2004), use of resistant maize hybrids is the most feasible means of controlling polysora rust. A hypersensitive type of resistance, conditioned by a single dominant gene, was highly effective in preventing yield loss and eliminated sporulation of secondary inoculum. However, races of *P. polysora* existed that limited their wider use. Slow rusting, a general form of resistance that results in a reduced rate of disease development without severely affecting the rust population, effectively controlled rust in Africa in the 1950s (Bailey *et al.* 1987).

According to CPC (2004), the disease can be controlled by chemicals. Foliar sprays and seed dressing with chemicals effectively delayed appearance of the rust and increased yield in the wet season in Nigeria. The application of fungicides including oxycarboxin, pyracarbolid, tridemorph and triforine satisfactorily prevented infection by southern and common rust in Taiwan. Oxycarboxin and pyracarbolid had curative efficacy because they inhibited the development of pustules and killed uredia in the lesions.

Maydis leaf blight (*B. maydis*) infection occurs from the seedling to generative stage of corn plant. This disease was found in all corn-sown areas in Palu Valley, the highest incidence being in the rainy season. The development of southern corn leaf blight is favored by high temperatures and prolonged periods of high humidity (CPC 2004; CYMMIT 2004). Spore production was higher at 30°C than at 22.5°C or 15°C. Spore release was favored by humidity and infrared radiation. Relative humidity and wind speed also affect the release of conidia (CPC 2004). Farmers control the disease with foliar fungicide application. Nevertheless, according to CPC (2004), cultural control and sanitary methods such as destroying or burying the crop residue can be beneficial. Lower levels of disease were observed in plowed plots than in tillage systems in which more the crop residue had been left on the surface. The germination of spores exposed on the soil surface was significantly greater than that of buried spores. Continuous maize culture increased the severity of the disease.

Banded leaf and sheath blight (BLSB) caused by *R. solani* is another important corn disease in Palu Valley. Infected plants were found in all study locations, and it seemed that higher disease incidence occurred during the rainy rather than the dry season. BLSB of corn has become increasingly severe and economically important in several countries of Asia (Sharma 2002). The use of chemicals to manage BLSB in corn is limited and has adverse ecological implications, while the use of resistant varieties has not progressed much because of the limited host genetic variability for resistance to *R. solani* (Sharma *et al.* 2002). One of the effective means of control against BLSB is biological control using *Bacillus subtilis* and the fungicide captan as seed treatment and foliar sprays with the fungicide validamycin (Muis and Quimio 2006).

The other corn diseases found in Palu Valley such as curvularia leaf spot (*C. lunata*), bacterial stalk rot (*E. chrysantemi* pv. *zeae*) and common smut (*Ustilago maydis*) were considered minor because their disease incidence was low. Curvularia leaf spot can be controlled by using bisdithane or dithane fungicides, and bacterial stalk rot by removing the infected plants or using streptomycin bactericide and common smut by removing infected plants.

Our survey also noted two diseases that infected the ear: aspergillus ear rot (*A. plavus*) and gray ear rot (*P. zeae*). Most of the disease incidence was found in stored corn. Of the two, *A. flavus* is considered an important ear/kernel disease of corn. It is a member of a group of fungi known as storage fungi which cause deterioration of grain or seeds of plant species stored when seed moisture content is in the range 13-20% (Christensen and Meronuck 1986 *in* CPC 2004). It also produces aflatoxin in the infected seeds which can cause death or symptoms of toxicity when ingested by animals or humans (Diener *et al.* 1987). Aflatoxin contamination is of major economic importance in cotton, maize, groundnut and tree nuts due to the infection of seeds in the field, but it may also develop in seeds and grain of other crop species when they are improperly stored (Diener *et al.* 1987). Surveys throughout the world emphasize the enormous problem presented by aflatoxin to human health and to the economics of crop production (CPC 2004). So far, there have been no single control measure taken by farmers in Palu Valley to control *A. flavus*. Montes-Belmont and Carvajal (2008) reported that essential oils of *Cinnamonum zeylanicum* (cinnamon), *Mentha piperita* (peppermint), *Ocimum basilicum* (basil), *Origanum vulgate* (origanum), *Teloxys ambrosioides* (the flavoring herb epazote), *Syzygium aromaticum* (clove) and *Thymus vulgaris* (thyme) caused a total inhibition of fungal development in maize kernels. Thymol and o-methoxycinnamaldehyde significantly reduced maize grain contamination. The optimal dosage for protection of maize varied from 3% to 8%. Combinations of *C. zeylanicum* with the remaining oils gave efficient control. A residual effect of *C. zeylanicum* was detected after 4 weeks of kernel treatment. No phytotoxic effect on germination and corn growth was detected with any of these oils. Aflatoxin contamination of corn can be reduced by using the nonaflatoxigenic *A. flavus* (CT3 and K49) (Abbas *et al.* 2006).

Conclusions

The corn diseases found in Palu Valley were downy mildew (*Peronosclerospora maydis*, maydis leaf blight (*Bipolaris maydis*), banded leaf and sheath blight (*Rhizoctonia solani*), polysora rust (*Puccinia polysora*), curvularia leaf spot (*Curvularia lunata*), bacterial stalk rot (*Erwinia chrysantemi* pv. *zeae*), common smut (*Ustilago maydis*), aspergillus ear rot (*Aspergillus plavus*), and gray ear rot (*Physalospora zeae*). The most important corn diseases in Palu Valley are downy mildew, maydis leaf blight, polysora rust, banded leaf and sheath blight, and aspergillus ear rot.

There is no single completely effective measure for controlling all corn diseases. However, losses can be minimized by following certain recommended practices. Corn diseases can be controlled by using disease-resistant hybrids or by applying fungicides to the seed. Other practices such as spraying plants with fungicide, adjusting soil fertility with fertilizers, and maintaining good drainage may also be used with certain diseases and in certain circumstances. Incorporating genetic resistance into agronomically desirable hybrids seems to be the most efficient and permanent means of controlling corn diseases. The treatment of seed corn with a fungicide either in a dust or slurry, may control seed rots and seedling diseases, but will control no other disease. Crop rotation and destruction of diseased plant parts have been suggested as control measures for certain plant diseases.

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Use of Arbuscular Mycorrhizae in Control of Sheath Blight (*Rhizoctonia solani*) on Maize

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Abstract. Sheath blight is a major disease of maize caused by Rhizoctonia solani. Arbuscular mycorrhizal (AM) fungi have have been reported to have the capacity to increase maize tolerance to the sheath blight infestation. A pot culture experiment was designed to study the effects of inoculation of maize by AM fungi (Glomus sp and Acaulospora mellea) on the development of sheath blight. The results indicated that inoculation with the AM fungi decreased sheath blight incidence. The decrease in intensity ranged from 21.9% to 40.8%. Inoculation of R. solani also reduced the percentage root infection and the number of spores produced by the mycorrhizae. The intensity of sheath blight was highest in plants inoculated with R. solani only but less in plants treated with R. solani plus mycorrhizae.

Key words: Glomus sp, Acaulospora mellea, Rhizoctonia solani

Introduction

Sheath blight caused by *Rhizoctonia solani* is an important disease of maize. *R. solani* infects the lower sheath, then spreads upward to the ears and can cause significant losses of harvested maize. This disease is found in many corn farms in southern Sulawesi and eastern Java (Rahamma *et al.* 1999; Soenartiningsih *et al.* 2006).

R. solani can also attack plants other than maize such as peanut, mung bean, soybean and sorghum. This disease can be controlled by pulling the infected plants, but that can be quite expensive when the number is large. It can also be controlled with fungicide, but this strategy is considered harmful to the environment.

Arbuscular mycorrhizae (AM) increase nutrient uptake by roots and thus aid plant growth (Stribley 1998). Mycorrhizal association has been proposed as an option in the management of soilborne pathogens (Perrin 1990). Association with AM fungi can lead to increased resistance due to the production of certain compounds such as phenol. Plants infected by mycorrhizae are often less colonized by pathogens and show reduced disease incidence. The present research was carried out to study the effect of inoculating corn with AM fungi *Glomus* sp and *Acaulospora mellea* on sheath blight incidence, root infection of mycorrhizae and spore density.

Materials and Method

The experiment was a three-factorial experiment arranged in a randomized complete design. The first factor of the experiment comprised the variety Wisanggeni and the line GM 30; the second was inoculation with mycorrhizae (*Glomus* sp and *A. mellea*); and the third was the inoculum quantity (weight) of mycorrhizae (10 g, 20 g and 30 g). Each treatment had three replications. Prior to use, the soil was fumigated with methyl bromide, placed in pots (5 kg soil per pot) and fertilized with urea 25 mg, KCl 25 mg and SP 36 12.5 mg per pot.

The inoculum of AM fungi, ie, *Glomus* sp and *A. mellea,* consisting of soil, root and spores (10–20 spores per gram of soil), was applied around the roots of maize seedlings at planting time. The *R. solani* pathogen was grown on potato dextrose agar incubated in a moist chamber (temperature 20–25°C) for 15 days after isolation. One gram of the culture containing mycelia and young developing sclerotia were used for inoculation around the lower sheath at 30 days after planting.

Assessment of infection

The effect of AM fungal symbiosis on disease development and severity was assessed 10–50 days after inoculation of *R. solani*. The rate of intensity of infection was determined as per the following formula:

$$I = \frac{Ó\,(n \times v)}{V \times N} \times 100\ \%$$

where

I = Intensity of infection
n = Number of plants in each infection category
v = Scale value of each infection category
V = Scale value of the highest infection category
N = Number of observed plants

Percentage root colonization by mycorrhizae

The extent of mycorrhizal colonization was assessed by recording the percentage of root pieces infected after clearing. The roots were cut into 1 cm pieces, and 100 root segments were examined. The percentage of mycorrhizal infection was calculated using the following formula (Kormanik *et al.* 1980).

$$\frac{\text{No. of roots with infection}}{\text{No. of roots observed}} \times 100\%$$

Isolation of mycorrhizal spores

Spores were isolated from the rhizosphere soil by wet-sieving and decanting (Gerdeman and Nicolson 1963). The spore density was calculated by direct count and represented as the number of spores 100 g^{-1} soil.

Results and Discussion

Intensity of sheath blight disease

The intensity of sheath blight of plants inoculated with mycorrhizae was significantly lower than that of noninoculated plants (Table 1). Inoculation with 10-30 g of AM fungi decreased sheath blight intensity by as much as 24.90–37.43 % in the Wisanggeni variety and the GM 30 line while in noninoculated maize AM fungal infection ranged from 42.19–47.67% after 50 days of inoculation by *R solani*. The average reduction in intensity of sheath blight ranged from 21.9% to 40.8%.

Increased tolerance of sheath blight in general is supposed to be the main effect of arbuscular mycorrhizae on the host plant. Furthermore, as also reported by Ascon and Barea (1996), AM association can reduce damage caused by soilborne plant pathogens. The ability of AM symbiosis to enhance resistance or tolerance in roots is not equal for the different AM fungi so far tested.

Percentage of root infection of mycorrhiza

Table 2 presents the percentage of root infection by mycorrhiza in plants that were inoculated with *R. solani* as well as AM fungi as compared with plants that were inoculated with only mycorrhizae. Plants inoculated with mycorrhizae only showed higher mycorrhizal root infection than plants that received both mycorrhizae and *R. solani*. The presence of *R. solani* suppresses mycorrhizal infection of the root. Reduction in root infection by *Glomus* sp and *A. mellea* due to *R. solani* ranged from 18.24% to 36.95%

Table 1. Intensity[1] of sheath blight on Wisanggeni and GM 30 corn varieties inoculated with *Glomus* sp and *A. mellea* at various inoculum weights.

Treatment			Intensity of sheath blight disease (%)				
Variety/ Lines	Mycorrhizal	Mycorrhizal inoculum weight (g)	10 days	20 days	30 days	40 days	50 days
			after inoculated R.solani				
Wisanggeni	*Glomus sp .*	0	8.77 a	19.81 a	29.90 a	37.74 ab	42.19 a
		10	3.42 c	6.85 d	13.44 d	21.49 c	32.95 bc
		20	0.00 d	1.23 e	7.36 e	13.50 d	24.90 c
		30	0.00 d	2.17 e	6.92 e	11.58 d	25.16 c
	A. mellea	0	9.22 a	20.60 a	30.80 a	38.95 ab	44.72 a
		10	7.04 b	8.30 de	16.43 cd	26.43 c	35.11 b
		20	0.53 d	4.38 ef	10.69 d	19.84 cd	27.43 c
		30	0.00 d	3.42 ef	10.55 e	15.43 d	26.80 c
GM 30	*Glomus sp .*	0	11.11 a	22.22 a	28.56 a	40.19 a	45.90 a
		10	8.71 ab	11.85 d	18.29 cd	24.96 c	35.12 b
		20	0.55 d	4.36 ef	13.44 d	16.31 d	27.85 c
		30	0.17 d	3.42 ef	11.70 de	17.54 d	28.13 c
	A. mellea	0	11.76 a	21.89 a	30.75 a	41.70 a 24.83 c	47.67 a
		10	6.04 b	11.51 d	20.16 c	18.28 cd	37.43 b
		20	1.23 d	4.39 ef	15.43 cd	17.37 d	29.70 c
		30	0.00 d	2.17 f	10.75 e		29.31 c

[1.] Values within the same column followed by the same letter are not significantly different as per Duncan's multiple range test (5%).

Table 2. Percentage of mycorrhizal infection[1] in Wisanggeni and GM 30 corn varieties that were inoculated with *Glomus* sp, *A. mellea* and *R. solani.*

Mycorhizal inoculum weight (g)	Mycorrhizal	Not *R. solani* / *R.solani*	MA fungi infection (%) Wisanggeni Variety	GM 30 Line	Average
10	*Glomus sp.*	Not *R. solani*	70.00 c	66.66 d	68.33
		R.solani	51.11 f	42.22 g	46.67
			60.56	54.44	57.5
	A. mellea	Not *R. solani*	60.00 e	52.00 f	56
		R.solani	46.66 g	36.66 h	41.63
			53.33	44.33	48.81
20	*Glomus sp.*	Not *R. solani*	86.66 a	73.33 bc	79.99
		R.solani	68.89 cd	53.33 f	61.11
			77.78	63.33	71.55
	A. mellea	Not *R. solani*	65.55 d	60.00 e	62.78
		R.solani	50.00 f	46.66 g	48.33
			57.78	53.33	55.55
30	*Glomus sp.*	Not *R. solani*	85.55 a	76.66 b	81.11
		R.solani	72.22 bc	60.00 e	66.11
			78.89	68..33	73.61
	A. mellea	Not *R. solani*	67.00 d	63.33 e	65.17
		R.solani	56.66 ef	46.66 g	51.66
			61.83	54.99	58.41
			65.03 d	56.46 f	

[1.] Values within the same column followed by the same letter are not significantly different as per Duncan's multiple range test (5%).

Table 3. Population of mycorrhizal spores on Wisanggeni and GM 30 corn varieties when inoculated with AM fungi (*Glomus* sp and *A. mellea*) as well as *R. solani.*

Mycorhizal inoculum weight (g)	Mycorrhizal	Not *R. solani* / *R.solani*	Spore population per 100 g/soil Wisanggeni Variety	GM 30 line	Average
10	*Glomus sp.*	Not *R. solani*	240.00 b	171.00 c	205.5
		R.solani	174.00 c	144.00 d	159
			207	157.5	182.25
	A. mellea	Not *R. solani*	156.00 c	129.00 d	142.25
		R.solani	105.00 de	99.00 e	102
			130.5	114	122.13
20	*Glomus sp.*	Not *R. solani*	280.00 a	247.00 b	263.5
		R.solani	216.00 b	178.00 c	197
			248	215.5	231.75
	A. mellea	Not *R. solani*	172.00 c	163.00 c	167
		R.solani	132.00 d	125.00 d	128.5
			152	144	148
30	*Glomus sp.*	Not *R. solani*	295.00 a	250.00 b	272.5
		R.solani	208.00 b	184.00 c	196
			251.5	217	234.25
	A. mellea	Not *R. solani*	189.00 c	159.00 c	174
		R.solani	124.00 d	138.00 d	131
			156.5	148.5	152.5
			190.92 b	166.08 c	

[1.] Values within the same column followed by the same letter are not significantly different as per Duncan's multiple range test (5%).

for the Wisanggeni variety, and from 26.68% to 57.88% for the GM 30 line.

R. solani significantly reduced the percentage of root length colonization by the AM fungi. This indicates that *R. solani* had a negative effect on mycorrhizal colonization (Morandi *et al.* 2002).

Mycorrhizal spores

Observations on spore population indicated that in maize plants inoculated with *Glomus* sp and *A. mellea,* the mycorrhizal spore population was higher than in plants that received both mycorrhizal and *R. solani* inocula (Table 3). This indicates that the presence of *R. solani* reduced the number of mycorrhizal spores significantly. In the combined treatment (mycorrhizae + *R. solani*) the mycorrhizal spore population was lower by 29.62-52.41% for Wisanggeni and by 15.21-38.76% for GM 30.

Conclusions

Inoculation with *Glomus* sp and *A. mellea* decreased sheath blight disease intensity by 21.9%, 37.7% and 40.8 % respectively. Inoculation of maize plants with mycorrhiza only – as compared with inoculation of both mycorrhiza as well as *R. solani* – showed higher mycorrhizal spore population and root infection, because the presence of *R. solani* suppresses mycorrhizal infection and spore formation.

Acknowledgement

We thank Prof. Dr. Ambarwati H. Tjokrosoedarmo and Prof. Dr. Nursamsi Pusposendjoyo of the faculty of Agricultural Gadjah Mada University. We are also grateful to Dr. John Bacobaon of the Indonesian Coffee and Cocoa Research Institute for his time and suggestions.

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Effect of Several Drying Methods on Infection of Maize by *Aspergillus flavus* in Gorontalo, Indonesia

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Abstract. Postharvest processing of maize can affect the quality of seed. If harvested maize has high moisture content and is not dried immediately, the chances of infection by the fungal pathogen Aspergillus flavus are very high. The fungus produces a mycotoxin that affects seed quality. Seed with high aflatoxin content can cause liver cancer in humans and adversely affect milk quality in animals. This study was conducted at the Indonesian Cereal Research Institute (ICERI) laboratory in 2005 to test the effect of seven different seed-drying methods (P1-P7) on A. flavus infection. Five hundred gram samples of maize seed were taken from each of these seven seed-drying treatments. Then 100 seeds of each were put in 10 sterile petriplates with wet filter paper, 10 seeds to every petriplate. The experiment was arranged in a randomized complete design, with seven treatments and 10 replications. The study was aimed at identifying drying methods that reduce A. flavus infection and maintain seed quality. The results indicated that the rates of A. flavus infection varied among the different drying treatments. Comulation of cobs after harvest for three and five days before drying stimulated the growth of A. flavus, and reduced the germination ability of the seeds. Cobs that were dried immediately following harvest resulted had low A. flavus infection and high germination ability.

Key words: Postharvest, A. flavus, aflatoxin, seed, corn

Introduction

Farmers usually plant maize in the early rainy season, so harvest is done under high rainfall conditions. This affects the moisture content of the grain. If not dried immediately after harvest, high moisture content (32-35%) in the cob (ear) can stimulate the growth of *Aspergillus flavus,* and also result in a high proportion (up to 15%) of broken seed at shelling (Tastra *et al*. 1990). Broken seeds are a good medium for *A. flavus* growth. The fungus produces aflatoxin in the seed. Echardi (1986) reported that when 100% broken yellow hybrid maize seeds and composite seeds were stored for four days at room temperature (30^{o}C), the aflatoxin content was 42 ppb and 26 ppb, respectively. According to the Food and Agriculture Organisation (FAO), the maximum permissible limit for aflatoxin in maize grain is 30 ppb (Beti and Ginting 1993). According to Hamilton (1986) and Wilson (1978), food contaminated by aflatoxin (>30 ppb) can cause deadly chronic diseases. Drying the grain is therefore imperative to prevent *A. flavus* infection. Purwadaria (1988) reported that drying the grain to decrease moisture content to 5.2-15.2% at harvest improved maize seed quality and quantity in Indonesia.

Maize grain can be dried in sunlight or by using a drying machine. Farmers in Indonesia dry maize grain by the roadside or in the household yard on a mat (83%), or on a concrete floor (6.8%) or by using a dryer (0.03%) (Subandi and Manwan 1990). Furthermore, Sarasutha *et al*. (2001) reported that farmers tend to dry the cobs by cutting the maize stem above the ear, opening the husk and then keeping the maize on the stalk to dry. Another method used is to hang the cobs from the attic of the house. These drying methods, however, leave the cobs susceptible to seedborne fungal infections.

The aim of our study was to identify drying methods that minimize the risk of *A. flavus* infection.

Method

The study was conducted in Tenilo in Gorontalo Provice of Indonesia in 2005. We collected 500 g samples of maize seed and subjected them to the following drying treatments.

P1 Maize plant kept in the field until 7 days of physiological maturity → harvest → peeling → sun-drying of the cob to decrease moisture content to 15-17%.

P2 Ears harvested after 7 days of physiological maturity → peeling → drying the cob to 15-17% moisture content in a dryer.

P3 Ears harvested after 7 days of physiological maturity → peeling → piling the cob for 3 days → shelling → drying the seeds in a dryer to 15-17% moisture content.

P4 Ears harvested after 7 days of physiological maturity → peeling → piling the cob for 5 days → shelling → drying the seeds in a dryer to 15-17% moisture content.

P5 Ears harvested the ear after 7 days of physiological maturity → peeling → sun-drying the cobs up to 15-17% moisture content.

P6 Harvest → peeling → shelling → drying with dryer to 15-17% water content.

From each sample, 100 seeds were taken randomly, then put in 10 petriplates, each with 10 seeds. The experiment was arranged in a randomized complete design, with 10 replications. Observations were done daily to see infection of seeds by the fungal pathogen. The percentages of infected seed were calculated as per the following formula.

$$I = \frac{a}{a + b} \times 100\%$$

I = Infection (%)
a = Number of infected seeds
b = Number of healthy seeds.

Results

The symptoms of an *A. flavus* infected cob can be seen in Figure 1.

Laboratory observations showed that the infection rate and germination percentage varied among the drying treatments. This was true also of nongerminated seed (Table 1).

All the treatments were infected by *A. flavus*. The infection could have happened between the harvest time and the drying process, whether on the drying floor when sun-dried or in the dryer tank if machine-dried. According to Goldblatt (1969), Butter (1974), Lillehoj and Hesseltine (1977) cited *in* Dharmaputra *et al*. (1998), *A. flavus* can infect maize before and after harvest. Furthermore, Widiastuti *et al*. (1988) reported that fungal infection of maize seed can begin in the field or even in storage.

In treatments P3 and P4, the percentage of infected seed was 56% and 68% respectively, significantly different

Figure 1. A maize cob with seeds infected by *A. flavus* and other fungal pathogens.

from the other treatments. The high rate of fungal infection in these two treatments was caused by the high moisture content (30%) in the cobs when they were piled for 3-5 days which can stimulate the development of *A. flavus*. Sumartini and Yusnawan (2005) have reported that piled maize cob may lead to lower grain quality because of fungal contamination.

The infection rates suffered by treatments P1 and P2 were 18% and 15% respectively, which were not significantly different from treatments P5 (10%), P6 (9%) and P7 (10%). The low infection rates may have been the result of immediate drying after harvest.

Table 1. *A. flavus* infection rate and germination percentage (average of seven samples per treatment[1]) of maize seed under different seed-drying treatments (P1-P7).

Drying treatment	Infected seed	Germination of noninfected seed (%)	Germination of infected seed (%)	Nongerminated seed (%)
P1	18b	82b	16a	2c
P2	15b	85ab	10ab	5c
P3	56a	44c	7b	49b
P4	68a	32d	13ab	55a
P5	10b	90ab	8ab	2c
P6	9b	91a	8ab	1c
P7	10b	90ab	9ab	1c
KK	10	12	8	9

[1.] Means followed by the same letter were not statistically significant at $P = 0.05$.

Table 2. Storage fungal development at different moisture levels in maize seed.

Pathogen	Moisture content (%)	Damage
Aspergillus resistensi	13.5-14.5	Blue flecks appear on seed; seed physiological quality decreases
A. glancus	14.0-4.5	Change of endosperm color from white to brown.
A. candidus	15.0-15.5	Brown color of endosperm, causing heat to 55°C, producing poison, dangerous to humans and animals.
A. ochraccus	15.0-15.5	Brown colour of endosperm causing heat, producing ochtoxin poison that is dangerous to humans and animals
A. flavus	18.0-18.5	Brown colour of endosperm producing heat, producing toxins that is dangerous to humans and animals
Penecillium	16.5-19.5	Brown colour of endosperm; seed become soft, reduces germination.

Source: Christensen (1972).

Moisture content is a factor that affects fungal development. At a moisutre content of 20% or more, development of the fungus is fast; in contrast, at a water content <13%, fungal growth is slow (Table 2).

Conclusions

- Piling of cobs after harvest and late drying can stimulate development of *A. flavus*.
- Drying immediately after harvest with a dryer or under the sun can prevent development of *A. flavus infection*.

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Downy Mildew Outbreak in West Kalimantan Province of Indonesia in 2007

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Abstract. Bengkayang Regency is a centre of maize production in West Kalimantan with 232 013 ha under maize out of a total cultivated area of 37 771 ha. Downy mildew (DM) in maize, caused by a fungus Peronosclerospora maydis, was widespread in the province in 2007. A survey was conducted on June 27, 2007 to assess the infection. The outbreak had seriously damaged maize in two districts, Sanggau Ledo and Tujuh Belas, with the percentage of infected plants ranging from 26% to 100% depending on the varieties planted. The variety Pioneer-21 had the lowest infection percentage with 26-34%, followed by Jaya-1 with 52-527%, a local variety with 64-84%, BISI-9 with 90% and C-7 with 95-100%. Infection was severe although seed treatment had been done in the outbreak area with fungicide Saronil 35 SD containing metalaxyl as an active ingredient, or Ridomil Gold containing mefenoksam at the rate of 350 g L^{-1}. At the time of visiting the DM outbreak area in West Kalimantan, we were of the assumption that P. maydis had developed resistance to the fungicide metalaxyl. Two experiments conducted in 2007 and 2008 both proved so.

Key words: Maize, downy mildew, Peronosclerospora maydis, outbreak

Introduction

Downy mildew (DM) has been known for a long time in Indonesia. The disease has been reported to be caused by three species of *Peronosclerospora,* ie, *P. maydis*, *P. philippineosis* (Semangun 1993), and recently *P. sorghi,* identified from the highlands of Berastagi North Sumatera and Junrejo East Java (Wakman 2007). The disease has been reported from almost all provinces of the country. The common control method for DM in Indonesia is seed dressing with a fungicide containing metalaxyl as the active ingredient. Ridomil 35 WP and Saromil 35 SD, both containing metalaxyl, have been applied to control DM in Indonesia since 1980 (Jasis *et al.* 1981)

Outbreaks of DM occurred in Indonesia in 2006 and 2007. In 2007 the Director of BPTP of West Kalimantan wrote a letter to the Director of ICERI, asking for a researcher to visit West Kalimantan to identify the cause of the outbreak. (Wakman *et al.* 2008). The DM outbreak had occurred in an area where maize seeds had already been treated with metalaxyl. This indicated the possibility that *P. maydis* had become resistant to metalaxyl in West Kalimantan. During our visit to the outbreak area in mid-2007, a quick and simple experiment was conducted to test that hypothesis.

One month later, information from West Kalimantan was received from Dr. Tatang Ibrahim, Director of AIAT (Assessment Institute for Agriculture Technology), that DM was observed in all the experiments that had been treated with the fungicide and the infection rate was 100%. This observation strengthened the indication that resistance of *P. maydis* to metalaxyl had already happened in West Kalimantan. The test of resistance of DM to metalaxyl was then repeated in 2008 with a dosage up to two or three times the recommended dosage. These results confirmed that *P. maydis* was resistant to metalaxyl in West Kalimantan.

Method

Field observation

Observations were recorded by the author in several maize fields in four districts in Bengkayang Regency on May 27, 2007. At each location, three separate rows of maize in farmers' fields were taken as the sample plots, and the number of DM-infected plants was recorded.

Labolatory observation

Leaf samples of DM-infected maize plants were collected in the afternoon, washed with tap water and then put in a glass with a little sugar solution (1%) until 20 pm. The leaves were taken out and put in a transparent plastic bag which was kept outside the house until 4:00 am. The conidia were collected from the leaf surface by adding 100 mL distilled water and shaking. The conidial suspension was poured into a beaker, which was refrigerated for ± 2 hours to delay germination. The precipitate conidia at the bottom of the beaker were collected with a pipette and put in a tube to which alcohol (96%) was added to get a final concentration of 70% alcohol. A slide was prepared using a drop of lactophenol blue to which a drop of the conidial solution was added and covered with a cover glass. The conidia were observed under a light microscope.

Field experiment 2007

The effectiveness of the fungicides Saromil and Ridomil Gold used in West Kalimantan was tested in a randomized design with three replications. Each replication was located at the center of a DM-affected maize plot. The experiment was planted on June 26, 2007. There were four treatments:

(T-1) = Saromil seed treatment as done by seed company
(T-2) = (T-1) plus 2.5 g Saromil + 10 mL water per kg seed
(T-3) = (T-1) plus 2.5 g Saromil + 50 mL water per kg seed
(T-4) = (T-1) plus Ridomil Gold

Each treatment was planted in one-row, 5 m plots with a spacing of 75 cm between rows and 20 cm within each row and 2 seeds per hill.

Field experiment 2008

The test of resistance of *P. maydis* to metalaxyl was conducted in Bengkayang Regency in West Kalimantan. The experiment was arranged in a randomized block design with four replications. The treatments were four rates of Saromil fungicide containing metalaxyl:

- Control (0 g)
- 2.5 g per kg seed (recommended dose)
- 5.0 g per kg seed (2 × recommended dose)
- 7.5 g per kg seed (3 × recommended dose)

The maize variety Srikandi Putih was used in the experiment. The variety was planted as a source of inoculum on April 18, 2008. Treatment plots were planted on May 24, 2008. Observation for DM was done 30 days after planting.

Results

Field observation

Downy mildew disease of maize in Bengkayang Regency was found in two districts, Sanggau Ledo and Tujuh Belas, with average incidence ranging from 26% to 100% (Table 1).

Laboratory observation

The conidia of *Peronosclerospora* sp isolated from infected maize plants in West Kalimantan were round-shaped, similar to the conidia of *P. maydis* common in Java.

Field experiment 2007

It was reported by the Director of BPTP of West Kalimantan that at six weeks after planting all the four treatments of the experiment were 100% infected by downy mildew (Table 2). This meant that the fungicide containing metalaxyl had not been effective in controlling downy mildew.

Field experiment 2008

Downy mildew infection was very high in all the four experimental treatments, even on plots treated with high doses of Saromil (Table 3). The percentage of DM-infected plants was higher for the treated plots than for the control plot. Increasing the dosage of Saromil tended to increase DM infection.

Table 1. Percentage of downy mildew-infected maize plants in Bengkayang, June 2007.

District	Sample number	Downy mildew %	Variety
Sanggau Ledo	1	52-57	Jaya-1
	2	95-100	C-7
	3	26-34	Pioneer-12
	4	28-34	?
	5	90	BISI-9
Tujuh Belas	6	30-40	?
	7	64-84	Local
	8	55-71	?

Table 2. Downy mildew (DM) incidence after four fungicidal treatments with variety C7 in West Kalimantan.

Treatment	DM incidence
Fungi treated by seed company (T1)	100
(T1) + Ridomil Gold (mefenoksan a.i.) (T2)	100
(T1) + Saromil (farmers' treatment) (T3)	100
(T1) + Saromil 2.5 g kg^{-1} seed (T4)	100

Table 3. Downy mildew (DM) incidence under four different dosages of Saromil.

Dosage per kg seed	DM (%)
0 g (control)	89.7
2.5 g	91.7
5.0 g	96.3
7.5 g	98.0

Conclusions

The downy mildew outbreak on maize in 2007 was found mainly in two districts, Sanggau Ledo and Tujuh Belas of Bengkayang Regency, in West Kalimantan. Disease incidence was intense in areas where the seed had been treated with fungicide, indicating that *P. maydis* had become resistant to fungicides containing metalaxyl as the active ingredient. A rapid field experiment conducted in 2007 and a resistance test done in 2008 both showed that *P. maydis* has become resistant to fungicides containing metalaxyl in West Kalimantan.

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Maize Pest Status and Their Natural Enemies in Maize Production Area of Donggala, Central Sulawesi, Indonesia

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Abstract. This experiment was aimed at understanding the species composition and status of maize pests in Donggala district of Central Sulawesi province in Indonesia in order to identify an appropriate control strartegy and make location-specific IPM recommendations. Observations were recorded in seven villages in three subdistricts on 0.5-1 ha plots of farmers' fields during May-Aug 2007. The observations were recorded visually using sweep nets. The results showed that the maize pests found in Donggala were almost the same as elsewhere in terms of structure, infection and distribution. The most important of the maize insect pests were Ostrinia furnacalis Guenee, Helicoverpa armigera Boddie and Atherigana sp. The second rung of important pests were Dalbulus maidis Delong and Walcott, Rhopalosiphum maidis Fitch and Nezara viridula L. Another pest, Nicentrites testaceipes, was also found in high numbers. The other maize pests found were not important. These were Spodoptera litura F., Plusia calsites Esp., Lamprosema indicata F., Locusta migratoria and Valanga sp. Their natural enemies were predators and parasites. There were 12 species of predators found in our survey: Menochilus sexmaculatus, Scymnus sp, Epilachna sp, Coccinella transversali, Ophinea sp, Rodolia, Chrysopa, Euborellia annulata and Lycosa pseudoannulata. One species of larval parasite was found from the order/family Hymenoptera/Ichneumonidae. We also found that egg parasites were more effective in controlling O. furnacalis population compared with larval parasites. The percentage of egg mass of O. furnacalis parasited was 96% in all locations.

Key words: Maize pests, natural enemies, species composition

Introduction

A number of insect pests are associated with maize in Indonesia. Baco and Tandiabang (1988) reported that 50 insect species attack the crop, some of them important, causing serious damage. Rejesus and Javier (1985) and Teetes *et al.* (1983) also reported similar results in other Asian countries. One of the most important of these pests is the Asian corn borer (ACB; *Ostrinia furnacalis*). Annual maize yield loss caused by insect pests in developing countries was reported to be 30% (Berger 1962), and this is mainly caused by ACB, ear borer and army worm.

The egg parasite of the Asian corn borer and ear borer is *Trichogramma evanescens* (Nonci *et al.* 2000; Gonzales and Cadapan 2001; Javier *et al.* 2001), while the pathogens found to be effective in controlling the larvae of ACB and ear borer are *Beauveria bassiana* (Bals) and *Metarrhizium anisopliae* (Metch) (Supriyatin 1993; Yasin *et al.* 2000; Baco 2000). Information about the occurrence of these natural enemies of insect pests in the maize production area of Central Sulawesi is limited. Against this context, we conducted an experiment to understand the species composition and status of maize pests in Donggala district of Central Sulawesi.

Materials and Method

The experiment was conducted (May-Aug 2007) in farmers' fields in seven villages in three subdistricts of Donggala: Jono Oge and Sidondo villages in Biromaru subdistrict; Bahagia, Petimbe and Bobo in Palolo; and Labuan Toposo and Labuan Panimba in Labuan. Samples were collected from 0.25-1 ha maize plots, including 50 samples from each location, 10 samples from every corner and 10 samples from the middle of the plots.

Observation on insect pest species and their natural enemies was done visuallyby using a sweep net. The collected insect eggs were kept in a petri dish containing wet filter paper and labeled. Larvae were placed in plastic boxes (7 cm in diamter; 8 cm height) and also labeled. The larvae were fed corn stalks. The parasited pests were observed under a microscope at the AIAT laboratory with the help of identification guides by Teetes *et al.* (1983), Ortega (1987) and van der Laan (1981). Observations were done daily and the species and number of parasites were recorded and identified using the identification guides.

The parasited eggs were counted as per the formula:

$$P = \frac{a}{b} \times 100\%$$

where
P = Parasited eggs
a = Number of parasited egg masses
b = Number of observed eggs

Results and Discussion

Insect pests found

The result of our observations on maize pests and their natural enemies in Donggala are presented in Table 1. The maize pests found in Jono Oge and Sidondo villages in subdistrict Biromaru were *Ostrinia furnacalis*, *Dalbulus maidis*, *Spodoptera litura, Mythimna separata*, *Rhopalosiphum maidis*, *Nezara viridula*, *Nicentritis testaceipes, Valanga spp, Locusta migratoria* and one species of locust from the family Tettigonidae. On the basis of the level of infection, the plant part damaged and distribution, it was found that *O. furnacalis, R. maidis* and *N. viridula* were the most important maize pests in Biromaru followed by *S. litura, N. testaceipes, M. separata, Valanga spp, L. migratoria,* and pests from the families Tettigonidae and Accaridae.

Ostrinia furnacalis, D. m*aidis* and *N. testaceipes* were the most important pests in the three villages in Palolo subdistrict. The other maize pests found were *H. armigera, R. maidis, L. indicata, Valanga* spp and *L. migratoria*.

In Labuan subdistrict, the pests found were *O. furnacalis, R. maidis*, *N. viridula*, *Atherigona* sp, *D. maidis*, *S. litura, M. separata, N. testaceipes*, V*alanga* sp and *L. migratoria. Ostrinia furnacalis, Atherigona* sp, *R. maidis, N. viridula, D. maidis* and *N. testaceipes* were the most important.

The occurrence of these pests is related to the availability of host plant, plant stage, and climate. Fourteen species of maize pests were found in the villages, six from the order Lepidoptera (*O. furnacalis, H. armigera, S. litura, M. separata, Plusia calcites* and *Lamprosema indicata,* one species (*Atherigona* sp) from the order Diptera, one species (*R. maidis*) from the order Homoptera, one species (*N. viridula*) from the order Heteroptera, one species (*D. maidis*) from the order Hemiptera and two species (*Valanga* sp and *Locusta migratoria*) from the order Orthoptera, and two unidentified species from the families Tettigonidae and Accaridae. In this observation there was one newly found species, *Necentritis testaceipes*, with a high population. This insect pest attacked the maize plant in an early stage and the highest population was usually found at the beginning of the silking stage. According to Teetes *et al.* (1983), its larvae consume the green part of the leaf, leaving only the epidermal tissue.

The status and spread of all the maize pests in Donggala were almost the same. The most important pest was *O. furnacalis*. It was found in all locations in high humbers – an average of 1.2-7.3 insects per plant. This pest is the most important maize pest in Indonesia and in some Asia and West Pacific countries (Nonci *et al.* 1996; Nafus and Schreiner 1987; Tseng 2000). The next highest pest population was of *D. maidis, R. maidis* and *N. viridula.* Economically, *D. maidis* inflicts a huge financial loss because besides being an insect pest itself, it also acts as a vector of maize stunt spiroplasma, maize stunt mycoplasma and maize rayado fino virus (Pitree 1970; de Oliveira *et al*. 2007). Most of the damage done by this pest occurs during the seedling stage (4-6 weeks after planting).

Table 1. Maize pest[1] composition[2] in Donggala, Central Sulawesi, Indonesia, May-Aug 2007.

Location		Corn pest species															
Sub	Valley	O.f.	H.a.	At.	D.m.	S.l.	M.s.	R.m.	N.v.	N.t.	P.c.	L.i.	A.	V.	L.m.	T.	S.i.
Biromaru	Jono Oge	+++	-	-	++	+	+	++	++	++	-	-	+	+	+	+	+
	Sidondo	++	-	-	++	+	-	++	++	+	-	-	-	+	+	-	
Palolo	Bahagia	+++	++	-	+++	-	-	++	++	+++	+	+	+	+	+	+	+
	Patimbe	++	+	-	+++	-	-	++	++	+++	-	-	-	+	+	-	-
	Bobo	++	+	-	+++	-	-	++	+	+++	-	-	-	+	+	-	-
Labuan	Labuan Toposo	+++	-	++	++	+	+	++	++	++	-	-	+	+	+	+	+
	Labuan Panimba	++	-	-	+	-	+	++	++	++	-	-	-	+	+	-	-

1. O.f. = *Ostrinia furnacalis;* H.a. = *Helicoverpa armigera;* At = *Atherigona* sp, D.m. = *Dalbulus maidis;* S.l. = *Spodoptera litura;* M.s. = *Mithymna separate;* R.m. = *Rhopalosiphum maidis;* N.v. = *Nezara viridula;* N.t. = *Nicentritis testaceipes;* P.c. = *Plusia calcites;* L.i. = *Lamprosema indicate;* A = Acrididae; V. = *Valanga* sp., L.m. = *Locusta migratoria,* S.i. = *Sesamia inference;* T. = Tettigonidae
2. - = Not found; + = Low population; ++ = Moderately high population; +++ = High population.

Table 2. Average egg mass of *O. furnacalis* parasited by *T. evanescens* in three subdistricts of Donggala, Central Sulawesi, Indonesia.

No.	Location		Parasited egg mass (%)
	Subdistrict	Village	
1	Biromaru	Jono Oge	96.00
2		Sidondo	89.80
3	Palolo	Bahagia	59.73
4		Petimbe	-
5		Bobo	77.23
6	Labuan	Labuan Toposo	75.00
7		Labuan Panimba	81.95

Due to its role as vector of the sugarcane mosaic virus, maize dwarf mosaic virus and maize leaf-fleck virus, *R. maidis* is considered an economically important insect pest too (Anonymous 2008).

Nezara viridula is a polyphagous cosmopolitan insect and an important pest of maize. It attacks the plant by sucking the fluid from young ears. The symptoms are indicated by rolled husk. The rolling is very clear as the ear develops, resulting in opened ears.

Atherigona sp was found in Labuan but not in the other locations because when observations were taken in Labuan the maize plants were still young (2 weeks) while in the other locations they were already 4-8 weeks old. According to Kardinan *et al.* (1993) and Iqbal *et al.* (1993), *Atherigona* sp is a pest that attacks young maize plants, especially during the rainy season.

Natural enemies

The natural enemies of these pests found in our observation in Donggala were one parasite and 12 predators. The predators were *Menochilus sexmaculatus, Scymnus* sp, *Epilachna* sp, *Ophionea* sp, *Solenopsis geminata, Rodolia* sp, *Afidenta gradaria, Euborellia annulata, Chrysopa* sp, *Lycosa pseudoannulata,* and *Atipena* sp. *Euborella annulata* has the potential to be developed as a biological control agent because it has a high predation rate (Sitomurang and Gabriel 1988; Gonzales et *al.* 1995; Nonci 2005). This predator is available in enough pressure in the field and potentially can suppress the pest population, especially lepidopterous pests.

It was found that egg masses of *O. furnacalis* and *H. armigera* were parasited by *Trichogramma evanescens*. According to Pabbage *et al.* (1999) and Nonci *et al.* (2001), *T. evanescens* was effective in attacking the egg masses of *O. furnacalis* and *H. armigera* both in the laboratory and in the field.

The percentage of egg mass of *O. furnacalis* parasited by *T. evanescens* in the Donggala maize production area is presented in Table 1. The highest percentage of egg mass parasited (96.0 %) was found in the village Jono Oge in the subdistrict of Biromaru due to which the number of plants damaged by the pest was low. The number of holes found was two to three holes per plant. This result is almost the same as that reported by Pabbage *et al.* (2001) who found a *T. evanescens* parasite rate of 91.96% in the filed.

Natural enemies in the field can be used by farmers to reduce the use of pesticides to control maize pests.

Conclusions

The most important insect pests of maize found in Donggala district was *Ostrinia furnacalis* Guenee, *Helicoverpa armigera* Boddie and *Atherigana* sp. Next in importance were *Dalbulus maidis* Delong and Walcott, *Rhopalosiphum maidis* Fitch and *Nezara viridula* L. *Nicentrites testaceipes* was also found in high numbers.

The other maize pests found were not important. These were *Spodoptera litura* F., *Plusia calsites* Esp., *Lamprosema indicata* F., *Locusta migratoria* and *Valanga* sp.

The natural enemies of these pests were predators and parasites. There were 12 species of predator found: *Menochilus sexmaculatus, Scymnus* sp, *Epilachna* sp, *Ophionea* sp, *Solenopsis geminata, Rodolia* sp, *Afidenta gradaria, Euborellia annulata, Chrysopa* sp, *Lycosa pseudoannulata,* and *Atipena* sp.

One species of larval parasites found was from the order/family Hymenoptera/Ichneumonidae.

Egg parasites were more effective in the control of *O. furnacalis* compared with the larval parasite. The percentage of egg mass of *O. furnacalis* parasited ranged from 59.73% to 96% in all locations.

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Inheritance of Resistance to Southern Corn Rust in Maize

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Abstract. The inheritance of resistance to southern corn rust in maize caused by Puccinia polysora Underw. was studied in nine crosses of three resistant inbreds and three susceptible inbreds. The resistant inbreds were Nei 9202, Tzi 15 and Kei 0306. The susceptible inbreds were Nei 9008, Nei 432001 and Ki 11. Generation mean analysis was employed to study the gene action of rust resistance. Six generations of each cross including the female parent (P_1), the male parent (P_2), F_1, F_2 and backcross progenies to both parents (BC_1 and BC_2) were grown in August 2004 at the National Corn and Sorghum Research Center, Pakchong district in Nakhon Ratchasima province of Thailand. Occurrences of rust on leaves at 80 days after planting were investigated under natural inoculums. The results revealed that additive gene action for rust resistance was dominant in most of the crosses. However, the epistatic effect was found in some crosses. Resistance to southern corn rust was controlled by dominant genes. The type of gene effect involved complete, partial and absence of dominance depending on the level of resistance of the parents. Kei 0306, the resistant inbred, gave higher levels of resistance to southern corn rust in F_1, F_2, BC_1 and BC_2 generations. Thus, this inbred would have potential for breeding resistance to southern corn rust in maize.

Key words: Maize, southern corn rust, inheritance

Introduction

Southern corn rust, caused by *Puccinia polysora* Underw., has enormous destructive potential on susceptible maize hosts. *P. polysora* generally infects maize leaves at the preflowering stage. It then forms numerous pustules as the plant develops. The disease becomes severe and the leaf and sheath tissues are killed, resulting in the plant becoming prematurely desiccated. Severe infection of southern corn rust occurrs in the late rainy season in warm and high humidity conditions. In Thailand, southern corn rust generally has been regarded as a major pathogen of maize. The first recorded rust epidemic in Thailand was in 1956 but the destruction was not severe. In 1984, it was reported that maize-growing areas in Nakhon Ratchasema province suffered severe losses because of corn rust on sweet corn susceptible hybrids, especially inbred lines (Pupiphat 1986). Raid *et al*. (1998) and Rodriguez-Ardon *et al*. (1980) reported that southern rust caused yield losses of approximately 39-45% on susceptible maize hybrids.

Ullstrup (1965) identified a single dominant gene, *Rpp9*, from a South African introduction (PT 186208) that conferred resistance to *P. polysora* race PP_9, isolated in Indiana. Futrell *et al*. (1975) identified another source of single-gene resistance to race PP_9, but the relationship of this gene to *Rpp9* was not investigated.

Scott *et al*. (1984) studied the inheritance of resistance found in lines from four populations. He reported that one or two gene models with varying degrees of dominance were used to explain the resistance. Zummo (1998) reported a variation of disease symptoms, ie, pustule incidence, tumescence and sporulation among maize genotypes exhibiting components of partial resistance. The objective of this study was to determine the inheritance of resistance to southern corn rust in Thailand using generation means analysis.

Materials and Methods

Population development

Inbred line Nei 9202 from the Department of Agriculture (DOA), Tzi 15 from IIGA and Kei 0306 from Kasetsart University (KU) were used as the resistant parents (P_1) and inbred lines Nei 9008 and Nei 432001 from DOA and Ki 11 from KU as the susceptible parents (P_2). Nine crosses were made: Nei 9202 × Nei 9008, Nei 9202 × Nei 432001, Nei 9202 × Ki 11, Tzi 15 × Nei 9008, Tzi 15 × Nei 432001, Tzi 15 × Ki 11, Kei 0306 × Nei 9008, Kei 0306 × Nei 432001 and Kei 0306 × Ki 11. The F_1 from these nine crosses were self-pollinated and also backcrossed to both parents to obtain the F_2, BC_1 and BC_2 generations. Six generations of each cross with resistance control NSX022031, Suwan

4452 and susceptible control CPDK 888 were evaluated in the field under natural inoculum at the National Corn and Sorghum Research Center, Nakhon Ratchasema province, northeastern Thailand.

A randomized complete block with three replications was used as the field statistical design. Each entry was planted in a six-row plot 5 m long. Spacing between the rows was 0.75 m and spacing between plants within each row 0.25 m.

Field screening

Visual estimates of rust severity were performed on the 5th leaf below the ear leaf, ear leaf and a flag leaf at 80 days after germination (grain-filling period). Individual plants were evaluated for each population on a 0-5 scale described by Gupta (1983) in which 0 = no uredia, hypersensitive flecks; 1 = very slight to slight infection, one or two to few scattered pustules on lower leaves only; 2 = moderate number of pustules on lower leaves only (light infection); 3 = abundant pustules on lower leaves; few on the middle leaves (moderate infection); 4 = abundant pustules on lower and middle leaves, extending to upper leaves (heavy infection); and 5 = abundant pustules on all leaves, plants may prematurely dry or be killed by the disease (very heavy infection).

Data analysis

Generation mean analyses were carried out on the original scale to determine the gene effects of rust resistance. The mean observation of each character consisted of components of generation means proposed by Hayman (1958) to include six parameters: m = mean; [d] = additive effects; [h] = dominance effects; [i] = additive × additive interactions; [j] = additive × dominance interactions; [l] = dominance × dominance interactions. Student's t-test was employed to test whether each gene effect deviated significantly from zero, as suggested by Mather (1971).

Results and Discussion

The mean scores for southern rust reaction of P_1, P_2, F_1, F_2, BC_1 and BC_2 populations of different crosses made during 2004 are shown in Table 1. The southern rust-resistant lines (P_1) Nei 9202, Tzi 15 and Kei 0306 had consistently low scores in the range of 0.72-1.04; and the susceptible lines (P_2) Nei 9008, Nei 432001 and Ki 11 had disease scores in the range of 3.33-4.43. The resistant parent Kei 0306 exhibited greater rust resistance than the other resistant parents. Susceptible parent Ki 11 showed high rust rating in every crossing, which would make it the most susceptible inbred.

F_1 and BC_1 mean disease scores of all crosses were lower than those of mid-parent [$MP = (P_1 + P_2)/2$] and tended toward the resistant parents. This shows that the rust resistance characteristic is controlled by a dominant gene. The dominance level differed among the crosses. The F_2 populations had lower disease scores than P_2 but the scores were close to mid-parent. The BC_2 populations had mean disease scores close to P_2's scores. The generation ranges from resistant to susceptible were BC_1, F_1, F_2 and BC_2, respectively.

Generation mean analysis was performed on sets of original data to estimate the genetic effects of southern rust reaction. Results from the generation mean estimates

Table 1. Means and standard errors of disease reaction of different populations of maize obtained from resistant × susceptible crosses.

Population	Nei 9202 x Nei 9008	Nei 9202 x Nei 432001	Nei 9202 x Ki 11	Tzi 15 x Nei 9008	Tzi 15 x Nei	Tzi 15 x Ki 11	Kei 0306 x Nei 9008	Kei 0306 x Nei 432001	Kei 0306 x Ki 11
	0.98±0.03	1.03±0.02	1.04±0.03	0.95±0.04	1.02±0.03	0.98±0.03	0.72±0.11	0.77±0.08	0.74±0.02
P_2	3.40±0.01	3.65±0.12	4.31±0.26	3.40±0.03	3.52±0.09	4.43±0.08	3.33±0.003	3.70±0.08	4.37±0.08
F_1	1.39±0.08	1.44±0.06	1.63±0.09	1.83±0.08	2.61±0.13	2.19±0.36	0.71±0.08	1.28±0.07	0.59±0.09
F_2	2.19±0.04	1.83±0.12	2.19±0.11	2.59±0.09	2.77±0.03	2.83±0.17	1.59±0.16	1.75±0.09	1.53±0.11
BC_1	1.50±0.07	1.17±0.09	1.31±0.14	2.19±0.09	2.28±0.25	2.28±0.06	0.88±0.14	1.08±0.17	0.70±0.15
BC_2	2.68±0.05	2.60±0.12	3.11±0.15	2.97±0.09	3.00±0.02	3.25±0.08	2.17±0.16	2.54±0.21	2.32±0.14
MP [1/]	2.19	2.34	2.68	2.18	2.27	2.71	2.03	2.24	2.56
CV(%)	4.73	7.53	11.03	5.15	7.78	11.52	14.62	12.06	11.24
	0.25	0.38	0.64	0.31	0.51	0.8	0.6	0.58	0.5

[1/] MP = Mid-parent.

Table 2. Estimates[1] of gene effects and standard errors for resistance to southern rust in nine crosses.

Crosses	m	d	h	i	j	l
Nei 9202 x Nei 9008	2.59±0.24**	-1.21±0.01**	0.22	-0.16	0.06±0.17	-0.39
Nei 9202 x Nei 432001	2.12±0.56**	-1.31±0.06**	0.82	0.22±0.56	0.06	0.56
Nei 9202 x Ki 11	2.60±0.61**	-1.64±0.13**	0.9	0.08±0.60	0.16	0.67
Tzi 15 x Nei 9008	2.22±0.44**	-1.23±0.03**	1.89±1.04	0.4	0.89±0.26**	-2.27±0.64*
Tzi 15 x Nei 432001	2.79±0.51**	-1.25±0.05**	0.10±1.52	-0.01	1.06±0.50*	0.76
Tzi 15 x Ki 11	2.97±0.72**	-1.73±0.04**	0.24±1.55	0.46	1.51±0.21*	0.06
Kei 0306 x Nei 9008	2.29±0.77**	-1.31±0.05**	0.61	0.51	0.03±0.44	0.71
Kei 0306 x Nei 432001	2.00±0.67**	-1.47±0.06**	1.56	0.24±0.67	0.01±0.56	0.73
Kei 0306 x Ki 11	2.64±0.59**	-1.82±0.04**	-0.88	0.51	0.39±0.41	0.33±0.94

1. * and ** indicate significance at 0.05 and 0.01 probability levels, respectively.

for southern rust resistance are presented in Table 2. There were significant differences among their generations of each cross. The results indicated highly significant additive (d) gene effect for southern rust resistance in all crosses. Interaction of the gene was not found in Nei 9202 and Kei 0306 donor parents, while additive × dominant gene effect (j) was responsible in generation involving Tzi 15 donor parent such as Tzi 15 × Nei 9008 (0.89±0.26**), Tzi 15 × Nei 432001 (1.06±0.50*) and Tzi 15 × Ki 11 (1.51±0.21*).

Conclusions

Rust resistance was inherited through additive gene action playing a role in increasing rust resistance. However, the epistatic effect was found in some crosses. Resistance to southern corn rust was controlled by a dominant gene. The type of gene effect involved complete, partial and no dominance depending on the level of resistance of the parents. Kei 0306, the resistant inbred, gave the highest levels of resistance to southern corn rust in F_1, F_2, BC_1 and BC_2 generations. Thus, this inbred would have potential for breeding resistance to southern corn rust in maize.

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A Simple and High Throughput Technique for Field Inoculation of E*xserohilum turcicum* in Maize

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Abstract. Turcicum leaf blight (TLB) is a major disease that causes losses up to 98% in cool and humid conditions. Disease management depends upon resistant sources. However, nonavailability of a foolproof technique makes it difficult to locate resistant sources. Among the existing techniques, the hypodermic method is better but tedious, and causes injury to plants. To find a better alternative technique for inducing field infection, a modified treatment of actual lesion powder was compared with existing methods in glasshouse conditions with a susceptible control (CM 202). The treatment was tested in the field for refinement at a hot-spot location (Barapani, Meghalaya, India; 1000 m msl). Infected leaves were collected from a TLB-affected maize field and actual lesion area was collected. The collected portions of leaves were dried in the shade and pulverized. Two pinches of powder were applied in the central whorl at four growth stages from 2-3 to 5-6 leaf. Both single and repeated applications were tried with two susceptible controls, RCM 1-2 (local) and CM 202 and a resistant control, CM 104. Powder applied at various stages of growth had a significant difference. However, repeat application of powder had no significant difference from single application. Final disease data were recorded on an individual plant basis at the silk drying stage. Powder applied at the 3-4 leaf stage was found effective in inducing field infection abundantly, reaching the score of 5.0 (on a 1-5 rating scale). Hence, a single application of powder collected from the lesion area and applied to maize leaf whorls at the 3-4 leaf stage was found sufficient to induce a full field infection.

Keywords: Inoculation, technique, Exserohilum turcicum, maize

Introduction

Turcicum leaf blight (TLB) is a major foliar disease throughout the world causing significant losses (Adipala *et al.* 1993) where high humidity, low temperature and cloudy weather conditions prevail. The disease is caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. In India the disease is prevalent in the Himalayan region during the rainy season, in peninsular India during both rainy and postrainy seasons and in the plains of eastern India, particularly Bihar, during the postrainy season. Some major maize-growing regions in the states of Bihar, Karnataka, Maharashtra and Andhra Pradesh have been noted as endemic areas for the disease, which reduced grain yields drastically (Kachapur and Hegde 1988; Harlapur *et al.* 2000).

At the hot-spot location chosen for this study, all predisposing conditions for disease appearance and development prevail and make it difficult to manage the disease during the cropping season. Maize is the second most important cereal crop after rice in Meghalaya state of India and disease management is purely dependant upon resistant sources. Nonavailability of easy and effective techniques makes it difficult for plant breeders to locate resistant sources from the pool of populations/genotypes. Among the existing techniques of inoculation, the hypodermic method is better but it is tedious and causes injury to plants, thereby attracting secondary infection by mold and bacteria. Hence, the present study was conducted with the objective of finding a better technique for inoculation under field conditions.

Materials and Method

Selection of genotypes

CM 202 was selected for a comparative study of the three existing methods, ie, hypodermic, suspension and whole infected leaf powder (WILP) with the treatment of actual lesion powder (ALP). The best result of the comparison study was refined at the hot-spot location (Barapani, Meghalaya, India; 1000 m msl) with three genotypes, CM 202 (established susceptible control) RCM 1-2 (local susceptible control) and CM 104 (established resistant control). These three genotypes were used for single as well as repeat inoculation.

Methods of planting and design

Five plants were maintained for each treatment of four in the comparative study. The recommended schedule of practices was followed maintaining appropriate plant density. The seeds were sown for the refinement study during June-Sep in row system with the recommended package of practices. Three replications were maintained throughout the of study.

Inoculum preparation

Highly infected leaves were collected from a TLB-affected maize field. The leaves were dried in the shade and pulverized for making WILP powder inoculum. In the ALP method, the actual lesion areas are cut from the infected leaves and processed as in the case of WILP. Inoculum for the hypodermic method (Elliot and Jenkins 1946) and the suspension method (Dutta *et al.* 2005) was prepared following standard methodology.

Inoculation

The plants were inoculated at four stages of growth in terms of leaves, ie, 2-3 leaf, 3-4 leaf, 4-5 leaf and 5-6 leaves in the comparison trial. Inoculum ($1.5\text{-}2.0 \times 10^4$ conidia mL^{-1}) in the suspension method was inoculated through an atomizer in the whorl as well as on the top leaves. In the hypodermic method, inoculum ($1.4\text{-}1.5 \times 10^4$ conidia mL^{-1}) was applied with a hypodermic syringe at the selected nodal portion of the stem. Two pinches (about 60 mg) of the WILP powder (0.25×10^4 conidia gm^{-1}) as well as ALP (0.65×10^4 conidia gm^{-1}) was applied in the central whorls and water was sprinkled. Finally the whorl was covered with polythene to create humidity. In the refinement trial, the four above stages were inoculated in two ways, the single and repeat.

Disease intensity

Disease intensity was recorded using a 1-5 rating scale suggested by Payak and Sharma (1983). A slight modification in the scale was made by using zero, which indicated no disease symptoms. A disease reaction score of 0.1-2.0 was considered as resistant, 2.1-2.5 as moderately resistant, 2.6-3.0 as moderately susceptible, 3.1-4.0 as susceptible and 4.1-5.0 as highly susceptible. Disease intensity was recorded on an individual plant basis at the silk drying stage. The average of three plants in the comparison study and ten plants in the refinement study was taken from the respective treatment in each replication.

Statistical analysis

Nonparametric tests (Seigel and Castellan 1988) were applied for comparison of different treatments. The Kruskal Wallis test was applied to compare different inoculation methods and inoculation stages. The Mann-Whitney U-test was applied to compare single and repeat inoculations for different maize varieties.

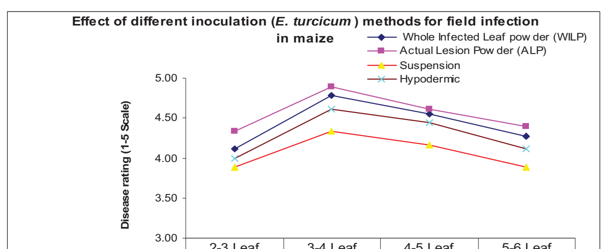
Results

Comparison of existing methods

Among the four existing methods of inoculation tested with the susceptible control CM 202, the modified treatment with actual lesion powder (ALP) was found best in creating field infections (Fig. 1) with a disease index of 4.89 followed by the WILP and hypodermic methods (4.78). The minimum disease index (4.33) was produced by the suspension method. The 3-4 leaf stage of plants was found suitable for inoculation which yielded the maximum disease index in almost all treatments followed by the 4-5 leaf stage.

Refinement of technique

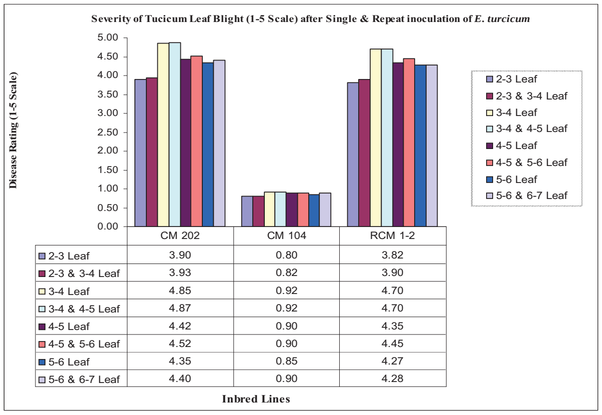
Powder applied at various stages of growth had a significant difference (Fig. 2). However, repeat application of the powder was not significantly different from a single application. The ALP applied at the 3-4 leaf stage produced sufficient disease (4.85) whereas in the repeat application at the 4-5 leaf stage, the same could produce a disease rating of 4.87 which was nonsignificant for CM 202. The disease intensity recorded for CM 104 (0.92) and RCM 1-2 (4.70) had no difference at the 3-4 leaf stage and repeated at 4-5 leaf stage. The single application of powder collected



	2-3 Leaf	3-4 Leaf	4-5 Leaf	5-6 Leaf
Whole Infected Leaf powder (WILP)	4.11	4.78	4.56	4.28
Actual Lesion Powder (ALP)	4.33	4.89	4.61	4.39
Suspension	3.89	4.33	4.17	3.89
Hypodermic	4.00	4.61	4.44	4.11

Growth Stages

Figure 1. Comparison of various inoculation techniques with the actual lesion powder (ALP) method on a 1-5 disease rating scale.



	CM 202	CM 104	RCM 1-2
2-3 Leaf	3.90	0.80	3.82
2-3 & 3-4 Leaf	3.93	0.82	3.90
3-4 Leaf	4.85	0.92	4.70
3-4 & 4-5 Leaf	4.87	0.92	4.70
4-5 Leaf	4.42	0.90	4.35
4-5 & 5-6 Leaf	4.52	0.90	4.45
5-6 Leaf	4.35	0.85	4.27
5-6 & 6-7 Leaf	4.40	0.90	4.28

Figure 2. Severity of turcicum leaf blight (1-5 disease rating scale) after single and repeat inoculation of *E. turcicum*.

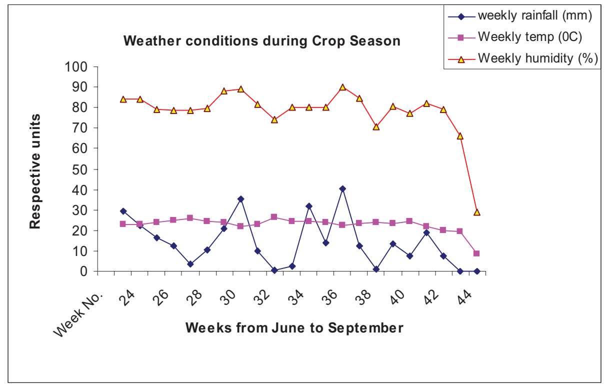


Figure 3. Weather conditions prevailing during the crop season.

from the ALP and applied in leaf whorls at the 3-4 leaf stage was found sufficient to induce a full field infection.

Test of significance

Results of the Kruskal Wallis test indicated that the different inoculation methods, ie, pulverized, AL pulverized, suspension and hypodermic methods differ significantly at 1% level of significance. While comparing the different inoculation stages by using the Kruskal Wallis test, it was found that different inoculation stages, ie, 2-3 leaf, 3-4 leaf, 4-5 leaf and 5-6 leaf, differ significantly at 1% level of the significance. The comparison of first inoculation and repeat inoculation by the Mann-Whitney U test indicated that there was no significant difference between the single and repeat inoculations for the genotypes CM 202, CM 104 and RCM 1-2.

Discussion

A number of different methods to induce field infection by *E. turcicum* have been reported (Ledencan *et al*. 2001). In this study, the actual lesion powder was found superior to other methods in terms of effectiveness, economy and ease of application. The efficacy of the powder was more than the other methods, only a negligible decrease in disease was noted as a result of the advanced stage inoculation. ALP applied to the leaf whorl resulted in the highest level of leaf blight despite a low conidial load. Disease severity was more than for any of the methods tried, which may be attributed to less competition for nutrients, oxygen, space and other unknown factors that increased the infectivity of conidia, resulting in more disease intensity.

Hypodermic inoculations were effective but their use resulted in serious mechanical injury to plants. Applications of liquid suspension of spores and mycelium in the leaf whorl gave desirable results but only with repeated application (Sharma 1999). The low level of leaf blight in the noninoculated control plots showed little natural infection in the beginning, resulting in slow spread of *E. turcicum*.

The climatic conditions required for TLB were congenial (Gowda *et al*. 1995) during the crop period from June to Sep (Fig. 3). Weekly rainfall was low (3.7 mm) and humidity (83.9 -88.1%) and temperature (22.0-25.5^{0}C) were consistent during initial crop growth. The climatic conditions were favorable from inoculation to the disease development. However, fluctuation in rainfall and humidity was noted during disease development. The higher humidity was more favorable for disease spread, giving more effective results in each treatment.

Our results show the usefulness of ALP in creating sufficient disease pressure required for meaningful resistance screening. This method has an additional advantage in that it can be used by investigators who do not have facilities for isolating and culturing the pathogen. Also, relatively nonskilled person may be employed in conducting most of the operations.

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Response of Maize Germplasm to Maize Weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae)

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Abstract. The maize weevil, Sitophilus zeamais Motsch., is the most important insect pest of stored products in the world including Indonesia. More than 50% grain damage has been reported for nontreated maize during storage on account of this pest. This study was conducted to evaluate the resistance response of maize germplasm to maize weevil infestation. A total of 44 lines/varieties, obtained from a maize breeding project, were examined by the Pest and Disease Laboratory of the Indonesian Cereal Research Institute (ICERI) in Maros, Indonesia during May-Sep 2007. Samples of each line/variety (25 g) were infested with 10 adults of 1-7 days old Sitophilus zeamais for 10 days. Each experiment was arranged in a randomized complete block design with three replications. We recorded the total number of adult maize weevils emerged, percentage of grain damage and weight loss at 90 days after storage. The result indicated that 9 lines/varieties were resistant to maize weevil with a percentage of damage ranging from 1.51% to 9.84%. These lines were Bualemo, Holotaupi, Lagaligo, Trunen B, MLG-5216, ACROSS8365, VIH-2, GM-26 and CIMCALI97ACHAPIASA3.

Key words: Maize, maize weevil, germplasm

Introduction

One of the causes of damage to and weight loss of food material, especially grain, during storage is storage pests such as *Sitophilus zeamais* (Motschulsky). The maize weevil (*S. zeamais*) is a beetle belonging to the Curculionidae family. It has a characteristic snout or rostrum projecting from the front of the head. The *S. zeamais* pest is about 2.5-4 mm long and is identified by its distinctive yellow to orange colouration on the wing covers. According to Kartasapoetra (1987), the form of the S. *zeamais* imago's head is long with a snout. The wings can expand finely, so that they are capable of flight.

The egg, larval and pupal stages of the weevil occur in the kernels and are rarely seen. Feeding is done within the grain kernel, and adults bore exit holes to emerge. Females drill a tiny hole in the grain kernel, deposit an egg in the cavity and then plug the hole with a gelatinous secretion. The egg hatches into a young larva which bores toward the center of the kernel, feeds, grows and pupates there. New adults bore emergence holes from the inside, then leave to mate and begin a new generation. The adults fake death by drawing their legs close to the body, falling and remaining still when disturbed. The larvae only develop at temperatures above 13°C (http://agspsrv34.agric.wa.gov.au).

Sitophilus zeamais is a serious pest of stored grain. They develop inside whole grain kernels as small, white, wrinkled, grub-like larvae. There generally are no external signs that the larvae have been feeding and growing inside the seed until after about one month when the adult weevils chew through the seed coat and emerge. They are frequently regarded as primary pests of grain since they are able to infest otherwise nondamaged grain. Infestations can result in reduction in weight and quality of grain as a result of the larvae feeding on the endosperm. Both larvae and adults feed on grain. Temperatures may be attained which actually kill the insects. Weevil-damaged grain can be readily recognized by the presence of large exit holes.

Weevil infestation may start in the field. Varieties with poor husk-covering properties are vulnerable to initial infestation (Giles and Ashman 1971). The main focus of improvement in maize breeding programs has traditionally been increased yield and field pest resistance. Since varieties are rarely assessed for resistance to stored-product pests, introduction of improved varieties has often been accompanied by increased susceptibility to stored-product pests (Kossou *et al.* 1993).

According to Pabbage *et al*. (1990), the percentage of grain weight loss after 6 months of storage can reach 80.3% when kept in a plastic bag.

The aim of this study was to evaluate the resistance response of maize germplasm to infestation by *S. zeamais*.

Materials and Method

This experiment was conducted at the Pest and Disease Laboratory of ICERI during May-Sep 2007. The grain samples were first stored in a freezer at –6°C for 14 days to remove (kill) any field infestations (live insects or eggs) by weevils or any other pests. A total of 44 accessions of maize germplasm were tested using the method described by Dobie (1974). Samples of 25 g each were stored in 150 mL plastic pots with screen ventilation. The samples were infested with 10 adults of *S. zeamais* (1-7 days old). After an oviposition period of 10 days, the weevils were removed. The samples were then left undisturbed under the experimental conditions until F_1 adults started to emerge about 30 days later. The emerging adults were removed daily and counted until all the F_1 adults had emerged. The experiment was arranged in a randomized complete design with three replications.

When no more maize weevils emerged, the damaged and healthy grain were counted and their weight was recorded. The percentage of grain damage was calculated as per the formula:

$$\text{Percentage of grain damage} = \frac{\text{number of damaged grain}}{\text{total number of grain}} \text{ x } 100\%$$

The grain weight loss percentage was calculated by the following formula according to Heines (1980):

$$\text{Percentage of grain weight loss} = \frac{(U \times Nd) - (D \times Nu)}{U\,(Nd + Nu)} \times 100\%$$

U = weight of healthy grain
Nd = number damaged grain
D = weight of damaged grain
Nu = number of healthy grain

Results and Discussion

The percentage of grain damaged (Table 1) by *S. zeamais* in 44 maize accessions ranged from 1.51% (Bualemo) to 51.67% (Panyipatan). The number of *S. zeamais* emerged adults were 1-5 for five of the 44 accessions, 7-14 for 15 accessions, 15-30 for 13 accessions, 31-58 adults for 6 accessions and 60-104 adults for 5 accessions.

There were 9 accessions with grain damage less than 10%: Bualemo, Holotaupi (2.31%), Lagaligo (6%), TrunenB (6.05%), MLG-5216 (6.51%), Across 8365 (8.09%), Vih-2 (9.14%), GM-26 (9.53%) and Cimcali97Achapiasa3 (9.84%). Sixteen accessions had grain damage percentages in the range of e”10 % to d”20%. Grain damage ranged from e”20% to d”30% in 13 accessions, from e”30 % to d”40% in 5 accessions. There was one accession with damage more than 50%. Dobie (1974) had reported that the susceptibility of a variety depends upon the number of eggs laid and the subsequent survival and growth of the larvae and pupae.

The percentage of grain weight loss due to S. *zeamais* in the 44 accessions ranged from 0% (MLG-5216) to 6.20% (Panyipatan). Grain weight loss was 0-2% for 25 accessions, e”2-3% for 12 accessions, e”3-4% for two accessions, e”4-5% for three accessions and e”5-for two accessions.

According to Kim *et al.* (1988), the external coat of structured maize kernel material can represent the primary factor of resilience to situating of maize weevil eggs. Kernel size is more important in determining resistance to *S. zeamais* with large kernels appearing to show greater resistance than smaller ones (Gudrups *et al.* 2001). The resistance of stored maize to pests has been attributed to a number of factors including kernel hardness (Dobie 1974; Serratos *et al.* 1987), kernel size and texture (Kossou *et al.* 1993), phenolic content (Arnason *et al.* 1994). According to Tipping *et al.* (1989), the resistance factors were principally influenced by general combining ability and maternal effects were not expressed in the F_1 generation of maize weevil.

The kernel color (Table 1) does not affect kernel damage, percentage of weight loss and number of *S. zeamais* emerged adults. Resistance to maize weevil is classified by percentage of kernel damage, weight loss and the number of *S. zeamais* emerged adults. Resistant maize accessions had kernel damage percentage less than 5%, weight loss less than 1% and 1-3 *S. zeamais* emerged adults. Intermediate varieties had 6-d”50% kernel damage, less than 4% weight loss and e”3-d”50 emerged adults. Susceptible varieties had a percentage of kernel damage less than 50%, weight loss of more than 4% and more than 50 emerged adults.

Conclusions

The varieties Lagaligo and Holotaupi showed more resistance to maize weevil than the other accessions evaluated. The number of *S. zeamais* emerged adults was small for both varieties (3 for Lagaligo and 1 for Holotaupi) with low grain damage of 6% for Lagaligo and 2.31% for Holotaupi and a low percentage of grain weight loss of 0.32% and 0.26%, respectively.

Table 1. Percentage of grain damage, grain weight loss and number of adult maize weevils (*S. zeamais*) after 90 days of infestation of 44 maize accessions, Maros, Indonesia, 2007.

No.	Lines/varieties	Color	*Sitophilus zeamais*	Damaged grain (%)	Grain weight loss (%)
1	Bualemo	White	1	1.51	4.13
2	Holotaupi	Yellow	1	2.31	0.26
3	Lagaligo	Yellow	3	6	0.32
4	SW-89A-121	White	4	12.41	0.97
5	MLG-5216	Brown	4	6.51	-1
6	Cimcali97ACHAPIASA3	Yellow	7	9.84	0.59
7	Palakka	White	7	13.66	0.98
8	GM-26	Yellow	8	9.53	1.45
9	Trunen B	Yellow	10	6.05	0.29
10	Pakelo	White	10	20.12	1.96
11	L.Poso	White	10	13.36	0.92
12	P.Marisa	Yellow	10	10.59	0.77
13	MLG-5073	Brown	10	13.95	1.39
14	Penjalinan	White	10	13.09	0.42
15	Srikandi Kuning-1	White	11	19.75	1.43
16	MLG-5194	Yellow	11	12.59	1.03
17	GM-27	White	11	14.96	1.91
18	MR-14	White	12	16.77	1.36
19	L.Palopo	Yellow	14	22.98	2.85
20	MLG-5215	White	14	13.93	2.79
21	Dalo-dalo	Brown	15	12.36	1.45
22	S99TLWQ-AB	Yellow	17	28.09	2.34
23	Sinematiali	Yellow	18	21.52	2.22
24	Permadi	Brown	19	21.45	2.67
25	L.Blora	White	19	12.36	1.45
26	Milo Pulo	Yellow	19	23.06	2.42
27	Harapan Baru	Yellow	20	15.27	1.75
28	MLG-5226	Yellow	23	19.11	1.8
29	MLG-5064	White	24	23.25	2.9
30	MR-10	Yellow	24	23.14	4.54
31	Kalipare	White	27	23.38	2.35
32	MLG-5200	Yellow	28	17.62	1.27
33	Tempx Trop YDQ	White	29	24.44	2.55
34	Obatama=Across 8363	Yellow	31	36.34	1.07
35	Across 8365	Yellow	33	8.09	1.26
36	VIH-2	Violet	34	9.14	1.42
37	Leinasi Kuning	Yellow	42	35.29	2.92
38	Jawa Kuning	Yellow	56	20.69	3.06
39	CIR-3-3-1	Yellow	58	23.61	2.57
40	Hideradab	Yellow	60	39.73	4.22
41	CIR/14/CIR	Yellow	66	30.33	5.87
42	Leinasi Putih	Yellow	72	35.72	2.87
43	Panyipatan	White	77	51.67	6.2
44	L.Kusa	Mixture	104	27.38	3.08

CIR/14/CIR and Panyipatan were the most susceptible to *S. zeamais*, the number of maize weevils for each variety was 66 and 77 adults respectively. Grain damage was 30.33% for CIR/14/CIR and 51.67% for Panyipatan, and percentage of grain weight loss was 5.87% and 6.20%, respectively.

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Influence of Storage Temperature and Duration on Egg and Pupal Development of *Chilo partellus*

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Abstract. The ability to store different insects at different stages of their life cycle for prolonged periods provides considerable flexibility to researchers in conducting their experiments. The present study was undertaken to determine the effect of storage temperature and duration on the development of eggs and pupae of Chilo partellus (Swinhoe). Two-day-old eggs were incubated at different storage temperatures (5, 10, 15, 23 and 33ºC) and durations (1, 3, 5 and 7 days). The pupae were exposed to 10ºC for 2 and 5 days to examine the effect on developmental period and adult emergence. The percentage egg hatch and incubation period were influenced by storage temperature and duration. Egg hatch ranged from 15.9% to 94.6% across temperatures and storage durations. When the eggs were stored at 5ºC for ³3 days, egg hatch was reduced (15.9-65.6%). However, hatchability was not affected after storage of eggs at 10ºC (except for 7 days), 15ºC and 33ºC. The egg incubation period was less (4.8 days) at 33ºC. However, the storage of eggs at 5, 10 and 15ºC increased the incubation period. The regression model for egg hatch explained only 72.2% variation (p=0.01), whereas forecasting of incubation period based on temperature, storage duration and temperature × storage duration was quite effective (R^2=0.91, p=0.01). It is safer to store C. partellus eggs at 10°C (for 3-5 days) or 15°C (for 3-7 days) to increase incubation period for 2-5 days without affecting egg hatch. The storage of pupae at 10ºC increased developmental period (11.7–15.5 days) without affecting adult emergence.

Key words: Chilo partellus, storage, egg hatchability, temperature, incubation period

Introduction

Spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is an important pest of rainy-season maize in South Asia (Hari *et al.* 2008). In India, it attacks maize in the early whorl stage and causes yield losses of 26-80% in different agroclimatic regions of the country (Panwar 2005). The use of resistant cultivars is a good alternative in pest management programs in developing countries. For developing resistant cultivars against *C. partellus*, large numbers of maize genotypes have to be artificially screened in a field environment. Resistance in maize to *C. partellus* is determined by artificially infesting the crop using egg masses at about the 2-week-old stage (Kumar 1992). The success of a screening programme relies on the timely supply of egg masses in adequate numbers at this specific stage of plant development. Moreover, being a key pest of maize, this pest is routinely used in research on insect-host plant interactions, insecticide resistance, transgenics, biological, physiological and behavioral studies.

It is desirable to manipulate various aspects of insect biology in order to have adequate numbers of insects that are at an appropriate stage of development for experimental purposes (Dhillon and Sharma 2007). Insects are poikilothermic organisms and ambient temperature has a major effect in determining their rate of development. They can be exposed to low temperatures to slow down the rate of development (Marrone *et al.* 1985). Extreme low temperatures, however, can have a lethal effect on their metabolic functions. Of the various developmental stages, eggs can be targeted for manipulating the insect biology (Dhillon and Sharma 2007). In the case of *C. partellus,* the effect of temperature on the adult stage has been reported (Shukla and Bains 1975) but information on the storage of eggs at low temperatures is lacking. We hypothesized that storage of *C. partellus* eggs at low temperature would delay hatching by a few days so as to meet the infestation requirement at an appropriate plant stage. Therefore, the objective of this study was to determine the effect of a range of storage temperatures and duration on the development and hatchability of *C. partellus* eggs. Further, the pupal stage was exposed to different low temperature-storage duration combinations to investigate the effect on pupal development.

Materials and Method

Exposure of C. partellus eggs to different storage temperature-duration combinations

The eggs of *C. partellus* were obtained from a laboratory culture maintained on a green gram-based artificial diet (Kanta and Sajjan 1989) The culture was maintained as described by Hari *et al*. (2008). The adults were kept in oviposition jars (15 × 10 cm dia) at 23±2°C and 70% RH and regularly provided with water. The inside of each oviposition jars was lined with wax paper to serve as the oviposition substrate. The wax paper sheets having egg masses were detached and divided into small pieces for experimental purposes. Two-day-old eggs were exposed to 5, 10, 15, 23 (control) and 33ºC for 1, 3, 5 and 7 days. These were placed on moist filter paper in plastic vials (6 × 4 cm dia). Each treatment combination was replicated five times in a completely randomized design and there was one egg mass (25-30 eggs) per replication. At the end of the exposure period, the eggs from different treatments were incubated at 23°C. The eggs were examined at 12 h intervals in order to determine the percentage egg hatch and incubation period.

Pupal exposure to different storage temperature-duration combinations

Fresh *C. partellus* pupae (<12 hours of age) taken from the laboratory culture were exposed to 10ºC for 2 and 5 days. The pupae in the control set were kept at 23°C. Each treatment was replicated five times by taking 10 pupae (male:female in a 1:1 ratio) per replication. The pupae were kept in glass jars (15 × 10 cm dia) lined with moist filter paper. At the end of the exposure period, the pupae were again exposed to 23°C and observed daily to record adult emergence. The data on pupal period and percentage adult emergence were recorded from the different treatment combinations.

Statistical analysis

The data on egg hatch, egg incubation period, pupal period and adult emergence were subjected to two-way analysis of variance (ANOVA) and different treatment means were separated using the least significant difference (LSD) test. The data on percent egg hatchability and adult emergence were arc sine transformed prior to statistical analysis. Since storage temperature and storage duration interact to influence egg hatch and incubation period, multiple regression was used to determine the combination of these variables for forecasting of incubation period and percentage hatchability.

Results ad Discussion

Exposure of C. partellus eggs to different storage temperature-duration combinations

The percentage egg hatch and incubation period were significantly influenced by storage temperature ($F_{4,80} = 9.78$, $p = 0.05$ for egg hatch; $F_{4,80} = 941.20$, $p = 0.01$ for incubation period), storage duration ($F_{3,80} = 7.56$, $p = 0.05$ for egg hatch; $F_{3,80} = 213.12$, $p = 0.01$ for incubation period) and their interaction ($F_{12,80} = 3.12$, $p = 0.05$ for egg hatch; $F_{12,80} = 38.51$, $p = 0.01$ for incubation period). Egg hatching varied from 15.9% to 94.6% in different treatment combinations (Table 1). When *C. partellus* eggs were stored at 5°C for ^{3}3 days, hatchability was reduced (15.9-65.6%). However, hatchability was not affected by storage of eggs at 10°C (except for 7 days), 15°C and 33°C. When the data were

Table 1. Effect[1] of different temperature-storage duration combinations on egg hatch and incubation period of *Chilo partellus* under laboratory conditions.

Storage temperature	Storage duration (days)							
	Egg hatch (%)				Incubation period (days)			
(°C)	1	3	5	7	1	3	5	7
5	90.6±5.8d	65.6±4.5bc	51.9±13.2b	15.9±8.7 a	10.0 ± 0.1 d	11.4 ± 0.2 e	14.6 ± 0.1gh	15.0 ± 0.2 hi
10	91.1±3.6 d	77.6±9.8cd	78.1±7.9cd	55.2±17.2bc	9.0 ± 0.1 bc	11.1 ± 0.4 e	14.2 ± 0.1 g	15.4 ± 0.6 i
15	94.6±1.2 d	92.5±6.0 d	88.7±4.3 d	78.8±10.2cd	10.1 ± 0.1 d	11.0 ± 0.1 e	13.0 ± 0.2 f	14.1 ± 0.1 g
23	87.7±4.5 d	81.5±7.4cd	78.6±5.2cd	85.1±9.6 d	9.0 ± 0.2 b	9.2 ± 0.2 bc	9.4 ± 0.2 c	9.1 ± 0.2 bc
33	88.5±0.7 d	87.8±1.9 d	88.6±0.8 d	91.4±1.6 d	4.8 ± 0.2 a	4.9 ± 0.1 a	4.8 ± 0.2 a	4.7 ± 0.2 a

[1.] Means ± SE; means with different letters are significantly different (LSD test).

pooled over storage durations, eggs stored at 5°C and 10°C had low egg hatch (Fig. 1a). At different temperatures, the storage of eggs for 1 day did not have any effect on hatchability (87.7-94.6%) (Table 1). When the data were pooled over storage temperatures, eggs stored for 1 day had higher egg hatch. However, storage duration of 7 days adversely affected the egg hatch (Fig. 2a). The regression model did not improve the precision of forecasting in egg hatch beyond 72.2%. Egg hatch (%) = 105.20 – 0.63 (temperature) – 10.80 (storage duration) + 0.40 (temperature × storage duration) ($R^2 = 0.72$, $p = 0.01$)

For *C. partellus* eggs stored at different temperatures, the egg incubation period ranged from 4.7 to 15.4 days (Table 1). At 33°C, the eggs took significantly less time (<5 days) to hatch. However, storage of eggs at 5°C, 10°C and 15°C increased the incubation period when compared with control. The eggs stored for ^{3}3 days at 5°C, 10°C and 15°C hatched 2.2-5.8, 1.9-6.2 and 1.8-4.9 days later than in control respectively. When the data were pooled over storage durations, eggs stored at 5°C, 10°C and 15°C had higher incubation period (12.0-12.7 days) than in control (9.2 days) (Fig. 1b). Similarly, when the data were pooled over storage temperatures, the storage duration had significant effect on incubation period (Fig. 2b). It is advisable to store *C. partellus* eggs at 10°C (for 3-5 days) or 15°C (for 3-7 days) to increase incubation period for 2-5 days without affecting egg hatch. On the other hand, storage of eggs at 33°C would reduce incubation period by 4.2-4.6 days than in control. The regression model for incubation period explained 91.4% variation {Incubation period (days) = 10.13-0.12 (temperature) + 1.28 (storage duration) - 0.43 (temperature × storage duration) ($R^2 = 0.91$, $p = 0.01$)}.

Fig 1. Effect of storage temperature on egg hatchability and incubation period of *Chilo partellus*

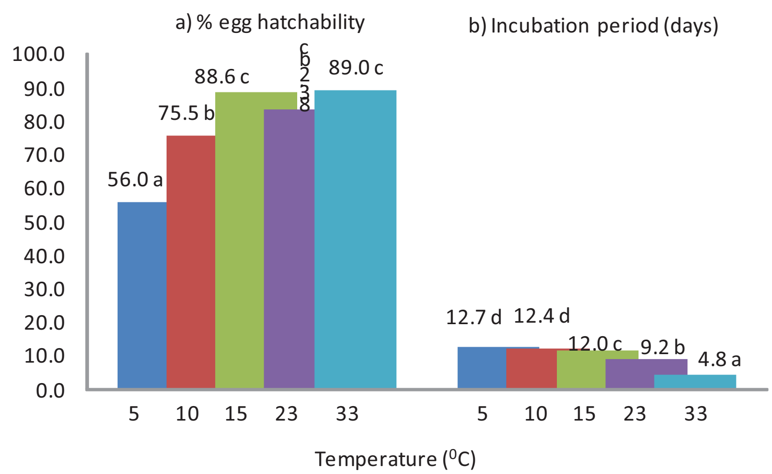
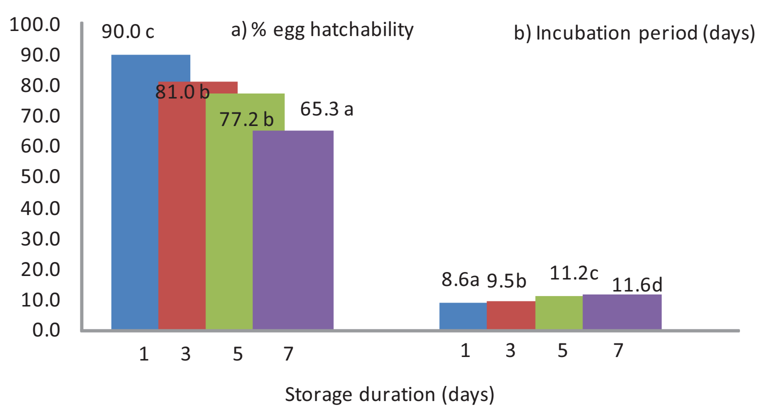


Fig 2. Effect of storage duration on % egg hatchability and incubation period of *Chilo partellus*



Pupal exposure to different storage temperature-duration combinations

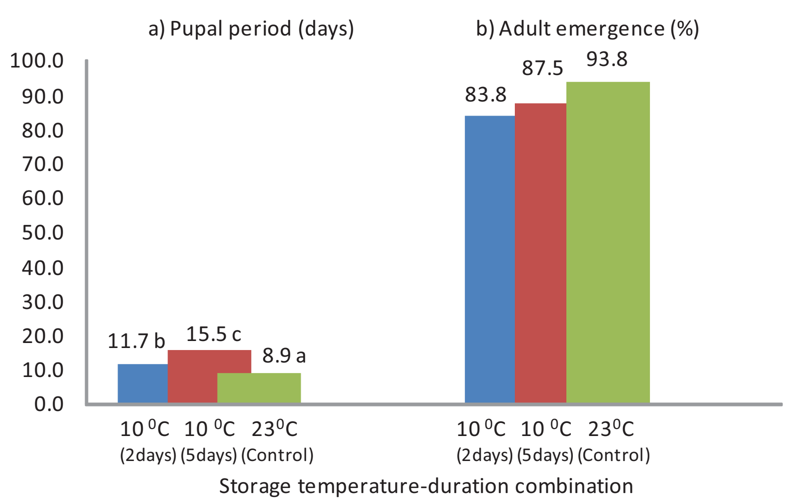
At 10°C, the pupae had higher developmental period than in control ($F_{2,24}$ = 130.10, p = 0.01) (Fig. 3a). Moreover, pupae stored at 10°C for 5 days had greater developmental period when compared with storage duration of 2 days. Adult emergence, however, was not affected by storage of pupae at 10°C (Fig. 3b).

The responses of insects to environmental extremes have an important bearing on their life cycles and survival. Cooling and freezing, and rates of temperature change influence physiological responses of insects including *C. partellus* (Sinclair *et al.* 2003). The results of our study suggest that temperature is an important regulatory factor in determining the abundance of *C. partellus* populations. The hatching of eggs was affected by storage at 5°C for ³3 days. When such a low temperature prevailed for a week, about 85% of the eggs did not hatch. In a recent study, Dhillon and Sharma (2007) revealed that storage of 2-day-old eggs of *Helicoverpa armigera* (Hubner) at 5°C for ³5 days was detrimental to egg hatch. According to Yokoyama and Miller (1989), the eggs of *Cydia pomonella* (Linnaeus) developed below 10°C, but the eggs died after storage at 0°C for 14 days. In our study egg hatch was not affected when stored at 10°C (except for 7 days) and 15°C. Dhillon and Sharma (2007) also reported that *H. armigera* eggs could be stored for 10 days at 10°C with a hatchability of >75%. On the other hand, a temperature of 33°C did not have an adverse effect on hatching of *C. partellus* eggs. Earlier, Shukla (1974) reported that survival of *C. partellus* eggs was 78.5% when kept at 32.5°C. He emphasized that eggs can better survive around 30°C. However, the exposure of *C. partellus* adults to 45°C for 2 hours resulted in low egg hatch (Shukla and Bains 1975).

When the eggs were stored at 33°C, the incubation period was less than 5 days. This implies that early requirement of *C. partellus* larvae could be met by storing the eggs at 33°C. This study corroborates the findings of Shukla (1974), who recorded mean duration of *C. partellus* eggs as 10.5 and 4.1 days, when kept at 20 and 32.5°C respectively. However the incubation period was higher, when the eggs were stored at 5, 10 and 15°C. Dhillon & Sharma (2007) reported that incubation period of *H. armigera* eggs could be increased by 3-6 days after storage at 10 or 15°C. From the present investigations, it is evident that *C. partellus* eggs could be stored at 10°C (for 3-5 days) or 15°C (for 3-7 days) to enhance incubation period for 2-5 days without affecting egg hatch.

Storage of *C. partellus* pupae at 10°C increased the developmental period (11.7–15.5 days). However, percentage adult emergence was unaffected after storage at low temperature. Our results, therefore, provide evidence that pupae can be stored at 10°C to delay adult emergence. The information generated here can be used for egg or pupal storage at an appropriate temperature for planning and execution of experiments involving *C. partellus*. Apart from the application of this information on storage at lower temperature to delay development, it could also be used for predicting emergence of neonate larvae or adults under different environmental conditions to design appropriate control strategies.

Fig 3. Effect of storage temperature and duration on pupal period and adult emergence of *Chilo partellus*



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Egg Parasitoid *Trichogramma chilonis* in the Management of Maize Stem Borers

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Abstract. These experiments were conducted in farmers' fields in Andhra Pradesh state of India during kharif (rainy season) in 2004/05, 2005/06 and 2006/07 to manage maize stalk borer Chilo partellus and during rabi (postrainy season) in 2006/07 to manage the pink stem borer, Sesamia inferens. The first treatment consited of farmers' practice. In the second, Trichogramma chilonis, an egg parasitoid, was released in the field by pinning egg cards to the under side of leaves uniformly in the field at the rate of 3 egg cards per 0.40 ha. We found that the egg parasitoid Trichogramma chilonis can be used effectively in managing stem borers C. partellus and S. inferens infestation in maize irrespective of the cultivars grown.

Introduction

Maize is infested by 139 insects at different stages of crop growth. Among them only stem borers are a major problem and require insecticidal intervention. Losses due to *Chilo partellus* (Swinhoe) during the rainy season range from 26.7% to 80.4%. Losses due to *Sesamia inferens* Walker range from 25.7% to 78.9% during the postrainy season (winter) in different agroclimatic regions of India (Chatterjee *et al*. 1969). Several workers have reported the use of insecticides for controlling borers. In general, farmers use endosulfan spray or carbofuran granules for controlling these pests. To minimize or avoid using insecticides, the egg parasitoid *Trichogramma chilonis* is an ideal alternative in managing stem borers at the egg stage itself. It is cost-efficient, environmentally safe and socially acceptable.

Method

Our experiments were conducted in farmers' fields in Andhra Pradesh during the *kharif* (rainy) seasons of 2004/05 2005/06 and 2006/07 for managing the maize stalk borer *Chilo partellus* and during *rabi* (postrainy) season of 2006/07 for managing the pink stem borer, *Sesamia inferens* (Table 1). The trial consisted of two treatments each planted over a 0.40 ha area in farmers' fields. The first treatment consisted of the farmers' practice. In the second, *T. chilonis*, an egg parasitoid, was released in the field by pinning egg cards to the under side of leaves. The egg cards were distributed uniformly in the field at the rata of 3 per 0.40 ha. The farmers spray endosulfan 35 EC at 3 mL L^{-1} of water 10-15 days after germination. Data on the number of plants infested, number of dead hearts formed and grain yield in the two treatments were recorded.

Results and Discussion

Data on percentage of plants infested and dead hearts formed and grain yield (Table 1) indicated that release of *T. chilonis* effectively controlled the borer in the egg stage itself compared to the farmers' practice of spraying endosulfan. During the rainy season of 2004/05 the percentage of plants infested and dead hearts formed in the egg parasitoid-released fields was 8.61% as opposed to 13.99% plants infested and 2.23% dead hearts formed in the farmers' practice with the NAC 6004 cultivar. Grain yield varied from 1262 kg in the former treatment to 1002 kg in the latter per 0.40 ha area. In the rainy season of 2005 the BH 2187 cultivar recorded 24.11% plants infested and 8.26% dead hearts in the farmers' practices treatment against 22.6% and 6.6% respectively in the egg parasitoid-released field. The trials were conducted with four cultivars in the rainy season of 2006 and two cultivars in the postrainy season of 2006. The percentage of plants infested and dead hearts in the four cultivars planted in the rainy season varied from 6.60% to 17.71% and 0.10% to 7.64% respectively in the egg parasitoid-released fields as against 10.38-20.67% and 0.10-7.64% respectively in the farmers' practice treatment. For the four cultivars in the farmers' practice, grain yield ranged from 2375 kg to 3310 kg in the egg parasitoid-released fields and from 2190 kg to 3164 kg in the farmers' practice fields. Grain yield was 136-185 kg more in the egg parasitoid-released fields than in the farmers' practice endosulfan-sprayed fields in the 2006 rainy season.

Table 1. Egg parasitoid *T. chilonis* in the management of maize stem borers.

Season	Cultivar	Farmers' practice of endosulfan-sprayed fields[1]			Egg parasitoid released fields**		
		% plants infested	% Dead hearts formed	Grain yield (kg acre^{-1})	% plants infested	% Dead hearts formed	Grain yield (kg acre^{-1})
Effect of egg parasitoid on *C. partellus*							
2004 *kharif*[3]	NAC 6004	11.76	2.23	1002	7.73	0.92	1262
Kharif 2005	BH 2187	24.11	8.26	-	22.6	6.6	-
Kharif 2006	HQPM 1	16.3	0.22	3164	6.6	0.10	3310
Kharif 2006	BIO 9681	17.3	2.53	2580	10.38	1.80	2625
Kharif 2006	CP 818	20.67	13.78	2190	17.71	7.64	2375
Kharif 2006	Rajkumar	18.51	6.53	2330	13.61	1.41	2540
Effect of egg parasitoid on *S. inferens*							
Rabi[4] 2006	BIO 9681	-	1.06	3728	-	0.52	3926
Rabi 2006	CP 818	-	0.45	3952	-	0.35	4186

1. Farmers sprayed endosulfan @ 3 mL L^{-1} at 10-15 days after germination.
2. Egg cards of *T. chilonis* released @ 3 cards per acre at 7 and 17 days after germination.
3. Kharif = Rainy season.
4. Rabi = Postrainy season.

During the postrainy season of 2006-07, the experiment was conducted with two cultivars, BIO 9681 and CP 818. The percentage of dead hearts varied from 0.35% to 0.52% in the egg parasitoid-released fields compared to 0.45-1.06% for the farmers' fields which received an endosulfan spray for managing S.*inferens*. Grain yield in the 0.40 ha area varied from 3728-3952 kg in the farmers' practice and from 3926-4186 kg in the egg parasitoid-released fields.

Conclusion

The egg parasiotoid *Trichogramma chilonis* can be used effectively in managing the stem borers *C. partellus* and *S. inferens* in maize irrespective of the cultivars grown.

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Chatterji S.M., Young W.R, Sharma G.C., Sayi I.V., Chhal B.S., Khare B.P., Rathore V.S., PanwarV.P.S. and Siddiqui K.H. 1969. Estimation of loss in yield of maize due to insect pests with special reference to borers. *Indian Journal of Entomology* 31(2):109-115.

Differential Response of CMLs and Their Hybrid Combinations to Pink Borer *Sesamia inferense* Walker

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Abstract. Two field experiments were conducted to identify CIMMYT Maize Lines (CMLs) and their hybrid combinations with the least susceptibility to pink stem borer under artificial infestation in the field. The first trial comprised of 28 inbred lines with two resistant checks, Winpink 4 (resistant control 1), CM 500 (resistant control 2), and one susceptible control, CM 300. The second trial was comprised of 45 yellow single crosses and Winpink 5 (resistant control 1), CM 500 (resistant control 2) and CM 300 (susceptible control). The lines and crosses were separated into four categories on the basis of the leaf injury rating (LIR): (i) Highly resistant: LIR less than the resistant controls; (ii) Moderately resistant: LIR more than resistant control 2 and less than 5.0; (iii) Susceptible: LIR above 5.0 and less than the susceptible control, CM 300; and (iv) Highly susceptible: LIR above the susceptible control. Of the 28 lines screened, 4 were found to be moderately resistant. CML 421 recorded an of 4.4, which was followed by CA03141 (4.60), CA03120 (4.60) and CA00106 (4.75). The crosses CML 429 × CML 474 (2.40) and CML 421 × CML 470 (2.70) were found to be highly resistant. As many as 38 crosses were found to be moderately resistant, recording an LIR of less than 5.0. Three crosses CML 427 × CML 470 (5.30), CML 425 × CML 429 (5.60) and CML 425 × CML 426 (5.70) were susceptible, recording an LIR less than the susceptible control CM 300 (6.20). Overall, the screening trials indicated that the inbred lines CML 421, CA0 3141, CA00106 and CA03120 and single crosses CML 429 × CML 474, CML 421 × CML 470 could be effectively utilized in the pink stem borer resistance breeding program.

Introduction

Insects attack maize throughout the cropping cycle, and the major insect pests attacking maize are the spotted stem borer *Chilo partellus* (Swinhoe) and the pink stem borer *Sesamia inferens* Walker. The former attacks the crop throughout India during the *kharif* (rainy) season while the later is restricted in peninsular India during the *rabi* (winter) season. The losses primarily due to *S. inferens* in *rabi* range from 25.7% to 78.9% (Chatterjee *et al.* 1969). Keeping in view the needs of resource-poor farmers and the ecological consequences of chemical control, there is a need to develop maize germplasm with moderate levels of resistance to insect pests.

Materials and Method

This study was conducted to identify CIMMYT Maize Lines (CMLs) and their hybrid combinations with the least susceptibility to pink stem borer under artificial infestation in field conditions. The study included two trials. The first involved 28 inbred lines with two resistant controls, Winpink 4 (resistant control 1), CM 500 (resistant control 2) and one susceptible control, CM 300. The second trial was comprised of 45 yellow single crosses and Winpink 5 (resistant control 1), CM 500 (resistant control 2) and CM 300 (susceptible control). All these inbreds and single crosses were obtained from the CIMMYT India program. The resistant and susceptible controls are locally adapted inbred lines. The trials were planted in a randomized block design with two replications in rows of 2.5 m with inter- and intrarow spacing of 75 cm and 20 cm, respectively. The trials were conducted during *rabi* 2005/06 at the Winter Nursery Experimental Farm, Hyderabad. Ten to twelve neonate larvae of pink stem borer were introduced into the whorl of plants using a larval dispenser. Four weeks after infestation, plants were scored for leaf injury rating (LIR) on a 9-point scale .The LIR scores were subjected to statistical analyses using the statistical software Windostat Version 8.0, and differences among the means were compared at $P = 0.05$.

Results and Discussion

Data on the LIR of 28 inbred lines and 45 crosses screened against *S. inferens* are presented in Table 1 and Table 2. The lines and crosses were grouped into four categories based on their LIR: (i) Highly resistant: LIR less

Table 1. Relative susceptibility of CIMMYT Maize Lines to pink stem borer.

Sl. No.	Pedigree	Mean LIR (1-9 scale)
Moderately resistant		
1	CML 421	4.4
2	CA03141	4.6
3	CA03120	4.6
4	CAOO106	4.8
Susceptible		
5	CA14513	5.1
6	CA03118	5.2
7	CML 472	5.4
8	CML 427	5.6
9	CA14502	5.6
10	P147-F2#105-2-1-B-1-B-B-B-B	5.7
11	CA14507	6.2
12	CA03139	6.4
13	CAOO106	6.6
Highly susceptible		
14	CA14701	6.8
15	CML470	6.9
16	Pop.31DMR-88-3#-B*13-B-B	7.2
17	P31 C 4S58-23-##-4-BBB-B-B	7.2
18	CA03130	7.4
19	CA14709	7.4
20	CML425	7.7
21	SW92145-2EV - 7 -3-8-B-B-B-B	7.7
22	CA14514	7.8
23	CML429	7.9
24	CA14524	7.9
25	CML426	8.0
26	CA14515	8.5
27	CA14509	8.5
28	CML474	8.6
29	Winpink 4 (Resistant control 1)	2.7
30	CM 500 (Resistant control 2)	2.5
31	CM 300 (susceptible check)	6.7
	SE (D)	1.4
	CD (0.05)	2.9

than the resistant controls; (ii) Moderately resistant: LIR above the resistant control 2 and less than 5.0; (iii) Susceptible: LIR above 5.0 and less than susceptible control CM 300; and (iv) Highly susceptible: LIR above the susceptible control, CM 300. Based on the above criteria, none of the lines was found to be highly resistant. Of the 28 lines screened, four were found to be moderately resistant. CML 421 recorded an LIR of 4.4, followed by CA03141 (4.60), CA03120 (4.60) and CA00106 (4.75). Nine lines were in the susceptible category, recording an LIR above 4.0 and less than the susceptible control, CM 300 (6.2). These lines were CA 14513 (5.1), CA0311 8(5.2), CML 472 (5.6), CA 14502 (5.6), Pop147-F2-#-105-2-1-B-1-B*-4 (5.7), CA14507 (6.2), CA03139 (6.4) and CA00106 (6.6). The remaining 15 were highly susceptible with LIRs more than the susceptible control. Among the highly susceptible lines, CML 474 recorded the highest LIR of 8.6.

Table 2. Relative susceptibility of yellow single crosses to pink stem borer.

Sl. No.	Pedigree	Mean LIR (1-9 scale)
Highly resistant		
1	CML 429 × CML 474	2.4
2	CML 421 × CML 470	2.7
Moderately Resistant		
3	CML 474 x Pop 147-F2-#-105-2-1-B-1-B*4	2.8
4	CML 429 × Pop.147-F2-#-105-2-1-B-l-B*4	2.9
5	CML 470 × Pop.147-F2-#-105-2-1-B-1-B*4	2.9
6	CML 470 × CML 472	3.0
7	CML 426 × CML 474	3.1
8	CML 421 × CML 472	3.2
9	CML 425 × CML 470	3.2
10	CAO0106 × Pop 147-F2-#-105-2-1-B-l-B*4	3.2
11	CML 472 × CML 474	3.3
12	CML 425 × CAO0106	3.4
13	CML 426 × CAOO106	3.4
14	CML 421 × CML 427	3.5
15	CML 421 × CML 474	3.5
16	CML 425 × CML 427	3.5
17	CML 421 × CML 425	3.6
18	CML 421 × CML 429	3.7
19	CML 426 × CML 427	3.7
20	CML 429 × CML 472	3.7
21	CML 426 × CML 429	3.8
22	CML 421 × CML 426	3.9
23	CML 426 × CML 472	3.9
24	CML 426 × Pop.147-F2-#-105-2-1-B-1-B*4	3.9
25	CML 470 × CML 474	3.9
26	CML 427 × CML 474	4.0
27	CML 425 × CML 474	4.1
28	CML 429 × CML 470	4.1
29	CML 421 × CAOO106	4.2
30	CML 425 × CML 472	4.3
31	CML 425 × Pop. 147-F2-#-1 05-2-1-B-1-B*4	4.3
32	CML 429 × CAOO106	4.3
33	CML 427 × CAOO106	4.4
34	CML 470 × CAOO106	4.4
35	CML 472 × CAO0106	4.4
36	CML 421 × Pop.147-F2-#-105-2-1-B-1-B*4	4.6
37	CML 427 × CML 429	4.7
38	CML 472 × Pop 147-F2-#-105-2-1-B-1-B*4	4.7
39	CML 427 × CML 472	4.8
40	CML 474 × CAOO106	4.8
Susceptible		
41	CML 427 × CML 470	5.3
42	CML 425 × CML 429	5.6
43	CML 425 × CML 426	5.7
Highly susceptible		
44	CML 427 × Pop 147-F2-#-105-2-1-B-l-B*4	6.4
45	CML 426 × CML 470	7.5
47	Winpink (resistant control 1)	2.8
48	CM 500 (resistant control 2)	2.9
46	CM 300 (susceptible control)	6.2
	SE (D)	1.2
	CD (0.05)	2.4

Forty-five single crosses involving CMLs 421, 425, 426, 427, 429, 470, 472, 474, CA00106 and Pop147-F2-#-105-2-1-B-1-B*-4 were screened against pink stem borer (Table 2). Two crosses, CML 429 × CML 474 (2.40) and CML 421 × CML 470 (2.70) were found to be highly resistant. As many as 38 crosses were moderately resistant, recording LIRs less than 5.0. Three crosses CML 427 × CML 470 (5.30), CML 425 × CML 429 (5.60) and CML 425 × CML 426 (5.70) were susceptible, recording LIRs less then the susceptible control, CM 300 (6.20). The crosses CML 427 × Pop147-F2-#-105-2-1-B-1-B*-4 (6.40) and CML 426 × CML 470 (7.50) were highly susceptible. Several workers have reported different levels of resistance/susceptibility of lines derived from CIMMYT-IR, MIRT, MBR and PT populations. (Bergvinson and Garcia Lara 2002; Panwar *et al*. 2001 ; Sekhar *et al.* 2004). Overall, the screening trials indicated that theI inbred lines CML 421, CA0 3141, CA00106 and CA03120 and single crosses CML 429 × CML 474, CML 421 × CML 470 could be effectively utilized in the pink stem borer resistance breeding program.

Conclusion

Of the 28 lines screened, four were found to be moderately resistant. CML 421 recorded the lowest leaf injury rating of 4.40 followed by CA03141 (4.60), CA03120 (4.60) and CA00106 (4.75). Among the 45 single crosses screened, two crosses CML 429 × CML 474 and CML 421 × CML 470 were found to be highly resistant. These two crosses recorded an LIR less than the resistant controls. Overall, the screening trials indicated that the inbred lines CML 421, CA03141, CA00106 and CA03120 and single crosses CML 429 × CML 474 and CML 421 × CML 470 could be effectively utilized in the pink stem borer breeding program.

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Gray Leaf Spot and Turcicum Leaf Blight Epidemics in the High Altitude Areas of Bhutan

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Abstract. Bhutan is a small country located on the southern slopes of the eastern Himalayas. It is a landlocked country sandwiched between Tibet (China) on the north and the Indian States of Assam, Arunachal Pradesh, Sikkim and West Bengal on the east, west and south. Over 79% of the population is engaged in agriculture. Maize ranks first among food crops in production, and is a major source of food especially for people living in high altitude areas. The crop is cultivated by 69% of the rural households for subsistence. The cultivation ranges from less than 300 m up to 3000 m owing to its capacity to adapt to different environments. Although maize has wide adaptability, areas lying 1200 m above sea level (masl) falling in the high- and mid-altitude areas are facing extremely serious problems from gray leaf spot (GLS) and turcicum leaf blight (TLB). Traditional varieties are most severely affected while improved varieties have shown some tolerance. Among the two diseases, GLS is the more damaging. It has caused significant economic damage in areas above 1800 masl. A total of 4193 households cultivating 4821.89 acres of maize were affected in 2007. Over 3000 households lost more than 50% of their production. The diseases were confirmed on the basis of technical inputs provided by an experienced plant pathologist, and appropriate strategies were developed to manage them. The immediate action to contain GLS and TLB was to spray the systemic fungicide Tilt (active ingredient propiconazole). For the long term, the maize research program has initiated the identification and adaptation of GLS-resistant lines using materials introduced from CIMMYT Colombia, Mexico, Zimbabwe and Nepal.

Introduction

Bhutan is a small country located on the southern slopes of the eastern Himalayas. It is a landlocked country sandwiched between Tibet (China) on the north and the Indian States of Assam, Arunachal Pradesh, Sikkim and West Bengal in the east, west and south. The country lies between latitudes 26°45'N and 28°10'N, and longitudes 88°45'E and 92°10'E. It has a total area of 38 394 sq km. The population of the country is 634 982 of which 79% are estimated to be engaged in agriculture. Maize (*Zea mays* L.) is a major food crop cultivated by 69% of the rural households for subsistence. It plays a critical role in ensuring household food security. Among food crops, maize ranks first in terms of the area cultivated and total production. Maize cultivation ranges from less than 300 masl up to 3000 masl owing to the crop's capacity to adapt to different environments. It is estimated that 80% of Bhutan's total maize production is consumed at the household level. This is valued at Nu. 353 million annually (Katwal *et al.* 2006). About 6% of the total production is sold, and is an important source of household income. The rest of the production is used as seed, processed into different products and fed to livestock. The maize production environment in Bhutan is broadly categorized into three zones mainly based on the altitude. The three production zones are: the subtropical maize production zone I (<1200 masl) or low altitude zone; subtropical maize production zone II (1200 -1800 masl) or mid-altitude zone; and the highland maize production zone (>1800 masl). These zones vary widely in their production potentials and constraints. Farmers in the highland and subtropical Zone II are facing a new, extremely serious problem of diseases, of which one had not been previously reported in the country (De Leon 2007). Gray leaf spot (GLS) and turcicum leaf blight (TLB) have become economically important diseases in Bhutan, especially in areas above 1200 masl. Serious outbreaks of these diseases were reported from 12 major maize-growing districts in 2006. Incidence of these diseases reached epidemic scale in 2007 and affected 4193 maize-growing households. The total area affected was 4821.89 acres and total production loss amounted to 6504.12 t (Royal Government of Bhutan 2007). The impact of these diseases, farmers' crop husbandry practices, disease management strategies and the disease reaction of the GLS-resistant line introduced from CIMMYT Colombia are discussed in this paper.

Materials and Method

1. An initial report of premature drying of maize was received from several maize-growing districts in 2006. Such reports mostly came from areas above 1500 masl.

Accordingly, field visits and rapid surveys were conducted in the affected areas by researchers from the National Plant Protection Centre (NPPC) and Regional Research Centres. The crop was in the late maturity stage and most of the lower leaves had completely dried. Some lesions of TLB were observed in the few upper leaves that were partially green. TLB was suspected of causing the damage. To assist the maize program in further confirming this problem, the assistance of a plant pathologist was sought for two weeks. Extensive field visits and transect walks were made jointly with maize researchers in the major affected areas to determine the level of disease incidence. Informal discussions were held with the affected farmers to understand their field and crop management practices. Infected leaf samples were collected for laboratory investigation and confirmation at the Renewable Natural Resources Research Centre (RNRRC), Wengkhar. The collected samples of GLS and TLB were incubated and observed under the microscope to identify and confirm the fungi. To contain the incidence of the diseases in the next season, spray of systemic fungicide Tilt (with the active ingredient propiconazole) was recommended. For the long term, identification and adaptation of GLS- and TLB-resistant lines has begun using materials introduced from Centro Internacional de Agricultura Tropical (CIAT) Colombia, Mexico, Zimbabwe and Nepal by planting at a disease hotspot site at Chaskar (1960 masl) in Mongar district in eastern Bhutan. The materials were planted in the third week of March. Regular monitoring of GLS and TLB was done and the diseases were scored on a 1-5 rating scale in which 1 = no lesions visible; 2 = few lesions seen on the two lower leaves; 3 = lesions visible on most of the leaves below the ear; 4 = many lesions visible on leaves above the ear; and 5 = all leaves dead.

Results and Discussion

Field visits and observations of spores under a simple microscope conducted with the help of the technical expert confirmed that the diseases were gray leaf spot (GLS) and turcicum leaf blight (TLB). Gray leaf spot caused by the fungus *Cercospora zeae-maydis* (Tehon and Daniels 1925) had previously never been reported in Bhutan. Turcicum leaf blight caused by *Exserohilium turcicum* (synonymous: *Helminthosporium turcicum*), locally known as *Sorsongma*, was present in the country but serious infection was noticed only after 2006.

Severe incidences of GLS were observed at elevations of 1800 masl and above. At these elevations, GLS was found to be causing much damage with some farmers losing almost 100% of their crop. At elevations 1700-1800 masl both GLS and TLB were prevalent. Below 1700 masl, TLB was more prevalent, but with incidences of lesser economic significance. GLS symptoms were also seen at 600 masl. However, the devastating effects of GLS were most seen in areas at and above 1700 masl. GLS and TLB incidences were confirmed in 12 major maize-growing districts. The most severe symptoms were observed in August when the crop was at the grain-filling stage. In some locations, maize plantings at 2-3 weeks after silking showed extremely high incidence of GLS. Field observations indicated that all the local varieties were highly susceptible to both GLS and TLB as compared to improved varieties. The three released varieties Yangtsipa (Suwan I), Khangma Ashom I (Suwan 8528) and Khangma Ashom II (Palmira 8529) showed slightly more tolerance to the disease.

Apparently, the main source of inoculum for GLS and TLB was infected crop residue, especially leaves and leaf sheaths left in the soil. Bhutanese farmers use maize stover for feeding animals, as animal bedding. They collect and heap residue in the field to be spread later at the time of planting. These practices seemed to greatly favor the development of the diseases. Farmers also feed the infected crop residues to cattle as fodder. The leftover residue from the feeding stall go into the compost yard which is later spread in the maize field. All these conditions present an ideal situation for disease development.

Further, in most maize-growing environments of Bhutan, cloudy weather with high humidity with extended periods of wetness prevail with the onset of the monsoon from late May onward. This favors development of GLS and TLB. The majority of Bhutanese farmers use locally made plows drawn by bullocks which do not penetrate very deep in the soil. These plows do not adequately incorporate the crop residue deep in the soil. Due to the lack of improved varieties suitable for high altitude zones, the majority of the households grow local varieties which are highly susceptible to the two diseases. Farmers also practise continuous cultivation of maize without crop rotation due to their limited landholdings and other suitable optional crops.

As the diseases are a serious threat to household food security, spraying of systemic fungicide Tilt (with the active ingredient propiconazole) was recommended as an immediate measure. Tilt 25 EC imported from India was recommended to be sprayed at 2 mL L^{-1} of water with a minimum of one spray two weeks before flowering. For the long term, maize breeding and selection has been initiated for identification and adaptation of GLS-resistant lines using materials introduced from CIMMYT Colombia, Mexico, Zimbabwe and Nepal at the disease hotspot site in Chaskar (1960 m asl) from the 2008 season. In total, 45 lines from CIMMYT Colombia, 23 lines from CIMMYT Mexico, 8 lines from CIMMYT Zimbabwe and 6 varieties from Nepal are being evaluated for tolerance to the diseases. Among the materials introduced from different sources, introductions from CIAT Colombia appear to be the most promising. A preliminary reaction of these lines to GLS and TLB is presented in Tables 1 and 2 below.

From these lines, 14 entries which received an average score of less than 2.5 for both GLS and TLB incidence have been selected and put in the off-season seed multiplication scheme at Lingmethang (640 masl). These entries are 1, 2, 3, 5, 6, 13, 15, 17, 21, 23, 25, 33 and 38. Once yield data is obtained, the two or three best GLS- and TLB-tolerant open-pollinated lines will be used in the half-sib recombination breeding scheme along with the released variety Yangtsipa as the male to develop new varieties tolerant to the two diseases. From the above selection, 6-7 best open-pollinated lines will be selected while the rest will be evaluated in multilocation trials.

Conclusions

High altitude farmers in Bhutan are serious problems of GLS and TLB. Although use of the insecticide Tilt seems to be quite effective, it use is not sustainable. It is expensive, can be damaging to the environment and also technically difficult to spray manually on the steep slopes

Table 1. Gray leaf spot incidence on 40 maize lines from CIMMYT, Colombia scored on different dates at the Chaskar disease hot spot, Bhtan.

Entry	Pedigree	GLS Score at different dates				
		23/7/08	9/8/2008	27/8/08	9/9/2008	Mean
1	Cap. Miranda 99Bact1F-1	1.3	2	2	2	1.8
2	Granada 01Phaeo1AS2	2	2	2.5	2.7	2.3
3	Turipana 01DMR 1D(1)	2	2.3	2.8	2.8	2.5
4	GLSIY01HG"B"	1.7	2	2	2.7	2.1
5	GLSIY01HG"A"	2	2.3	2.5	3.2	2.5
6	GLSIY01/SPMAT	2	2	2.5	2.8	2.3
7	Celaya OOHGYA	2	2	2.3	2.7	2.3
8	S98TLY-1 B	1.7	2.3	2.7	2.7	2.3
9	ACROSS S9624-1	1	2	2.4	2.7	2
10	S97TLYGH"AyB"(1)	2.3	3	3.2	3.2	2.9
11	SOOTLYQ-AB	2	2.3	3	3.2	2.6
12	S99TLYQGH"B"	2	2.7	3.2	3.3	2.8
13	Menegua 01 Phaeo	1.7	2	2.3	2.7	2.2
14	Granada 03Poly1A(SA3)	2	3.7	4	4	3.4
15	Granada 03Poly1A(SA4)	2	2.3	2.7	3	2.5
16	Granada 03Poly1A(x18)	1.3	2.3	3.2	3.8	2.7
17	Villavicencio 03Asp1C(QPM)	2	2.3	2.7	3.2	2.5
18	Villavicencio 03Al2A	2	2.7	3.2	3.3	2.8
19	Villavicencio 03Al1A	2.3	3.3	3.5	3.8	3.3
20	Villavicencio 03Cog1C	1.3	2	2.5	2.8	2.2
21	Villavicencio 03Asp1C(LET-EARLY)	1.7	2	2.8	3.3	2.5
22	Villavicencio 03Asp1C(LET-LATE)	2	2.3	2.7	2.7	2.4
23	Villavicencio 03Phaeo1A(SA4)	2	2	2.5	2.7	2.3
24	Villavicencio 03Phaeo1A(Pobs Am)	2.3	3.3	4	4	3.4
25	Villavicencio 03Phaeo1A(Elites)	2	2.3	2.8	3	2.5
26	Villavicencio 03Phaeo1A	2	2.7	3	3	2.7
27	Menegua 03Gloeo1C(S1Q)	2	3	3.2	3.5	2.9
28	Menegua 03Gloeo1A	2	3	3.2	3.5	2.9
29	Menegua 03Gloeo1A(7x8)S2	2	3	3.5	3.5	3
30	Menegua 03Gloeo1C(S3)	2	3	3	3.3	2.8
31	Menegua 03Gloeo1ACLA-1	1	3	3.2	3.3	2.6
32	Menegua 03Gloeo1AC	2	2.7	3.2	3.3	2.8
33	Cimcali 03HCG1A	1.7	2.3	2.8	3	2.5
34	CimcaliI97Achap1ASA4	1.7	3	3	3.3	2.8
35	S03TLYQ AB05	1.3	2.3	2.7	2.8	2.3
36	S99TLYQ GHAB	1.7	2.7	2.8	3.2	2.6
37	Cimcali 05BRoya1	2	3	3.7	4	3.2
38	ICA V305	2	2	2.7	2.8	2.4
39	Corpoica H112	1.3	2.3	2.5	2.7	2.2
40	CML02450/CML451	1.3	2	2.3	2.8	2.1

Table 2. Turcicum leaf blight incidence on 40 maize lines from CIMMYT, Colombia scored on different dates at the Chaskar disease hot spot, Bhutan.

Entry No	Pedigree	TLB Score at Different dates				
		23/7/08	9/8/2008	27/8/08	9/9/2008	Overall mean
1	Cap. Miranda 99Bact1F-1	2	2	3	3	**2.2**
2	Granada 01Phaeo1AS2	2	2	3	3	**2.3**
3	Turipana 01DMR 1D(1)	2	2	3	3	**2.5**
4	GLSIY01HG"B"	1	2	2	2	**1.9**
5	GLSIY01HG"A"	1	2	2	2	**1.6**
6	GLSIY01/SPMAT	1	1	2	2	**1.4**
7	Celaya OOHGYA	2	3	4	4	**3.1**
8	S98TLY-1 B	1	1	2	2	**1.4**
9	ACROSS S9624-1	2	2	2	2	**2**
10	S97TLYGH"AyB"(1)	2	3	4	4	**3.2**
11	SOOTLYQ-AB	1	2	2	2	**1.8**
12	S99TLYQGH"B"	1	2	2	2	**1.6**
13	Menegua 01 Phaeo	1	1	2	2	**1.5**
14	Granada 03Poly1A(SA3)	1	1	2	2	**1.5**
15	Granada 03Poly1A(SA4)	1	2	2	2	**1.7**
16	Granada 03Poly1A(x18)	1	2	2	2	**1.8**
17	Villavicencio 03Asp1C(QPM)	1	2	2	2	**1.8**
18	Villavicencio 03Al2A	2	2	2	2	**2.2**
19	Villavicencio 03Al1A	1	2	2	2	**1.8**
20	Villavicencio 03Cog1C	2	3	3	3	**2.6**
21	Villavicencio 03Asp1C(LET-EARLY)	1	1	2	2	**1.3**
22	Villavicencio 03Asp1C(LET-LATE)	2	3	3	3	**2.9**
23	Villavicencio 03Phaeo1A(SA4)	2	2	2	2	**1.7**
24	Villavicencio 03Phaeo1A(Pobs Am)	1	2	2	2	**1.7**
25	Villavicencio 03Phaeo1A(Elites)	1	2	2	2	**1.6**
26	Villavicencio 03Phaeo1A	1	2	2	2	**1.8**
27	Menegua 03Gloeo1C(S1Q)	2	3	3	3	**2.5**
28	Menegua 03Gloeo1A	2	2	2	2	**1.8**
29	Menegua 03Gloeo1A(7x8)S2	2	2	2	2	**1.9**
30	Menegua 03Gloeo1C(S3)	1	1	2	2	**1.3**
31	Menegua 03Gloeo1ACLA-1	1	2	2	2	**1.5**
32	Menegua 03Gloeo1AC	2	3	4	4	**3**
33	Cimcali 03HCG1A	1	2	2	2	**1.8**
34	CimcaliI97Achap1ASA4	1	2	2	2	**1.8**
35	S03TLYQ AB05	2	2	2	2	**2**
36	S99TLYQ GHAB	1	1	1	1	**1.3**
37	Cimcali 05BRoya1	2	2	2	2	**1.7**
38	ICA V305	2	2	3	3	**2.3**
39	Corpoica H112	1	1	2	2	**1.4**
40	CML02450/CML451	1	1	2	3	**1.8**

where most of the maize crop is grown in Bhutan. The most sustainable option appears to be the use of resistant varieties. Therefore, there is an urgent need to test and develop resistant varieties. This could only be achieved through technical guidance and support from the global and regional maize programs.

Acknowledgement

I am grateful to the Council of RNR Research of Bhutan of the Ministry of Agriculture and the Royal Government of Bhutan for allowing me to participate in the 10th Asian Regional Maize Workshop by making funds available from the RNR Research Systems Support Project funded by SDC Helvetas. I am also grateful to CIMMYT for accepting my paper.

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Resistance evaluation of quality protein maize (QPM) genotypes to seedling fly (*Atherigona* sp.)

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Abstract. Seedling fly (*Atherigona* sp.) is one of the biotic factors causing unsteadiness of maize production in Indonesia. The use of the resistant variety is more effective and efficient in controlling this pest. The study of resistance evaluation of the QPM genotypes is a part of the technology components to create the resistant variety to seedling fly. The study aimed to evaluate the resistance of QPM genotypes to seedling fly. The research was carried out on Muneng Experimental Farm, Probolinggo, East Java from August till December 2006. Fifty genotypes of white and yellow QPM genotypes including Srikandi Putih-1 as resistant check and Bayu as susceptible check for the white QPM genotypes and Srikandi Kuning-1 as resistant check and Lamuru as susceptible check for the yellow QPM maize genotypes were evaluated and arranged on simple lattice design 10 x 10 with 2 replications. The result showed that 4 of white QPM genotypes had 0% of infestation i.e. MSQ-P1(S1)-C1-11, MSQ-P1(S1)-C1-12, MSQ-P1(S1)-C1-44, and MSQ-P1(S1)-C1-45, while Srikandi Putih-1 as resistant check and Bayu as susceptible check had 0% and 20% of infestation, respectively, and 3 of yellow QPM genotypes i.e. MSQ-K1(S1)-C1-16, MSQ-K1(S1)-C1-35, and MSQ-K1(S1)-C1-50 had 0% of infestation.

Introduction

Special corn is a kind of maize having certain superiority such as Quality Protein Maize (QPM) where its endosperm contains higher protein quality compared to normal corn. The quality protein maize can improve nutrient value of babies and their mothers (Vasal, 1994; Pixley and Bjarnason, 1993). In several African countries, corn flour of QPM is made as babies' food, while it is served as typical food in South America named Tortilas (Sproule *et. al,* 2002).

In Indonesia, the quality protein maize is also improved to support national programs i.e. food diversity and food safety. ICERI researcher had improved and evaluated several QPM genotypes. One of evaluated QPM that had been conducted was its resistance to the major corn pests particularly seedling fly.

Seedling fly (*Atherigona* sp.) is one of the biotic factors causing unsteadiness of maize production in Indonesia. It could damage corn plantation up to 100% (Kalshoven, 1981; CPC, 2001). The efforts to control this pest involve the use of egg parasitoid like *Trichogramma* spp., change of planting time, crop rotation, and application of insecticides. But the use of the resistant variety is more effective and efficient in controlling this pest.

The study of resistance evaluation of the QPM maize genotypes is a part of the technology components to create the resistant variety to seedling fly. The study aimed to evaluate the resistance of QPM maize genotypes to seedling fly.

Material and Methods

The research was carried out on Muneng Experimental Farm, Probolinggo, East Java from August till December 2006. Fifty two genotypes of S2-S4 white and yellow QPM maize including Srikandi Putih-1 as resistant check and Bayu as susceptible check for the white QPM maize genotypes and Srikandi Kuning-1 as resistant check and Lamuru as susceptible check for the yellow QPM maize genotypes were evaluated and arranged on simple lattice design 10 x 10 with 2 replications. Each genotype was treated by matalaksil and carbofuran to prevent downy mildew disease and pest infestation and planted on 2.5 m of single row plot. Planting distance was 75 cm x 20 cm with two seeds/hill, and then remained one plant per hill at 3 weeks after planting. About two weeks before planting the evaluated genotypes, the susceptible variety was planted as border crop and seedling fly infestation source to the evaluated genotypes. Parameters observed were infestation (percentage of infestation (%) indicated resistance level of QPM genotypes), plant height (cm), and ear height (cm). Percentage of infestation was determined by formula, as fallow:

$$\text{Percentage of infestation (\%)} = \frac{\text{Number of crops infested by seedling fly}}{\text{Number of crops planted}} \times 100\%$$

Result and Discussion

Resistance level of white QPM genotypes to seedling fly was varied ranged from 0 to 25%. There was one genotype that had 25% of resistance level i.e. MSQ-P1(S1)-C1-23, while MSQ-P1(S1)-C1-1, MSQ-P1(S1)-C1-4, MSQ-P1(S1)-C1-18, MSQ-P1(S1)-C1-38, and MSQ-P1(S1)-C1-39 similarly with Bayu as susceptible check had 20% of it (Appendix Table 1).

Table 1 showed that there were four genotypes of QPM which had 0 percent of seedling fly infestation. MSQ-P1(S1)-C1-11, MSQ-P1(S1)-C1-12, MSQ-P1(S1)-C1-44, and MSQ-P1(S1)-C1-45 were similar to the resistant check (Srikandi Putih-1). Table 1 also showed couple parameters.

While Resistance level of yellow QPM genotypes to seedling fly was varied ranged from 0 to 45%. MSQ-K1(S1)-C1-31, MSQ-K1(S1)-C1-43, and MSQ-K1(S1)-C1-46, had 30% of seedling fly infestation MSQ-K1(S1)-C1-27 were 35% and MSQ-K1(S1)-C1-33 were 45%. Those genotypes had higher resistant level compared to Lanuru as susceptible check (25%) (Appendix Table 2).

MSQ-K1(S1)-C1-16, MSQ-K1(S1)-C1-35, and MSQ-K1(S1)-C1-50 were categorized resistant genotypes, because their resistant level were 0 percent. They were lower than Srikandi Kuning-1 as resistant check (15%) (Table 2). Table 2 also showed couple parameters of yellow QPM genotypes.

Tabel 1. Resistant Genotypes of White QPM to Seedling Fly (*Atherigona* sp.)

Genotypes	Infestation (%)	Plant Height (cm)	Ear Height (cm)
MSQ-P1(S1)-C1-11	0	166.3	88.3
MSQ-P1(S1)-C1-12	0	172.5	82.5
MSQ-P1(S1)-C1-44	0	161.2	76.3
MSQ-P1(S1)-C1-45	0	154.3	76.3
Srikandi Putih-1 (Resitant Check)	0	182.7	80.5
Bayu (Susceptible Check)	20	187.5	87.7

Tabel 2. Resistant Genotypes of Yellow QPM to Seedling Fly (*Atherigona* sp.)

Genotypes	Infestation (%)	Plant Height (cm)	Ear Height (cm)
MSQ-K1(S1)-C1-16	5	175.2	79.8
MSQ-K1(S1)-C1-35	5	172.3	77.5
MSQ-K1(S1)-C1-50	5	167	66.7
Srikandi Kuning-1 (Resitant Check)	15	174.2	80
Lamuru (Susceptible Check)	25	164.3	87.8

Conclusion

There were four genotypes of white QPM i.e. MSQ-P1(S1)-C1-11, MSQ-P1(S1)-C1-12, MSQ-P1(S1)-C1-44, and MSQ-P1(S1)-C1-45 and three genotypes of yellow QPM i.e. MSQ-K1(S1)-C1-16, MSQ-K1(S1)-C1-35, and MSQ-K1(S1)-C1-50 categorized resistant to seedling fly.

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Appendix Table 1. Seedling fly Infestation on White QPM Genotypes, and Plant and Ear Height of White QPM

Genotypes	Infestation (%)	Plant Height (cm)	Ear Height (cm)
MSQ-P1(S1)-C1-1	20	185.8	90.5
MSQ-P1(S1)-C1-2	5	161.7	77.7
MSQ-P1(S1)-C1-3	10	186.8	95
MSQ-P1(S1)-C1-4	20	179.8	96.7
MSQ-P1(S1)-C1-5	10	156.3	75.2
MSQ-P1(S1)-C1-6	10	162.5	77.3
MSQ-P1(S1)-C1-7	15	160	85.2
MSQ-P1(S1)-C1-8	5	170.2	85.3
MSQ-P1(S1)-C1-9	10	165	88.7
MSQ-P1(S1)-C1-10	15	166.7	91
MSQ-P1(S1)-C1-11	0	167.3	88.3
MSQ-P1(S1)-C1-12	0	172.5	82.5
MSQ-P1(S1)-C1-13	5	166.3	85.2
MSQ-P1(S1)-C1-14	10	165.3	70.2
MSQ-P1(S1)-C1-15	10	173.8	84.2
MSQ-P1(S1)-C1-16	10	159.7	68.3
MSQ-P1(S1)-C1-17	5	73.9	43
MSQ-P1(S1)-C1-18	20	162.2	71.3
MSQ-P1(S1)-C1-19	10	157.7	66.3
MSQ-P1(S1)-C1-20	20	172.8	80.5
MSQ-P1(S1)-C1-21	10	174.2	74.2
MSQ-P1(S1)-C1-22	10	164.8	84.2
MSQ-P1(S1)-C1-23	25	158	82.3
MSQ-P1(S1)-C1-24	10	166.7	75.5
MSQ-P1(S1)-C1-25	15	153.5	72.3
MSQ-P1(S1)-C1-26	10	157	72.7
MSQ-P1(S1)-C1-27	5	177.5	102.2
MSQ-P1(S1)-C1-28	10	153.3	74.8
MSQ-P1(S1)-C1-29	10	176.8	93.2
MSQ-P1(S1)-C1-30	5	171.7	65.7
MSQ-P1(S1)-C1-31	15	150	78.5
MSQ-P1(S1)-C1-32	5	180.5	93.7
MSQ-P1(S1)-C1-33	5	164.2	74.5
MSQ-P1(S1)-C1-34	10	152.8	66.3
MSQ-P1(S1)-C1-35	15	164.7	81.8
MSQ-P1(S1)-C1-36	5	181.5	91.2
MSQ-P1(S1)-C1-37	10	171.5	97.3
MSQ-P1(S1)-C1-38	20	178.7	74.8
MSQ-P1(S1)-C1-39	20	163.2	69
MSQ-P1(S1)-C1-40	15	165.8	69.8
MSQ-P1(S1)-C1-41	20	166.3	89.7
MSQ-P1(S1)-C1-42	15	165.8	81.5
MSQ-P1(S1)-C1-43	10	156.3	76.3
MSQ-P1(S1)-C1-44	0	161.2	76.3
MSQ-P1(S1)-C1-45	0	154.3	67.2
MSQ-P1(S1)-C1-46	15	166	83.2
MSQ-P1(S1)-C1-47	20	161.8	81.7
MSQ-P1(S1)-C1-48	10	183.8	94.5
MSQ-P1(S1)-C1-49	10	172.8	92
MSQ-P1(S1)-C1-50	15	166.2	80.3
Srikandi Putih-1 (Resitant Check)	0	182.7	80.5
Bayu (Susceptible Check)	20	187.5	87.7

Appendix Table 2. Seedling fly Infestation on Yellow QPM Genotypes, and Plant and Ear Height of White QPM

Genotypes	Infestation (%)	Plant Height (cm)	Ear Height (cm)
MSQ-K1(S1)-C1-1	25	170	79.8
MSQ-K1(S1)-C1-2	10	168.8	75.3
MSQ-K1(S1)-C1-3	15	160.5	84.8
MSQ-K1(S1)-C1-4	20	171.7	74.8
MSQ-K1(S1)-C1-5	10	154.5	81
MSQ-K1(S1)-C1-6	15	140.7	65
MSQ-K1(S1)-C1-7	15	152.2	70.5
MSQ-K1(S1)-C1-8	20	162.8	76.7
MSQ-K1(S1)-C1-9	20	167.3	77.7
MSQ-K1(S1)-C1-10	25	153.5	75.5
MSQ-K1(S1)-C1-11	10	157.7	62.2
MSQ-K1(S1)-C1-12	10	166.8	76
MSQ-K1(S1)-C1-13	15	165.8	88.3
MSQ-K1(S1)-C1-14	15	157	69.2
MSQ-K1(S1)-C1-15	20	167.3	67.5
MSQ-K1(S1)-C1-16	5	175.2	79.8
MSQ-K1(S1)-C1-17	10	160.5	74.3
MSQ-K1(S1)-C1-18	15	168.5	75.2
MSQ-K1(S1)-C1-19	10	159.5	68.7
MSQ-K1(S1)-C1-20	20	162.8	84.7
MSQ-K1(S1)-C1-21	20	160.5	83.8
MSQ-K1(S1)-C1-22	20	150.7	73.8
MSQ-K1(S1)-C1-23	10	182.7	87.3
MSQ-K1(S1)-C1-24	25	191.3	103.2
MSQ-K1(S1)-C1-25	10	151.3	62.3
MSQ-K1(S1)-C1-26	15	165.5	77.3
MSQ-K1(S1)-C1-27	35	158.2	74.5
MSQ-K1(S1)-C1-28	25	166.7	62.2
MSQ-K1(S1)-C1-29	15	139.5	58.5
MSQ-K1(S1)-C1-30	20	149.5	75.7
MSQ-K1(S1)-C1-31	30	144.8	69.7
MSQ-K1(S1)-C1-32	15	155.2	71
MSQ-K1(S1)-C1-33	45	166.3	73.5
MSQ-K1(S1)-C1-34	15	164.2	68.7
MSQ-K1(S1)-C1-35	5	175.3	77.5
MSQ-K1(S1)-C1-36	10	159.2	78
MSQ-K1(S1)-C1-37	10	180.3	77.5
MSQ-K1(S1)-C1-38	20	168.5	78
MSQ-K1(S1)-C1-39	15	149	70.2
MSQ-K1(S1)-C1-40	15	168.3	78.7
MSQ-K1(S1)-C1-41	20	172	67.5
MSQ-K1(S1)-C1-42	20	172.5	87.7
MSQ-K1(S1)-C1-43	30	160.5	70.3
MSQ-K1(S1)-C1-44	15	161.3	69.7
MSQ-K1(S1)-C1-45	10	164.5	79.3
MSQ-K1(S1)-C1-46	30	155.5	65.5
MSQ-K1(S1)-C1-47	10	151	66.33
MSQ-K1(S1)-C1-48	15	158.3	78.17
MSQ-K1(S1)-C1-49	20	168.8	81.5
MSQ-K1(S1)-C1-50	5	167	66.7
Srikandi Kuning-1 (Resitant Check)	15	174.2	80
Lamuru (Susceptible Check)	25	164.3	87.8

Earwig (*Euborellia annulata*) as the promising biological control agent against asian corn borer

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Abstract. Maize is a cereal holding important role in fulfilling feed and food need in the world. The maize demand either the world or Indonesia always increase. Therefore, Indonesian government and farmers attempt to improve their maize production. Nevertheless, the maize pests (Asian Corn Borer, *Ostrinia furnacalis*) often become constraint factor to maintained. *O. Furnacalis* damage could be up to 80% of yield losses. However, this pest can be controled by utilizing natural enemy. The objective of this study was to give information about earwig as the promising biological control agent against Asian corn borer. This paper is a review of several studies carried out by researchers of Indonesian Cereals Research Institute (ICERI) The results reported that the use of alterntive diet such as the dog biscuit gave significant effect to the development of this natural enemy and earwig gave significant effect in controling Asian Corn Borer (ACB). It can be concluded that *E. annulata* promises as biological contol agent because of easy to understand biology, easy to mass produce, and effective to control ACB.

Keywords : Earwig, Promising Bio-control Agent, Asian Corn Borer

Introduction

Maize is a cereal holding important role in fulfilling feed and food need in the world. The maize demand either the world or Indonesia always increases. USDA (2007) reported that production, consumption, and world maize stock were 706,236 MT, 680,472 MT, and 125,914 MT, respectively. Meanwhile, national maize production and consumption of maize were 6,500 MT and 7,200 MT, respectively. Based on the data, the Indonesian government and farmers have to be attempted to improve the maize production.

In 2007, Indonesian government proclaimed three food crop priority i.e. rice, maize, and soybean which are expected self sufficient until 2015, so that they can fulfill national demand. Particularly, maize was expected self-sufficient in 2007 with production target as many as 13.54 million ton (Biro Perencanaan Departemen Pertanian, 2006).

Nevertheless, the target can not sometimes be accieved due to abiotic and biotic constraints. The most abiotic constraint is low availablity of soil nutrient and the biotic contraint is weeds, diseases, and pests (Subandi *et al,* 1988). One of the maize pests is Asian corn borer (*Ostrinia furnacalis*) and its damage could be up to 80% of yield losses.

Several practices to control this insect pest have already been utilized. The most popular farmer's practice is the use of insecticides. Unfortunately, these have negative impact not only on the environment but on human beings as well.

One control practice to control this pest that does not cause negative environmental effect is the use of biological control agents such as parasite, fungi, virus, nematode or predator. The predator as a biological control agent has several advantages. These advantages are: 1) non-polluting meaning that the use of predator is environmentally safe and acceptable; 2) in application, it can be combined with other bio-control agents; and 3) easy to mass produce. Earwig (*Euborellia annulata*) is one of predators that can be applied as biological control agent against *O. furnavalis*. The objective of this study was to give information about earwig as the promising biological control agent against Asian corn borer. This paper is a review of several studies carried out by researchers of Indonesian Cereals Research Institute (ICERI).

Biology of Earwig

Study of earwig biology has been conducted in Laboratory of Pests and Diseases, Indonesian Cereals Research Institute (ICERI), Maros, South Sulawesi since 2004. The study results carried out by ICERI researches explained that the female adult of *E. annulata* laid its eggs in colony with oval shape and white color (Fig. 1).

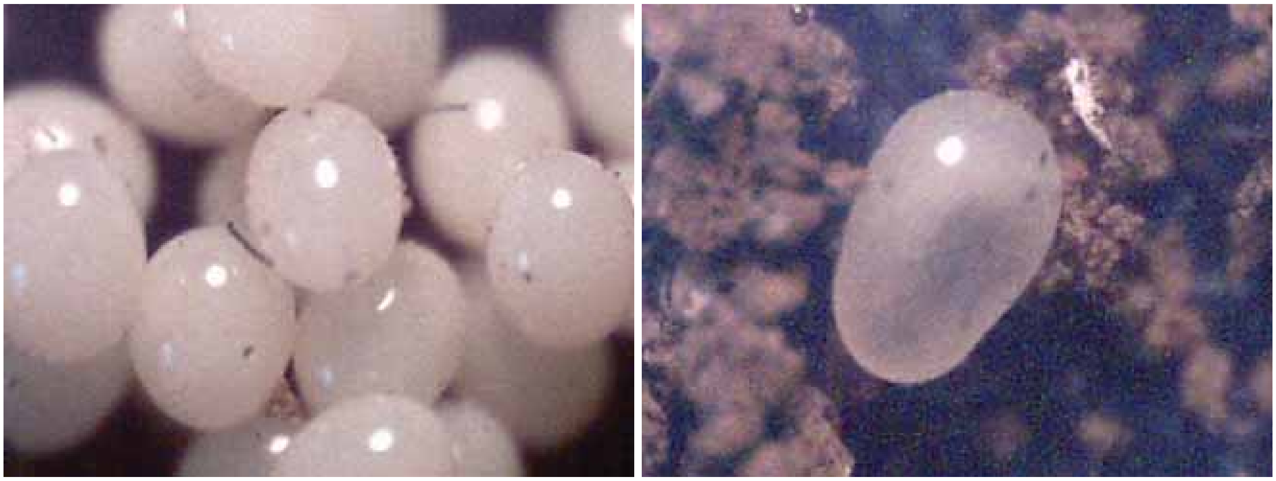

Figure 1. Egg colony of earwig (*E. annulata*) laid by female adult (a) with oval shape and white color (b) (Redrawn from Nonci, 2004)

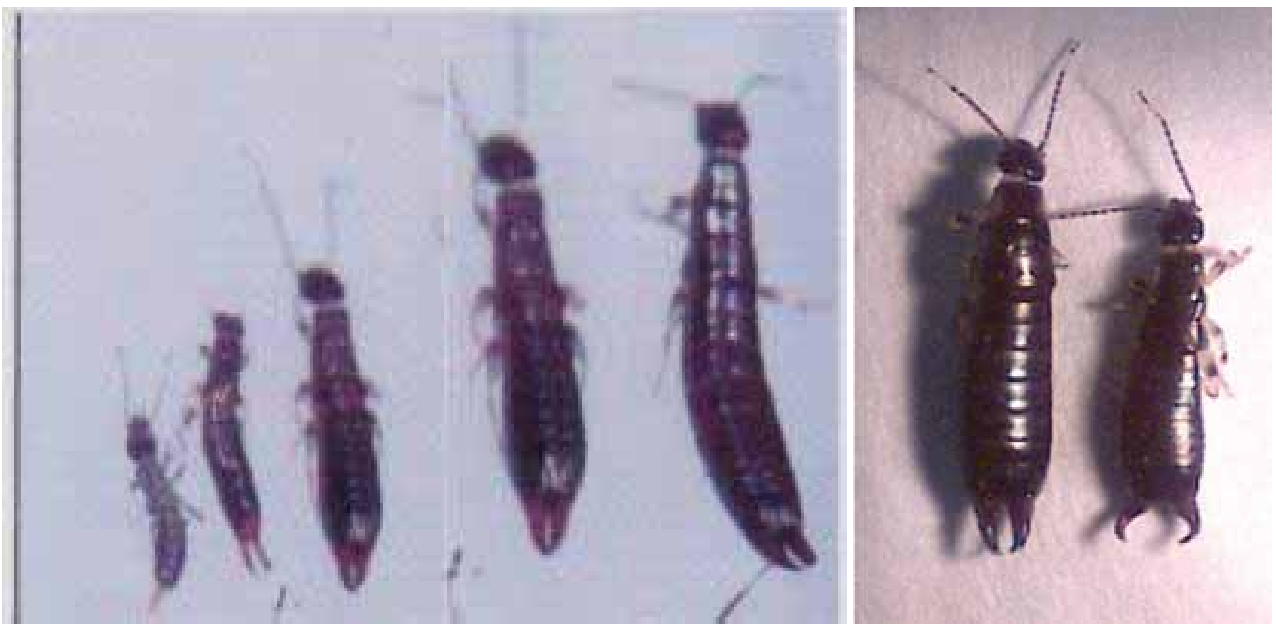

Figure 2. (a) Nymph of earwig (*E. annulata*) consisted of five instars (from first instar at the left side to fifth instar at the rigth side), (b) adult of earwig (Rosina and Adnan, 2007).

Meanwhile, nymph consists of 5 instars and male and female adult can be distinguished by seeing their forceps (Fig. 2 **).**

At biology of *E. annulata* feed on four diet types, egg stadia and mortality of earwig feed on dog biscuit were 6.6 days and 5.94%, respectively. They were lower than other diets (Table 1). It indicated that the egg of this predator reared on dog biscuit hatched faster and much more than other diets.

Table 1. Egg stadia and mortality laid by female of *E. annulata* feed on four diet types.

Diet	Egg Stadia ($\overline{X}$ ± SD, Days)	Egg Mortality (± SD, %)
Dog biscuit (Pedigree)	6,6 ± 0,89	5,94 ± 1,78
Cob	7,4 ± 0,89	11,46 ± 3,08
Chicken manure	7,6 ± 0,55	12,06 ± 5,89
Tribolium larvae	7,4 ± 0,55	10,57 ± 4,30

Source: Rosina and Adnan, 2007

Table 2. Nymph instar stadia of *E. annulata* feed on four diet types.

Diet	Nymph Stadia (± SD, Days)				
	Instar I	Instar II	Instar III	Instar IV	Instar V
Dog biscuit (Pedigree)	7,4 ± 0,55	7,2 ± 1,6	7,1±1,68	7,1±1,68	7,1±1,65
Cob	7,6 ± 0,55	7,28 ± 2,28	7,19±3,05	7,6±3,43	7,6±3,28
Chicken manure	7,6 ± 0,55	7,43 ± 3,35	7,77±3,91	7,23±3,81	7,23±3,76
Tribolium larvae	7,6 ± 0,55	7,45 ± 3,09	7,53±3,71	7,6±3,87	7,6±3,83

Source: Rosina and Adnan, 2007

Furthermore, Instar I, II, III, IV, and V of earwig feed on dog biscuit were 7.4, 7.2, 7.1, 7.1, 7.1 days, respectively (Table 2). They required time (day) faster than reared on other diets. It means that nymph stadia of earwig reared on pedigree are faster than feed on other diets. According to Morallo and Punzalan (2002) reported that the number of nymph and nymph stadia varied depended on food availability and ingredient.

Life span of earwig male and female that are given dog biscuit were 34.6 and 41.6 days, respectively (Table 3). Feed on other diets was longer than feed on dog biscuit. It indicates that the adult of earwig has longer time to predate Asian corn borer.

Mass Production of Earwig

The use of dog biscuit (pedigree) in mass producing earwig gave higher egg colony and number (Table 4)

The number of first instar emerged from the eggs of earwig feed on dog biscuit was higher than the other diets and number of the survived instar V of E. annulata was also higher compared to the others (Table 5). Table 6 showed that number of males and females emerged from the last instar of earwig feed on dog biscuit were significantly different compared to the others. They indicated that the dog biscuit as alternative diet could be utilized for mass producing.

Table 3. Longetivity of *E. annulata* male and female feed on four diet types.

Diet	Longetivity (± SD, Days)	
	Male	Female
Dog biscuit (Pedigree)	34,6 ± 9,50	41,8 ± 10,62
Cob	30 ± 11,16	35,6 ± 11,33
Chicken manure	27,2 ± 11,75	31,8 ± 10,31
Tribolium larvae	31,6 ± 10,53	33,8 ± 9,88

Source: Rosina and Adnan, 2007

Table 4. Average of egg colony and number of egg of *E. annulata* feed on four diet types

Diet	Mean of Egg number	Mean of Egg colony
Dog biscuit (Pedigree)	8.72	169.37
Cob	0.92	20.66
Chicken manure	0.46	8.26
Tribolium Larvae	0.46	10.56

Source: Rosina and Adnan, 2007

Table 5. Number of nymph first instar emerged from the egg of *E. annulata* feed on four diet types and number of nymph survived until instar V

Diet	Nymph Stadia (± SD, Days)				
	Instar I	Instar II	Instar III	Instar IV	Instar V
Dog biscuit (Pedigree)	208.39	175.79	113.8	97.31	71.6
Cob	79.87	64.26	38.09	15.61	8.72
Chicken manure	41.54	30.49	12.85	12.39	7.8
Tribolium larvae	55.99	46.82	23.87	16.07	11.48

Source: Rosina and Adnan, 2007

Table 6. Number of adult emerged from the last instar of *E. annulata* feed on four diet types

Jenis Pakan	Number of adult	
	Male	Female
Dog biscuit (Pedigree)	19.74	56.92
Cob	5.97	11.93
Chicken manure	3.67	7.80
Tribolium Larvae	5.97	11.02

Source: Rosina and Adnan, 2007

Table 7. Percentage of plant damage

Treatments	Observation	
	10 WAP	12 WAP
0 adult/ha	37.57	47.57
1000 adults/ha	25.58	35.64
5000 adults/ha	14.68	24.68
10000 adults/ha	2.72	4.22
20000 adults/ha	3.22	4.58

Source: Adnan, 2007

Tables 4, 5, and 6 indicated that dog biscuit (pedigree) can become a promising alternative diet in mass producing earwig. In addition, dog biscuit is easy to get and has reasonable price. Then, it can be concluded that earwig is easy to rear or mass produce.

Effectiveness of Earwig in Controling Asian Corn Borer (*O. furnacalis*)

In applying 20,000 adult of earwig/ha, percentage of plant damage at 10 week after planting and 12 week after planting (WAP) was 3.22 and 4.58%, respectively (Table 7). It was lower than the others.

Table 8. Average of Hole number and length (cm)

Treatments	Observation	
	Σ Hole	Hole length (cm)
0 adult/ha	12.00	7.79
1000 adult/ha	9.50	6.79
5000 adult/ha	5.50	5.25
10000 adult/ha	2.25	2.30
20000 adult/ha	2.50	2.64

Source: Adnan, 2007

Table 8 showed that average of hole number on plots applying 20,000 adult of earwig/ha was less than other treatments. Moreover, average of hole length was lower on plots applying 20,000 adult of earwig/ha than others.

Tables 7 and 8 indicated that earwig was effective to control Asian corn borer and could be applied as biological control agent.

Conclusion

Earwig (*E. annulata*) was one of predators that promise as biological control agent of Asian corn borer (*Osrinia furnacalis*).

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Perspective performance of the entomopathogenic fungi, *Beauveria bassiana*, as an integrated pest management (IPM) component in corn pest handling

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Abstract. The use of fungi *B. bassiana* as biological control agent of corn pests have been excessively done especially for corn borer and earworm. This fungus can suppress corn borer with mortality up to 84%. Three components i.e. pathogen, environment, and food are the factor influencing white muscardine growth. Entomopathogenic fungi *B. bassiana* is ecto- and endoparasite. Spore or conidia of this fungus infects target insect through cuticle, mouth, insect digestion and respiratory. Stroage effect to fungal germination showed that viability above 90% noticed on 2, 3 month storage, 12 days storage on PDA media and on corn media. Virulent test of several *B. bassiana* isolates (Bb-1, Bb-3, Bb-6, Bb-7, Bb-23, Bb-24, Bb-27, Bb-30, Bb-31, Bb-32, Bb33, Bb-34, Bb36, Bb-40, Bb-41, Bb-46, Bb-47, Bb-90, Bb-51, and Bb-52) revealed difference of mortality range of corn borer. 100% of mortality is noticed on isolates Bb-27, Bb-32, and Bb-34, mortality 80 – 90% is detected on Bb-23, Bb-24, Bb-30, Bb-31, Bb-36, and Bb-46, and the others are ranged 60 – 70%. The research result indicated that on cascading treatment, existence of *B. bassiana* hyphae in root and stalk just seemed on month V – XII, while it appeared in leaf on month VI – XII. Furthermore, on soaking treatment, it had appeared on month III – XII for root observation, month V – XII for stalk, and month VI – XII for leaf. At observation 7 DAI, almost all concentration giving mortality of larval instar 2, 3, 4, and 5 were significantly different to control. on observation bore number per stalk and tassel damage per planting, all of concentrations were significantly different to control at either 7 or 9 WAP. Similarly on observation of bore number/plant, drill length, and ear length, all of examined concentrations were significantly different to control, but on yield inspection, only concentration 5 x 10^7 conidia/ml was significantly different to control. The result above indicated the promising potency of *B. bassiana* especially in controlling corn borer by selecting strain having high virulence (Bb-27, Bb-31, and Bb-34) with concentration 5 x 10^7 conidia/ml. it is preferably applied at egg laying period of *O. furnacalis* (3 WAP, further application is 2 week interval). The achieved application result is better, if the relative humidity under plant canopy is <70%. The chance of *B. bassiama* applied as seed treatment can suppress infestation of corn borer due to endophyt characteristic possessed by it.

Keywords: Perspective Performance, B. bassiana, Corn Pest Handling

Introduction

The use of fungi *B. bassiana* as biological control agent of corn pests have been excessively done especially for corn borer and earworm. This fungus can suppress corn borer with mortality up to 84% (Bing and Lewis, 1993). In Maros, South Sulawesi, application of *B. bassiana* on corn plant with concentration 5 x 10^7 conidia/ml, interval 2 days/week/month was effective to suppress corn borer infestation and much better compared to use of carbofuran with concentration 3% (application dosage 5.33 kg/h), as well as it increased 80.7% of yield (Surtikanti, *et. al.*, 1997).

Yasin, *et. al.* (1997) found that inoculation of *B. bassiana* with concentration 5 x 10^5, 5 x 10^6, and 5 x 10^7 conidia/ml was effective to control corn borer larvae. Utilization of this fungus as one of IPM components become more important because several study results reported that *B. bassiana* was useful to hold back corn pests in the field.

According to Steinhaus (1967), the fungus *B. bassiana* was successful to infect insect pests. This fungus infected target insect through cuticle, mouth, insect digestive and respiration system. *B.bassiana* is easy to culture, simply mass production without sophisticated tool and relatively cheaper to culture (Soehardjan and Sudarmadji, 1993).

Favorable Factor for Fungus Growth

Three components i.e. pathogen, environment, and food are the factor influencing white muscardine growth. The environmental factors affected are temperature, humidity, and light intensity. For temperature factor, *B. bassiana* can be growth at 20 – 30°C with optimal growth 23 – 25°C. Critical temperature that can hamper is 5°C and 35°C and the fungus will die at 50°C. The optimal relative humidity for fungus development is 70 – 100%. Marcandier and Khachatourians (1978) reported that *B. bassiana* gave higher mortality of coffee borer at 98% relative humidity compared to at 0%, 50%, and 75%. In directed sunlight condition, conidia or spore of white muscardine can only survive 1 – 2 days. The conidia or spore covered from sunlight can survive longer and has high viability.

Killing Mechanism of The Fungus To Target Insect

Entomopathogenic fungi *B. bassiana* is ecto- and endoparasite. Spore or conidia of this fungus infects target insect through cuticle, mouth, insect digestion and respiratory (Tanada and Kaya, 1983). Its spore penetrates cuticle layer between head capsule and thorax, as well as among body segments. Initial mechanism is started by developing spore, it, then, forms hyphae and releases enzyme chitinase, protease, and lipase that help disentangling insect cuticle. Penetration period is for 12 – 24 hours. Mycelia grow in epidermis, then spread to a whole body. The infected insect is signed by slow movement, motionless, and dead. The insect body become mummification and is blanket by white hyphae.

Results of *B. bassiana* study technology

Storage Effect to *B. bassiana* Pathogenicity

Stroage effect to fungal germination showed that viability above 90% noticed on 2, 3 month storage, 12 days storage on PDA media and on corn media. The observation result of conidia number seemed that the number of conidia before stored reached density 88 x 10^6 conidia/ml. After 1 month storage, its density arrived at 99.2 x 10^6 conidia/ml. Increasing of conidia number on treatments 2, 3, 4, 5, 6, 7, 8, and 9 is same (108 x 10^6 conidia/ml). Pathogenicity of this fungus on observations 2, 4, 6, and 8 days after infection (DAI) is relatively low (0.00 – 57.77%), but on observations 10 and 12 DAI, pathogenecity increases up to 96.67% (Table 1).

Virulent Test of Several B. bassiana Isolates

Virulent test of several *B. bassiana* isolates (Bb-1, Bb-3, Bb-6, Bb-7, Bb-23, Bb-24, Bb-27, Bb-30, Bb-31, Bb-32, Bb33, Bb-34, Bb36, Bb-40, Bb-41, Bb-46, Bb-47, Bb-90, Bb-51, and Bb-52) revealed difference of mortality range of corn borer. 100% of mortality is noticed on isolates Bb-27, Bb-32, and Bb-34, mortality 80 – 90% is detected on Bb-23, Bb-24, Bb-30, Bb-31, Bb-36, and Bb-46, and the others are ranged 60 – 70%. On germination test of white muscardine, >90% of viability is detected on treatment of alginate pellet storage duration 2 months and the others are ranged between 50 and 80%. On mortality test, alginate pellet storage duration 2 months is able to eradicate larva 83 – 97%, while mortality on alginate pellet storage duration 4 and 6 months is noticed 43 - 83%. The study result showed that *B. bassiana* isolates Bb-27, Bb-32, and Bb-34 have high virulence (Table 2).

Table 1. Viability and Conidia Number of *B. bassiana*, and Mortality of *Ostrinia furnacalis* at 2, 4, 6, 8, 10, and 12 DAI

Treatments	Viability (%)	Number of conidia/ml	Observation (DAI)					
			2	4	6	8	10	12
			Mortality (%)					
1	90.60ab	99.2 x 10^6	16.67a	26.67a	36.67a	50.77a	73.33ab	93.33ab
2	98.83ab	108 x 10^6	13.33ab	20.00a	33.33a	50.75a	70.00ab	90.00bc
3	90.51b	108 x 10^6	13.33ab	20.00a	30.00ab	48.85a	66.67b	83.33bc
4	90.37b	108 x 10^6	6.67abc	13.33ab	26.67ab	37.22b	50.00c	76.67cd
5	81.79c	108 x 10^6	3.33bc	6.67bc	16.67b	30.79bc	33.33d	60.00d
6	62.02d	108 x 10^6	3.33bc	3.33bc	6.67c	23.85cd	20.00e	26.67e
7	46.30e	108 x 10^6	0.00c	0.00c	0.00d	15.17de	16.67ef	23.33e
8	40.00f	108 x 10^6	0.00c	0.00c	0.00d	6.49e	10.00f	13.33e
9	95.00a	108 x 10^6	20.00a	26.67a	36.67a	48.85a	80.70a	96.67a
10	94.50ab	108 x 10^6	16.67a	23.33a	36.67a	50.77a	76.67ab	96.67a

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
DAI = Days After Infection
1 = 1 month storage
2 = 2 month storage
3 = 3 month storage
4 = 4 month storage
5 = 5 month storage
6 = 6 month storage
7 = 7 month storage
8 = 8 month storage
9 = 12 days storage on PDA
10 = 12 days storage on maize media
Source : Yasin *et. al.*, (2000)

Efficacy of B. bassiana against larva O. furnacalis in Laboratory

At observation 1 day after inoculation (DAI), all of *B. bassiana* concentrations giving mortality of larval instar 2, 3, and 4 were not significantly different to control, but on larval instar 5, concentration 5 x 10^6 and 5 x 10^7 conidia/ml detected significantly different to control. Whereas, at observation 7 DAI, almost all concentration giving mortality of larval instar 2, 3, 4, and 5 were significantly different to control (Table 3).

Table 2. Viability of *B. bassiana* and Mortality of Corn Borer *O. furnacalis*) caused by Treatment of alginate pellet storage duration 2, 4, and 6.

Treatment	Viability (%) on Storage Duration (Month)			Mortality (%) on Storage Duration (Month)		
	2	4	6	2	4	6
Bb-27	92ns	79ab	65bc	90ns	73ab	63a
Bb-30	90	76b	60de	87	73ab	56a
Bb-31	91	77b	67ab	90	77ab	67a
Bb-32	90	76b	62cd	90	80ab	63a
Bb-34	92	81a	70a	97	83a	67a
Bb47	90	75b	57e	83	70b	43b
CV	12.2	12	13.1	9.1	8.2	11.4

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
Source: Yasin and Wakman (2003)

Table 3. Mortality of *O. furnacalis* Larvae on several concentrations examined at 4 observation interval.

Concentration(conidia/ml)	Mortality of Larval instar			
	2	3	4	5
At 1 DAI				
5 x 10^7	7.50a	5.00a	7.50a	22.50a
5 x 10^6	2.50a	5.00a	12.50a	10.00ab
5 x 10^5	5.00a	7.50a	5.00a	2.50b
5 x 10^4	2.50a	7.50a	2.50a	2.50b
5 x 10^3	2.50a	2.50a	2.50a	5.00b
Control	0.00a	0.00a	0.00a	0.00b
At 3 DAI				
5 x 10^7	30.00a	17.50a	37.50a	32.50a
5 x 10^6	10.00ab	10.00ab	17.50b	10.00b
5 x 10^5	12.50ab	17.50a	20.50ab	17.50b
5 x 10^4	5.00ab	10.00ab	17.00bc	10.00b
5 x 10^3	10.00ab	5.00ab	10.00bc	7.50bc
Control	0.00b	0.00bc	0.00c	0.00c
At 5 DAI				
5 x 10^7	45.00a	45.00a	50.00a	47.50a
5 x 10^6	30.00a	37.50a	40.00a	27.50b
5 x 10^5	25.00ab	37.50a	35.00a	17.50bc
5 x 10^4	7.50c	17.50b	22.50b	10.00c
5 x 10^3	10.00bc	5.00b	17.50b	7.50cd
Control	0.00c	0.00c	0.00c	0.00d
At 7 DAI				
5 x 10^7	62.50a	55.00a	57.50a	55.00a
5 x 10^6	45.00ab	30.00b	30.00b	27.50b
5 x 10^5	35.00b	47.50b	47.50b	20.00bc
5 x 10^4	17.50c	12.50c	12.50c	10.00cd
5 x 10^3	10.00c	12.50c	12.50c	2.50de
Control	0.00d	0.00d	0.00d	0.00e

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
DAI = Day After Inoculation
Source: Yasin *et. al.*, 1997

Efficacy of B. bassiana against *O. furnacalis* in Field

The examination of several *B. bassiana* concentration against *O. furnacalis* indicated that scoring of leaf laceration on all treatments was not significantly different to control at 2, 3, and 5 week after plating (WAP), nevertheless, on observation bore number per stalk and tassel damage per planting, all of concentrations were significantly different to control at either 7 or 9 WAP. Similarly on observation of bore number/plant, drill length, and ear length, all of examined concentrations were significantly different to control, but on yield inspection, only concentration 5 x 10^7 conidia/ml was significantly different to control (Table 4).

Table 4. Leaf Laceration Scoring, Bore Number, Tassel Damage, Drill Length, Ear length, and Yield.

Treatment(conidia/ml)	Scoring of Leaf Laceration Caused by *O. furnacalis* Infestation		
	Observation Interval (WAP)		
	2	3	5
5 x 10^7	2.21ns	0.81ns	1.69a
5 x 10^6	2.35	0.86	1.99ab
5 x 10^5	2.25	0.81	2.11ab
5 x 10^4	2.21	0.95	2.52b
Carbofuran	2.19	0.86	2.53b
Control	2.10	0.86	2.35ab
CV	16.20	10.00	19.50

Treatment(conidia/ml)	Bore Number and Percentage of Tassel damage Caused by *O. furnacalis* Infestation, Bontobili, 1996			
	7 WAP		9WAP	
	Bore Number/Stalk	Tassel Damage(%)	Bore Number/Stalk	Tassel Damage(%)
5 x 10^7	1.01a	0.00a	1.05a	0.00a
5 x 10^6	1.01a	1.04a	1.11a	2.61ab
5 x 10^5	1.01a	1.17a	1.08a	1.92ab
5 x 10^4	1.00a	1.91a	1.09a	2.45ab
Carbofuran	1.01a	1.27a	1.12a	1.79a
Control	1.17b	4.86b	1.31a	5.37b
CV	5.00	21.11	8.00	24.18

Bore Number, Drill Length, and Ear Length, Bontobili, 1996

Treatment(conidia/ml)	Bore Number/Plant	Drill Length (cm)	Ear Length (cm)
5 x 10^7	1.17a	5.57a	12.83a
5 x 10^6	1.19a	5.85a	12.34a
5 x 10^5	1.20a	6.07a	12.20a
5 x 10^4	1.20a	7.53ab	12.08ab
Carbofuran	1.13a	5.40a	12.72a
Control	1.49b	8.25b	10.61b
CV	7.00	20.10	8.20

The Effect of Several *B. bassiana* Concentration and Cabofuran 3% to Ear Damage and Yield, Bontobili, 1996

Treatment (conidia/ml)	Ear Damage Scoring	Yield (t/h)
5 x 10^7	1.88a	2.53a
5 x 10^6	1.99bc	2.05ab
5 x 10^5	2.31bc	2.02ab
5 x 10^4	2.61b	1.59b
Carbofuran	2.13bc	2.06ab
Control	3.38c	1.40b
CV	18.40	21.80

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
Source : Surtikanti, *et. al*., (1997)

Conclusion

The result above indicated the promising potency of *B. bassiana* especially in controlling corn borer by selecting strain having high virulence (Bb-27, Bb-31, and Bb-34) with concentration 5 x 10^7 conidia/ml. Because *B. bassiana* requires time in developing before killing larvae, it is preferably applied at egg laying period of *O. furnacalis* (3 WAP, further application is 2 week interval). The achieved application result is better, if the relative humidity under plant canopy is <70%.

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White muscardine (*Beauveria* spp.), a pledging natural enemy, against asian corn borer (*Ostrinia furnacalis*): a physiological characterization study

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Abstract. The improvement of the corn production often got constraint such as pest infestation. The pest often attacking the corn planting in Indonesia is Asian corn borer (*Ostinia furnacalis* Guenee). One control practice against Asian corn borer that probably causes minimal negative environmental effect is the use of biological control agents such as predators, parasites, nematode, virus, or fungi. White muscardine (*Beauveria* spp.) is one of the effective fungi in suppressing corn borer. But, several study results indicated that its virulence was different on every *Beauveria* isolate. The virulent difference of *Beauveria* spp. isolates hypothesized correlated to the physiological difference of them. Therefore, the objective of this research is to know the physiological characterization of *Beauveria* spp. isolates associated to their virulence. The study was carried out in the laboratory and screen house of Indonesian Cereals Research Institute from 2004 to 2005. *Beauveria* spp. Isolates were collected from different part of Sulawesi Island. Those collected isolates were then purified in the laboratory. The physiological characterization was based on the ability of each isolate to produce toxin Beauvericin, that conducted by thin layer chromatography and toxin detection was done by using UV light with 254 nm wave length. 18 isolates i.e. B07, B22, B23, B24, B27, B30, B31, B32, B33, B34, B36, B40, B41, B46, B47, B50, B51, and B52 were examined their virulence in the screen house. Maize variety Lagaligo was used. The detection result of toxin beauvericin indicated that there were 3 isolates i.e. B52, B07, and B41 which did not show stain on the chromatograph paper. Those isolates were still expected producing toxin, in spite of in low concentration. *Beauveria* spp. isolates B27, B32, B33, B34, B36, and B47 indicated bigger stain compared to the other isolates. It remarked that those isolates produced much more toxin compared to the other isolates. The result showed that population density of corn borer ranged 12 – 19 larvae per 2 stalks. The observation result showed that the number of infected insect on isolates B27, B32, B34, and B36 at 3 WAP ranged from 3 to 6 larvae/2 stalks much higher compare to isolates B07, B46, and B52 that were only ranged 1 - 2 larvae/2 stalks. Similarly, it occurred on further examination like at 5, 7, 9, 11 WAP, and harvesting. Isolate B32 was isolate which was very effective to infect *O. furnacalis* larvae with scoring value 4.17, while the lowest scoring was B07 (1.17 of scoring value). percentage of leaf laceration ranged from 2.76% to 28.97%. Examination of tassel damage was done at 7 WAP. It seemed that treatments B27, B32, and B34 had low intensity i.e. 6%, 5.67%, and 5.67%, respectively. The result of drill length on stalk was varied among treatments. Isolates B32, B34, B36, and B47 had drill length from 0.67 cm up to 1.33 cm and were significantly different to B07, B46, and B51 with 4.3 – 5 cm of length, while control gave 6 cm of length. it can be concluded that Isolates B27, B30, B32, B34, and B36 have high virulence to corn borer (*O. furnacals*).

Keywords : Beauveria spp., A pledging natural enemy , Asian corn borer, A physiological characterization study

Introduction

In 2003, The maize productivity rate in Indonesia was 3.2 t/h, while the study results of the corn productivity reached 4.5 – 8.0 t/h. Therefore, the farmers have chance to increase their maize production. The improvement of the corn production, however, often got constraint such as pest infestation.

The pest often attacking the corn planting in Indonesia is Asian corn borer (*Ostinia furnacalis* Guenee). Several practices to control this insect pest have already been utilized and the application of insecticides is the most popular farmer's practice. Unfortunately, the use of insecticides has negative impact both on the environment and on human health.

One control practice against Asian corn borer that probably causes minimal negative environmental effect is the use of biological control agents such as predators, parasites, nematode, virus, or fungi.

White muscardine (*Beauveria* spp.) is one of the effective fungi in suppressing corn borer. But, several study results indicated that its virulence was different on every *Beauveria* isolate. Soenartiningsih (1999) reported that the mortality of *O. furnacalis* caused by infection of *Beauveria* spp. reached 82.5%, while Yasin, *et. al.* (1999) recorded that *Beauveria* spp. could only infect corn borer up to

62.5%. Endang and Yohanes (2000) examined four of *Beauveria* isolates from the same host (*H. hampei*) but different isolate source locations. The results of their study showed that *Beauveria* gave different respond to the *H. hampei* larvae. Similarly, Feng and Johnson (1990) reported that every *Beauveria* spp. isolate had different killing ability to the target pest. The virulent difference of *Beauveria* spp. isolates hypothesized correlated to the physiological difference of them. Therefore, the objective of this research is to know the physiological characterization of *Beauveria* spp. isolates associated to their virulence.

Materials and Methods

Date and Place

The study was carried out in the laboratory and screen house of Indonesian Cereals Research Institute from 2004 to 2005.

Collection of Beauveria spp. Isolates

Beauveria spp. Isolates were collected from different part of Sulawesi Island. Those collected isolates were then purified in the laboratory. The purification was done by isolating the fungus from the hosts. The fungus was then put in 100 ml of dH_2O. The isolate suspension was diluted up to 10^{-5}. It was cultured on PDA, then incubated for 48 hours. The emerged single colony was transferred to the other PDA. All of isolates were used in physiology and virulence study.

Physiological Characterization of Beauveria spp. Isolates

The physiological characterization was based on the ability of each isolate to produce toxin Beauvericin. The purified isolates was cultured on the liquid Czapex Dox + Yeast Media for 7 days with room temperature and without light. 0.75 ml of Filtrat sample was poured in Erlenmeyer and mixed with 0.5 ml of methanol : chloroform solvent (2 : 1 v/v), then centrifuged for 10 minutes 1000 rpm. Supernatant was air dried on block heater with temperature 40°C, then added 100 µl of methanol : chloroform solvent (2 : 1 v/v). 20 µl of sample was poured on the oven dried chromatograph paper (type 60 F254 Merck, Darmstadt, Germany). The carrier soluble was used chloroform : methanol : acetate ethyl (18 : 1 : 1). Chromatography was done for 5 minutes or until the carrier soluble reached 1 cm from up border of paper, then the paper was got by forceps and air dried. Toxin detection was done by using UV light with 254 nm wave length. The purified beuvericin toxin was utilized as standard (Sigma, Germany).

Virulence Test of Beauveria spp. Isolates in Screen House

18 isolates i.e. B07, B22, B23, B24, B27, B30, B31, B32, B33, B34, B36, B40, B41, B46, B47, B50, B51, and B52 were examined their virulence in the screen house. The 18 isolates were selection results of 52 isolates found.

Pail (volume 9 gallons) was fulfilled 10 kg of soil, then planted maize (variety lagaligo). Each pail was planted 2 crops. The use of fertilizer with dosage 200 kg/h urea, 100 kg/h SP36, and 100 kg/h KCl was done instantly after planting and 100 kg/h urea was given at 5 weeks after planting (WAP). At 1 WAP, the *Beauveria* spp. suspension with concentration 10^6 conidia/ml and dosage 50 ml/pail was sprayed on the plant.

At 2 WAP, 20 new emerged larvae of *O. furnacalis* were infested and put on the rolled leaf. The plant and larvae were covered by gauze cloth. Leaf laceration caused by *O. furnacalis* was examined at 3 and 5 WAP, tassel damage was observed at 7 and 9 WAP, while examination of hole number per stalk was done at 9 and 11 WAP. Observation of bore length was done at harvesting, and the number of infected larvae was inspected at 3, 5, 7, 9, and 11 WAP. Each isolate as treatment was replicated 3 times so that number of pail utilized was 54 buckets. Leaf laceration valued by scoring 1 to 9 based on Guthrie *et. al.* (1980), as fallows at table 1.

Tassel damage counted by formula, as fallows:

$$P = \frac{a}{a+b} \; 100\%$$

P = Percentage of Tassel Damage
a = Amount of Infested Tassel
b = Amount of No Infested Tassel

Table 1. Scoring Value of Leaf Laceration Caused By Corn Borer

Score	Value (%)
1	0
2	1 – 10
3	11 – 20
4	21 – 30
5	31 – 40
6	41 – 50
7	51 – 60
8	61 – 70
9	71 - 100

Result and Discussion

Physiological Characterization of Beauveria spp. Isolates

The detection result of toxin beauvericin indicated that there were 3 isolates i.e. B52, B07, and B41 which did not show stain on the chromatograph paper. Those isolates were still expected producing toxin, in spite of in low concentration (Fig 1.). The other 15 isolates produced toxin beauvericin with varied concentration remarked by the presence of the stain appearance difference on chromatograph paper, and which was parallel with standard stain position. *Beauveria* spp. isolates B27, B32, B33, B34, B36, and B47 indicated bigger stain compared to the other isolates. It remarked that those isolates produced much more toxin compared to the other isolates. According to Tanada and Kaya (1983) and Riyanto and Santoso (1981), *Beauveria* spp. produced not only toxin beauvericin but also secondary metabolic i.e. beauverolide, bassianolide, isorolide, ocsalat acid, and color substance. Riyanto and Santoso (1991) proposed that toxin beauvericin damaged cell membrane structure and caused death of insect. Beauvericin also injured the main function of hemolymph, caused the change of cell nucleic and influenced cell movement in the cell row (Tanada and Kaya, 1983). The result indicated that there was significant correlation between mortality of *O. furnacalis* larvae and toxin produced. *Beauveria* spp. isolates producing much more toxin such as B32, B34, B36, etc. have high ability to kill larvae. Fungus *Beauveria* spp. formed toxin beauvericin on culture media and host insect. If beauvericin is succeeded to extract, purified, and then applied on insect pest, it can caused death of the insect. According to Grove and Pople (1980), beauvericin was too effective to control *A. agepty* larvae. While according to Tanada and Kaya (1983), beauvericin produced from culture media and if it was inoculated on silkworm, it will damage the main function of hemolymph. Fig. 1 showed that *Beauveria* spp. besides produced mycotoxin beauvericin, also generated other secondary metabolic. Kind of secondary metabolic was not detected yet, because of unavailability of standard.

Virulence Test of Beauveria spp. Isolates in Screen House

The result showed that population density of corn borer ranged 12 – 19 larvae per 2 stalks. At 3 WAP, the plant sprayed by isolate suspensions (B27, B30 and B32) had population density 12 – 13 larvae per 2 stalks much lower compared to other isolates such as B07, B41, and B52 with population density 17 larvae per 2 stalks, while

Figure 1. Production of Toxin Beauvericin of Several *Beauveria* spp. Isolates on Liquid Czapek Media. Bigger stain on the HPLC paper, higher toxin concentration produced by each of *Beauveria* spp. isolates.

population density on control reached 19 larvae per 2 stems. (Table 2). At 5 WAP, the population density on each treatment was increasingly varied. At treatments B30, B32, B34, and B36, it was lower i.e. 4 – 9 larvae/2 stalks, meanwhile, B07, B22, and B46 gave population density of *O. furnacalis* 16 larvae/2 stalks. At 7 WAP, isolates B32, B34, and B36 had lower density around 8 – 9 larvae/2 stalks and they were significantly different to B07, B46, and B51). The others had 18 – 21 larvae per 2 stalks and control gave up to 24 larvae/2 stems.

Similarly, it occurred on further observation such as at 9 and 11 WAP. The density difference of every treatments occurred, probably because of the virulent difference of each isolate. Isolates B32, B34, and B36 were proved having highest killing ability in the laboratory test. The high virulence of those probably caused by those ones especially B32 and B34 have faster sporulation ability, as well as other characteristics such as toxin production and other compound playing role in killing insect

Lower density on isolates B27, B32, B34, and B36 was appeared because of higher insect death caused by infection of *Beauveria* spp. The observation result showed that the number of infected insect on isolates B27, B32, B34, and B36 at 3 WAP ranged from 3 to 6 larvae/2 stalks much higher compare to isolates B07, B46, and B52 that were only ranged 1 -2 larvae/2 stalks. Similarly, it occurred on further examination like at 5, 7, 9, 11 WAP, and harvesting (Table 3). Based on Table 3, Isolate B32 was isolate which was very effective to infect *O. furnacalis* larvae with scoring value 4.17, while the lowest scoring was B07 (1.17 of scoring value).

This study also observed leaf laceration caused by infestation of *O. furnacalis*. At 3 WAP, percentage of leaf laceration ranged from 2.76% to 28.97%. The treatments getting lowest infestation were appeared at isolates B27, B32, and B34 wit intensity 3.40%, 3.23, and 2.76%, respectively (Table 4). Meanwhile, *Beauveria* spp. isolates sowed highest intensity were seemed at isolates B07, B46, and B52 with 17.67%, 22.50%, and 18.53% of intensity, respectively. Intensity on control was up to 28.17%.. At 5 WAP, the plant sprayed *Beauveria* spp. isolates had leaf laceration varied from 9.5% to 32.33%, and control had been up to 53.33%. Based on the data, amount of the one isolates utilized were effective to control corn borer. The treatments of *Beauveria* spp. isolates having low leaf damage were B27, B32, B34, and B36 with intensity 11.17%, 9.5%, 10.50%, and 10.67%, respectively. Low leaf damage was probably caused by low population density of corn borer larvae.

Table 2. Population Density of *O. furnacalis* Larvae per 2 Stalks on Effectiveness Study of Several *Beauveria* spp. Isolates. ICeRI Secreen House, Maros.

Isolates	Observation (WAP)					Score Means
	3	5	7	9	11	
B 07 (Aphids)	17 ab	16 ab	18 c	24 b	10 c	2,2
B 22 (Aphids)	16 ab	15 ab	16 cd	18 bcd	8 c	2,7
B 23 (*O.furnacalis*)	16 ab	12 b	14 d	16 cd	9 c	3
B 24 (*O.furnacalis*)	15 ab	11 b	12 ef	17 bcd	12 c	3,7
B 27 (*O.furnacalis*)	12 b	11 b	8 e	12 de	3 d	3,7
B 30 (*O.furnacalis*)	13 ab	8 c	12 d	20 bc	8 c	3,2
B 31 (*H. armigera*)	16 ab	12 b	14 de	14 c	6 c	3
B 32 (*O.furnacalis*)	12 b	4 d	8 e	12 de	3 d	4,1
B 33 (*N. lugens*)	17 ab	14 ab	14 f	18 bcd	18 ab	2,5
B 34 (*O.furnacalis*)	15 ab	9 c	8 e	10 de	12 c	3,6
B 36 (*O.furnacalis*)	14 ab	8 c	9 e	12 de	8 c	2,7
B 40 (*H. armigera*)	15 ab	13 ab	12 e	20 bc	14 b	3
B 41 (*N. virescens*)	17 ab	13 ab	10 e	18 bcd	12 c	2,1
B 46 (*O.rhinoceros*)	18 ab	16 ab	20 b	22 bc	10 c	3
B 47 (*O.furnacalis*)	15 ab	11 ab	10 e	8 e	9 d	3
B 50 (*H. armigera*)	15 ab	12 b	12 ef	16 de	14 b	3
B 51 (*O.furnacalis*)	17 ab	15 ab	18 c	24 b	16 ab	3,9
B 52 (*N. lugens*)	17 ab	14 ab	16 cd	12 de	10 c	2,3
Control	19 a	18 a	24 a	30 a	21 a	1
CV (%)	21,1	24,10	17,20	23,90	25,7	

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
WAP = Week After Planting

Table 3. The Amount of O. furnacalis Larvae infected Beauveria Spp. per 2 Stalks on Effectiveness Study of Several Beauveria spp. Isolates. ICeRI Secreen House, Maros.

Isolates	Observation (WAP) 3	5	7	9	11	Score Means
B 07 (Aphids)	2 cde	2 bc	2 cd	2 de	2 de	1,17
B 22 (Aphids)	3 bc	2 bc	3 bc	3 cd	2 de	2
B 23 (*O.furnacalis*)	4 abc	3 ab	4 abc	4 cd	3 cd	3,72
B 24 (*O.furnacalis*)	4 abc	3 ab	4 abc	5 bc	5 bc	3,88
B 27 (*O.furnacalis*)	6 a	5:00 AM	6 a	7 ab	6 ab	3,5
B 30 (*O.furnacalis*)	5 ab	4 ab	5 ab	6 bc	6 ab	2,42
B 31 (*H. armigera*)	4 abc	4 ab	5 ab	5 bc	6 ab	3,08
B 32 (*O.furnacalis*)	6 a	5:00 AM	6 a	8:00 AM	8:00 AM	4,17
B 33 (*N. lugens*)	3 bcd	2 bc	3 bc	5 b	5 bc	2,42
B 34 (*O.furnacalis*)	5 ab	5:00 AM	6 a	7 ab	7 ab	3,25
B 36 (*O.furnacalis*)	5 ab	4 ab	5 ab	6 ab	6 ab	3,25
B 40 (*H. armigera*)	4 abc	2 bc	4 abc	4 c	2 de	2,47
B 41 (*N. virescens*)	3 bcd	2 bc	3 bc	3 cd	2 de	2,17
B 46 (*O.rhinoceros*)	1 de	2 bc	2 cd	2 de	2 de	1,42
B 47 (*O.furnacalis*)	4 abc	3 ab	4 abc	5 bc	4 cd	2,72
B 50 (*H. armigera*)	4 abc	3 ab	4 abc	5 bc	3 cd	2,63
B 51 (*O.furnacalis*)	3 bcd	2 bc	2 cd	2 de	1 e	2,58
B 52 (*N. lugens*)	2 cde	2 bc	2 cd	2 de	2 de	1,5
Control	0 e	0 c	0 d	0 e	0 e	1
CV (%)	24,3	26,2	33,9	30,5	33,2	

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
WAP = Week After Planting

Table 4. Percentage of Leaf Laceration caused by *O. furnacalis* Larvae on Effectiveness Study of Several *Beauveria* spp. Isolates. ICeRI Secreen House, Maros.

Isolates	Observation (WAP) 3	5	Score Means	Isolates	Observation (WAP) 3	5	Score Means
B 07 (Aphids)	17,67 c	30,83 b	2,5	B 36 (*O.furnacalis*)	3,90 e	10,67 d	4,5
B 22 (Aphids)	11,83 d	29,90 bc	3,25	B 40 (*H. armigera*)	8,00 d	20,00 c	3,5
B 23 (*O.furnacalis*)	9,67 d	20,67 c	3,5	B 41 (*N. virescens*)	11,87 d	25,67 bc	3,5
B 24 (*O.furnacalis*)	9,23 d	22,33 c	3,5	B 46 (*O.rhinoceros*)	22,50 b	32,33 b	2
B 27 (*O.furnacalis*)	3,40 e	11,17 d	3,5	B 47 (*O.furnacalis*)	10,17 d	23,00 c	3,5
B 30 (*O.furnacalis*)	5,50 e	12,33 d	4,5	B 50 (*H. armigera*)	11,00 d	23,00 c	3,5
B 31 (*H. armigera*)	8,33 d	20,67 c	4,5	B 51 (*O.furnacalis*)	13,87 d	27,00 bc	3,25
B 32 (*O.furnacalis*)	3,23 e	9,50 d	3,5	B 52 (*N. lugens*)	18,53 c	29,20 bc	2,75
B 33 (*N. lugens*)	8,23 d	26,00 c	4,5	Control	28,17 a	53,53 a	1
B 34 (*O.furnacalis*)	2,76 e	10,50 d	4,5				
CV (%)	15,2	16,9			15,2	16,9	

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
WAP = Week After Planting

Observation of bore number was done at 9 and 11 WAP. Based on the study result at 9 WAP, the number of bores formed were ranged from 1 to 5 hole per stalks (Table 5). Low number of bores was found at isolates B23, B27, B30, B32, B34, and B36 with 1 hole/stalk of each isolate. High number of bore occurred at treatments B07, B46, B51, and B52 with 3 holes/stalk of each isolate. At 11 WAP, several examined isolates were effective to control corn borer, it seemed by low number of bores. Those meant isolates were B07, B32, and B36 with each isolate 1 hole/stalk, while control had 6 holes/stalk.

According to Nafus and Schreiner (1987), larval instar I up to III of *O. furnacalis* feed on still young rolled leaf or

Table 5. Amount of bores caused by *O. furnacalis* Larvae on Effectiveness Study of Several *Beauveria* spp. Isolates. ICeRI Secreen House, Maros.

Isolates	Observation (WAP)		Score Means	Isolates	Observation (WAP)		Score Means
	3	5			3	5	
B 07 (Aphids)	3,00 b	3,00 b	2,17	B 36 (*O.furnacalis*)	1,00 c	1,00 c	3,5
B 22 (Aphids)	2,00 bc	2,00 bc	2,5	B 40 (*H. armigera*)	2,00 bc	2,00 bc	2,83
B 23 (*O.furnacalis*)	1,00 c	2,00 bc	2,67	B 41 (*N. virescens*)	2,00 bc	3,00 b	2,67
B 24 (*O.furnacalis*)	2,00 bc	2,00 bc	2,5	B 46 (*O.rhinoceros*)	3,00 b	4,00 b	2
B 27 (*O.furnacalis*)	1,00 c	1,00 c	3,17	B 47 (*O.furnacalis*)	2,00 bc	2,00 bc	3,17
B 30 (*O.furnacalis*)	1,00 c	3,00 b	2,83	B 50 (*H. armigera*)	2,00 bc	3,00 b	2,67
B 31 (*H. armigera*)	2,00 bc	2,00 bc	2,5	B 51 (*O.furnacalis*)	3,00 b	3,00 b	2,17
B 32 (*O.furnacalis*)	1,00 c	1,00 c	3,5	B 52 (*N. lugens*)	3,00 b	3,00 b	2,17
B 33 (*N. lugens*)	2,00 bc	3,00 b	2,33	Control	5,00 a	6,00 a	1
B 34 (*O.furnacalis*)	1,00 c	1,00 c	3				
CV (%)	26,7	22,7			26,7	22,7	

Means with the same letter in the column are not significantly different at 5% level of significance by DMRT
WAP = Week After Planting

leaf surface sheltered by the opened leaf. Larval instar III till VI began to bore maize stalk. On the high intensity, there were more than 1 larva in stalk. Nonci (2000) reported that 9 holes could be found in 1 stalk and each hole was found 1 larva.

After larvae were succeeded through in the stalk, the larvae continued to do activity until forming pupa and it would go out after emerging from pupa. Larva of corn borer that was in the stalk feed on inside stalk web. It caused formation of tunnel inside the stalk. The length of tunnel ranged 0.5 cm up to couple cm depended on the activity of corn borer.

Conclusions

Based on the result above, it can be concluded that:

1. There is difference of toxin production from each isolate.
2. Isolates producing higher beauverin toxin have higher virulence.
3. Isolates B27, B30, B32, B34, and B36 have high virulence to corn borer (*O. furnacails*).

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Section 5

Maize Agronomy

Promotion of Zero Tillage in Maize through Farmer-Participatory Approach in Bihar, India

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Abstract. Every time a field is plowed, soil life is disturbed, and organic matter and water are lost. Millions of hectares of agricultural land could be saved from this loss if farmers could be convinced about the benefits of conservation agriculture. There is need for a paradigm shift in the way soil is cultivated. Efforts were made in this direction in the Indian state of Bihar. Ten farmer-participatory trials were conducted with zero-till maize during winter (seeded first week of November 2006) and summer (first week of July 2007) in four districts of the state: Samastipur, Muzaffarpur, Begusarai and Purnea. Farmers harvested an average yield of 5.0 t ha^{-1} with zero till as compared to 3-5 t ha^{-1} under the conventional system in summer. During the summer of 2007, however, rains at flowering damaged the crop and yields were affected due to poor seed set. The yield advantage in summer was due to sowing on time (no field preparation needed), less irrigation time (if required) and higher input use efficiency due to precise placement of fertilizer and seed. Farmers reported that yield variations during summer were normally due to loss of time and resources in field preparation and highly unpredictable weather. Yields in winter (8.0 t ha^{-1}) were similar under zero and conventional till. Farmers using zero till reported savings in terms of field preparation, labor for weeding and irrigation water during winter due to optimum growing conditions. They could plant earlier and save Rs. 1200-1500 $acre^{-1}$. The farmers' observation was that zero-till maize saves precious time and money besides ensuring high input use efficiency.

Key words: zero till, farmer-participatory approach

Introduction

Under the rainfed conditions and in the low-input cropping systems prevalent in India, summer maize yields are low, averaging 1980 kg ha^{-1}. The main causes of this are biotic and abiotic stresses. Healthy soil is one of the most important assets of the farmer. Sustaining soil productivity requires perennial action by soil organisms on organic materials. This optimizes soil porosity and movement of roots, water and gases in the root zone. Soil is more quickly formed and self-renewed from the top downward than by slow addition from the bottom upward. Loss of porosity diminishes the soil's infiltration capacity and water-holding potential, factors that provide insufficient organic substrates for soil organisms and accelerate oxidation of soil organic matter and hinder the self-recuperation of soil (Shaxson 2006).

The Food and Agriculture Organization (FAO) predicts that if soil losses continue unchecked, the potential rainfed crop production will decline by about 15% in two decades in Africa, about 19% in Southeast Asia, and more than 41% in Southwest Asia (Kelly 1983). When soil losses are higher than the natural soil regeneration rate, sustainable agriculture is not possible. Recent studies show that soil erosion is a selective process, with the most fertile soil particles taken away. Eroded soil sediments usually contain several times more nutrients than the soils they originated from (Stocking 1988). Zero-till planting is a truly sustainable production system that promises to address the problem of soil erosion and may hold out benefits to farmers in the tropics and warmer areas of Africa and Asia. The reason for this is that "no technique yet devised by mankind has been anywhere near as effective at halting soil erosion and making food production truly sustainable as no-tillage" (Baker *et al.* 1996).

Zero-till planting cuts production costs by 15-20% by eliminating the 4-8 tillage operations practised in conventional tillage systems. An added benefit: the incidence of most annual weed species decreases with zero-till seeding, wheat-maize rotation and crop residue retention. This in turn reduces herbicide use. In contrast, weed populations persist with tillage and monocropping despite intensive weed control. Where zero-till seeding, monocropping and residue removal are practised, however, new, difficult-to-control weeds are appearing. Results of trials based on 10 years of careful monitoring at CIMMYT Mexico provide strong justification for delivering suitable technologies to farmers in the Altiplano of Central Mexico and similar regions (Govaerts *et al.* 2005). Mexico as a whole faces the problem of soil erosion due to strong winds, rain and flooding with the onset of the monsoon rains toward the end of June or in the first week of July every year. Farmers therefore do not invest in the summer crop due to the unpredictable weather and the time that has to be expended in field preparation, which further exposes the crop to the vagaries of nature. To address similar problems, farmer-participatory trials on zero-till maize were conducted in four districts of Bihar, India, to sensitize farmers of the region to the benefits of conservation agriculture.

Material and Methods

The trials were conducted on one-acre plots. Conventional-till plots were prepared by farmers as per their local practice (6-8 passes of the cultivator followed by planking for fine tilth) and no-till plots were established where crop residue had been retained after the previous harvest. Spacing was kept at 70 cm row-to-row and 20 cm plant-to-plant for both planting systems. Weeds were controlled with the herbicide glyphosate –(preplanting) followed by atrazine (immediately after sowing). One application of carbofuran 3G (Furadan) placed in whorls was given to control stem borer. Quality Protein Maize (Shaktiman 4 from Rajendra Agriculture University seeded in the winter) and normal maize (Pioneer 30R77 seeded in the summer) cultivars were used in the study. Farmers followed their normal management practices for the different crop seasons in both conventional- and zero-till conditions. Fertilizers were applied in the ratio of N70:P24:K12 kg per acre in winter and N32:P16:K8 kg per acre in summer using urea (46% N), diammonium phosphate (DAP, 18% N and 46% P) and muriate of potash (MOP,- 60% K}. Observations were recorded for: 1. yield; 2. time taken for sowing; 3. time taken for irrigation; 4. weed control, and 5. production costs.

Results

Due to proper placement of seed and fertilizers through the tyne openers of the planters, the differences in germination were very clear to the farmers. The zero-till field had uniform germination with a healthy plant appearance compared to plants in the conventional-till field which was characterized by a mix of healthy and nonhealthy plants and poor germination.

Time taken for sowing

It took on average an hour and a half to sow as well as place the basal fertilizers in the zero-till plots whereas it took six workers eight hours to sow the conventional-tilled plots. Preparation of the latter plots required six cultivator operations with fertilizer broadcasting in the last operation followed by planking, making it an energy- and time-consuming process.

Time taken to irrigate For the conventional-tilled plot, it took on an average 7-8 hours to cover the whole area as compared to 4.5-6.0 hours depending on the soil type for the whole zero-till area. This meant a cost saving in terms of time as well as money since irrigation is performed with diesel pumpsets in northern Bihar. Also, precious groundwater is saved, which can be used to put more area under irrigated agriculture.

Weed control

Weeds were effectively controlled with a preplanting application of the herbicide glyphosate (0.4 k.g.a.i acre^{-1}) during maximum weed pressure to kill most of the weeds (annual and perennial). This was followed by pre- or postemergence application of atrazine (10-15 days after germination) at the rate of 0.5-0.8 k.g a.i acre^{-1} in 150-200 litres of water for both the herbicides.

Yield

Yields were monitored across the sites and were observed to be higher in the summer (kharif, or monsoon-sown crop) under zero till due to better utilization of fertilizers, risk avoidance due to timeliness of agricultural operations, and better root establishment and anchoring. Yields as high as 3-4.0 t ha^{-1} were observed in winter under both practices and 2.0-2.5 t ha^{-1} in summer in spite of unfavorable weather (Fig. 1a, 1b). The differences will be even more evident if the full package of conservation agriculture-based resource-conserving technologies is adopted over a period of time.

Discussion

Farmers observed that the zero-till seeding technology ensured timeliness of operations, input use efficiency and immediate economic returns in terms of savings in land preparation, seeding cost and less irrigation time leading to saving of fuel. It was found in these farmer-participatory trials that farmers could save more than Rs 2000 per ha on field preparation costs (Fig. 2). They also saved money in labor (Rs 1000 per ha) and Rs 70-105 per irrigation (2-3 hours' saving in irrigation time) in winter and summer as and when irrigation was applied. It was felt by farmers that zero-till technology is advantageous as they could save Rs 5000-6000 per ha.

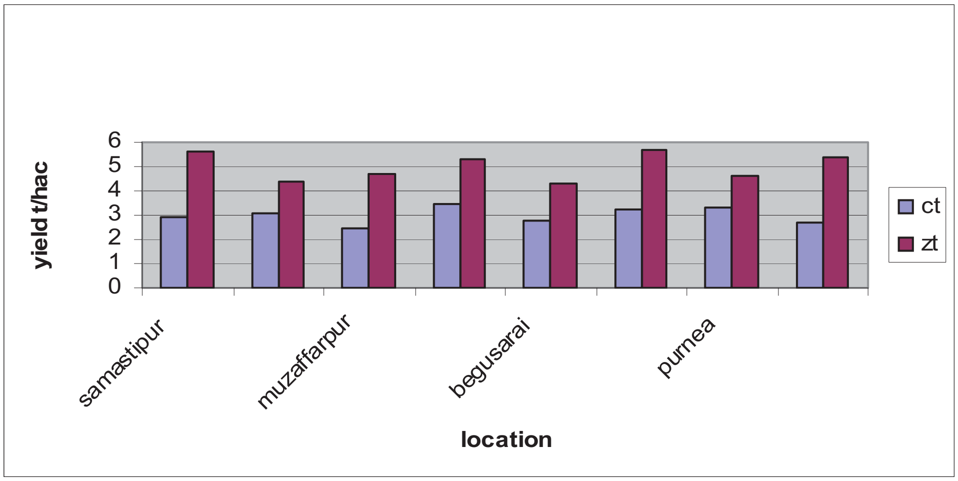

Figure 1a. Yield of maize under conventional (ct) and zero-till (zt) planting in summer 2006.

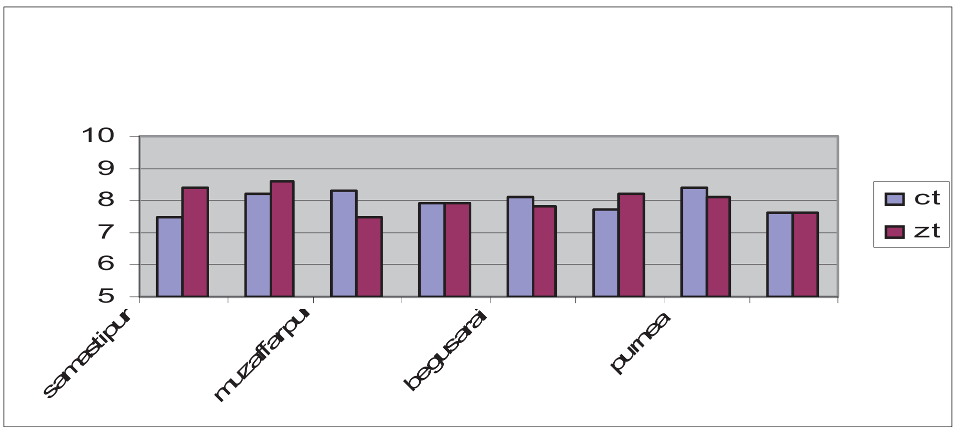

Figure 1b. Yield of maize under conventional (ct) and zero till (zt) in winter 2007.

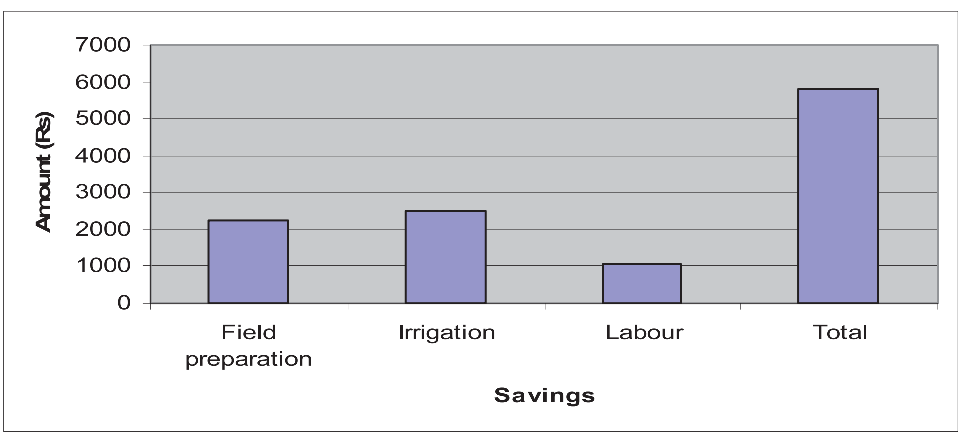

Figure 2. Savings (per ha) in no-till maize in Bihar, India.

Suggestions by farmers

The lack of availability of low-cost, good-quality seed of high-yielding hybrids/varieties of different maturity groups coupled with drought and waterlogging tolerance especially in summer was one of the constraints identified by farmers. They tend to apply very little or no fertilizer during summer due to the unpredictable weather, so low nitrogen-efficient genotypes will play a vital role in improving India's maize productivity since a major part of the maize area in the country is sown during summer. Lack of training and local nonavailability of zero-till seeders were the other constraints to the spread and adoption of this technology. So efforts to promote local availability of quality equipment, low nitrogen-efficient genotypes coupled with drought and waterlogging tolerance will go a long way in promoting conservation agriculture-based resource-conserving technologies.

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Enhancing Productivity of Maize-based Cropping System through Integrated Weed Management under Rainfed Conditions

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Abstract. During the rainy season maize is infested by a variety of weeds, leading to losses ranging from 34% to 67% or sometimes even more. A field experiment with different integrated weed management (IWM) practices was conducted during 2004, 2005 and 2006 with the objective of identifying appropriate IWM for enhancing productivity of maize-based cropping systems under rainfed conditions. Pooled results for the three years showed that IWM markedly reduced weed density and weed dry matter at 35 DAS and 50 days after sowing (DAS). Manual or mechanical weeding at 20 DAS and 35 DAS gave a weed control efficiency (WCE) of 44.24% and 49.91%, respectively, compared to 86.97% under weed-free control. Similarly, PE atrazine @ 0.75 kg ha^{-1} for a pure stand of maize gave a WCE of 62.61%. Inclusion of soybean as an intercrop in paired maize rows in a 2:2 row ratio (30/90 cm) and treatment with PE Pendimethalin @ 1.0 kg ha^{-1} followed by one hand weeding at 35 DAS resulted in a significant reduction in weed count, which in turn produced the least weed dry matter (41.46 gm m^{-2}) against 115.87 gm m^{-2} obtained under nonweeded control at 50 DAS. Further, this system showed significant superiority in terms of WCE (64.20%), giving rise to significant improvement in yield attributes and yield of the crops in the cropping system and producing the highest maize equivalent yield of 3211 kg ha^{-1} with a B:C ratio of 1.98. This was significantly superior to the other IWM practices. Decreased weed counts and weed dry matter in this IWM system seem to have been the result of relatively less space being available for growth of weeds, leading to weed smothering up to the critical crop stage. Thus, maize intercropped with soybean in a 2:2 row ratio (30/90 cm) with PE Pendimethalin @ 1.0 kg ha^{-1} followed by one hand weeding at 35 DAS can be the appropriate IWM system for enhancing productivity of maize-based cropping systems under rainfed conditions.

Introduction

Being a wide-row crop, maize can provide a conducive environment for luxuriant growth of weeds during the rainy season, causing yield losses ranging from 33%-67% (Sreenivas and Satyanarayana 1994). Adoption of a suitable intercropping pattern involving summer (*kharif)* pulses and maize not only ensures additional returns but also reduces weed density by very quickly covering the interrow spaces thereby not allowing weeds to utilize the resources. Paired-row planting of maize + soybean in a 2:2 row ratio maintained at 30/90 cm leaves more space (90 cm) to soybean but relatively littleto weeds. However, intercropping alone is not sufficient to overpower weeds during the rainy season. Therefore, integration of different methods of weed control were attempted in the present investigation with the objective of identifying more efficient and economically viable models of integrated weed management (IWM) for enhancing productivity of maize-based cropping systems under rainfed conditions in the state of Rajasthan, India.

Materials and Methods

This field study was carried out at the Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT). Banswara, Rajasthan for three consecutive years: summer 2004, 2005 and 2006. The soil of the experimental plot was clay loam in texture with a pH of 7.8. Heavy rains during the initial part of the rainy season are a characteristic feature of this region favouring luxuriant growth of weeds followed by a terminal drought. As a consequence, maize yields are reduced under this rainfed agroecological system. Maize hybrid PEHM-2 and soybean variety JS-335 were used as the test crops in this experiment. Different IWM practices including manual, mechanical, chemical and agronomical methods and their combinations (Table 1) were evaluated in a randomized block design with three replications. Maize as a pure crop was planted at 60 x 25 cm while paired-row maize intercropped with soybean in a 2:2 row ratio was planted at 30/90 cm. Both maize and soybean were sown simultaneously with fertilizer quantities calculated on the basis of the area occupied by each crop. Need-based plant protection measures were undertaken. Sampling techniques for all growth and yield characters, including estimation of maize equivalent yields, were followed as per standard procedure. The treatments were evaluated on the basis of LAI (leaf area index) of maize at 50 DAS, weed density and dry matter at 35 and 50 DAS, %WCE at 35 and 50 DAS, number of grain/cob and the maize equivalent yield. The benefit:cost ratio was computed to assess the economic viability of the IWM technology. The crop was maintained rainfed during all the years of the investigation.

Results and Discussion

Pooled data for the three years presented in Table 1 show that IWM practices markedly reduced weed density and weed dry matter at 35 DAS and 50 DAS compared with control (Fig. 1). Manual weeding at 20 DAS and 35 DAS gave a weed control efficiency (WCE) of 44.24% at 50 days after planting. Similarly, at the same stage, intercultivation at 20 DAS and 35 DAS gave a WCE of 46.63% and 49.91%, respectively, compared to 86.97% as determined under weed-free conditions (Fig. 2). Similarly, PE Atrazine @ 0.75 kg ha^{-1} for a pure stand of maize gave a WCE of 36.66% and 62.61%, respectively at 35 DAS and 50 DAS. Inclusion of soybean as an intercrop in paired rows in a 2:2 row ratio (30/90 cm) treated with PE Pendimethalin @ 1.0 kg ha^{-1} followed by one hand weeding at 35 DAS produced a significant reduction in weed count which in turn resulted in the least weed dry matter (41.46 g m^{-2}) against 115.87 g m^{-2} obtained in the nonweeded plot at 50 DAS (Fig. 1).

Further, this IWM system showed significant reduction in the weed count which resulted in a significantly superior WCE of 64.20% against 86.97% for the weed-free plot. Reduced weed counts and increased WCE in this IWM treatment significantly improved yield attributes and grain yield especially in terms of the number of grain/cob which increased by 56.81% over the weedy check (254.70 grain/cob). Under this IWM system, the maize crop was favoured significantly, and produced the highest maize equivalent yield of 3211 kg ha^{-1} as compared to 1786 kg ha^{-1} for the weedy check plot. The maximum B:C ratio of 1.98 (Table 1 and Fig. 3) was also obtained with this IWM system, which was significantly superior to the rest of the IWM practices.

Inclusion of soybean as an intercrop under rainfed conditions in paired-row planting (2:2 row ratio) allowed relatively less space for growth of weeds from a very early stage of crop growth due to quick coverage of ground, more shading and the smothering effect. Furthermore, the system is additive, having an additional 50% population of soybean in the maize stand with better spatial use of resources and mutual cooperation between species (C3 &

Table1. Effect of IWM treatment on weed density, weed dry matter, weed control efficiency (WCE, %), yield and yield attributes and B:C ratio of a maize-based cropping system under rainfed conditions in Rajasthan, India.

Treatment Details	Weed density (nos/m^2)		Weed Dry meter (g/m^2)		Maize LAI at 50 DAS	% WCE	
	35 DAS	50 DAS	35 DAS	50 DAS		35 DAS	50 DAS
T_1: Weedy check (Control)	14.41* (207.02)	15.79 * (248.92)	70.06	115.87	2.42	0	0
T_2: Manual weeding at 20 &35 DAS	12.94 (166.99)	11.05 (121.62)	54.93	64.6	3.38	21.57	44.24
T_3: Inclusion of legume intercrop (Maize+Soybean2:2)	12.66 (160.01)	12.68 (160.37)	54.82	59.4	3.15	21.71	48.7
T_4:T_3 *fb* PE pendimethalin (1kg/ha)	12.37 (152.51)	12.48 (155.21)	47.21	53.49	3.37	32.68	53.73
T_5:T_4 *fb* one hand weeding at 35 DAS	12.4 (153.25)	10.36 (106.85)	48.33	41.46	3.64	30.89	64.2
T_6: Sole maize *fb* inter cultivation at 20 DAS	12.82 (163.80)	13.06 (170.11)	54.73	61.75	3.43	21.93	46.63
T_7: Sole maize *fb* inter cultivation at 20 & 35 DAS	12.89 (165.69)	11.10 (122.65)	55.27	58.07	3.71	20.97	49.91
T_8: T_6 *fb* removal of weeds manually from interspace	12.51 (156.10)	12.80 (163.33)	48.94	67.76	3.92	30.14	41.56
T_9: Sole maize *fb* PE Atrazine (0.75 kg/ha)	11.27 (126.53)	11.60 (134.10)	44.34	43.3	3.96	36.66	62.61
T_{10}: Sole maize (weed free)	4.8 (22.66)	4.78 (22.41)	13.54	15.13	4.37	80.67	86.97
SEm $\pm$	0.15 (3.31)	0.15 (3.10)	2.25	1.72	0.12	3.19	1.42
C. D. at 5%	0.44 (9.83)	0.45 (9.20)	6.67	5.1	0.37	9.47	4.23

* Square root transformation: □x + 0.5

Figure 1. Effect of IWM on weed density and weed dry matter at 50 DAS.

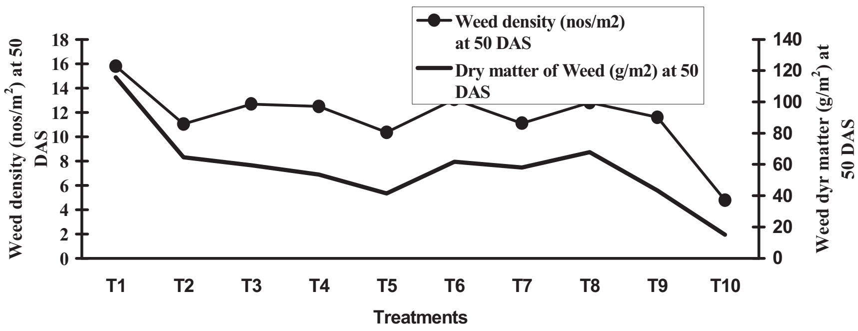


Figure 2. Effect of IWM on % WCE and number of grain/cob.

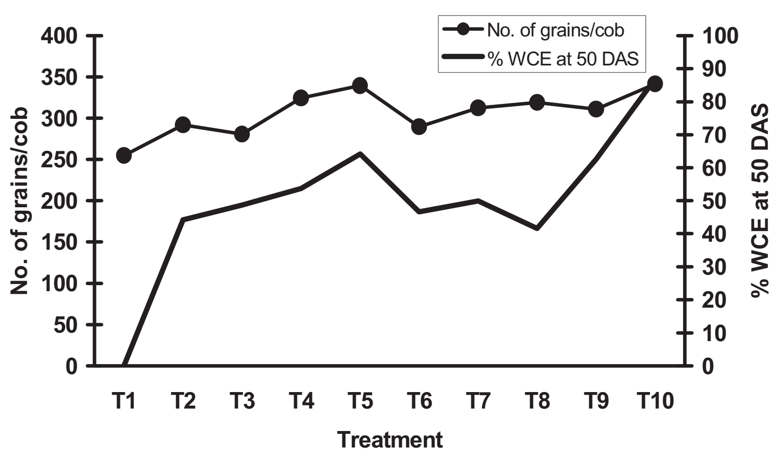
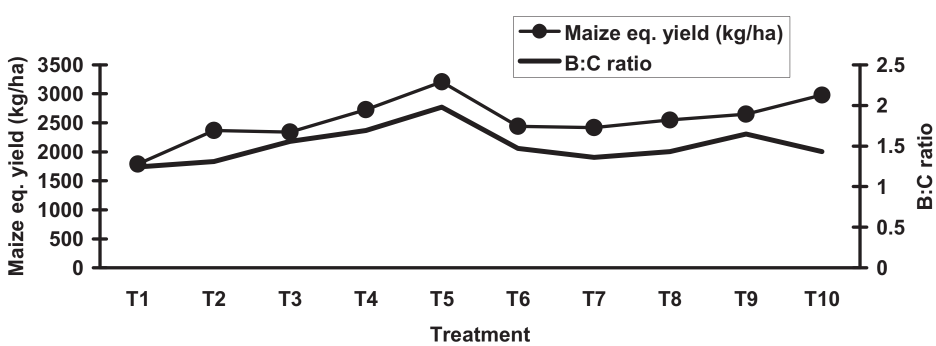


Figure 3. Effect of IWM on maize equivalent yield and B:C ratio.



C4), which seem to be responsible for the suppression of weeds (Prasad and Rafey 1996). The use of PE Pendimethalin @ 1 kg ha^{-1} kept weed seed germination under check right from the initial stages (Thakur 1994) followed by one hand weeding at 35 DAS. This treatment reduced the weed count and weed dry matter and increased the WCE to 64.20% at the 50-day crop stage. This may have helped to enhance yield attributes and crop yields in the system.

Conclusion

Maize intercropped with soybean in paired rows in a 2:2 row ratio (30/90 cm) with PE Pendimetalin @ 1.0 kg ha^{-1} followed by one hand weeding at 35 DAS can be an appropriate and economically viable IWM system for enhancing productivity of a maize-based cropping system under rainfed conditions.

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Soil Moisture Conservation for Improving Maize Yields through Participatory Micro-watershed Approach in the Foothills of the Shivaliks

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Abstract. The Shivaliks are the foothills of the Himalayas. This area has been neglected for a long time and now faces a constant threat of soil erosion and flooding. The Shivaliks cover an area of over 20 000 km^2 in the Indian states of Himachal Pradesh, Punjab, Haryana and Jammu & Kashmir. Nearly 70 m ha of land in these ranges, mainly on the Himalayan slopes, are critically degraded and need immediate attention. The prime cause of the backwardness of this region is reduction in soil fertility and productivity due to massive soil erosion. Crop yields in the foothills are quite low. The situation requires efficient management of land and rainwater for conserving in situ. It was observed from studies that four plowings are necessary to obtain optimum wheat yield per unit of land. An increase in soil moisture content has been observed from 6.7% in the absence of plowing to 19.4% with four plowings. On-farm experiments with maize in the lower Shivaliks showed that soil moisture storage increased 9.3%, 12.7% and 16.8% and plant height increased 18.1%, 33.1% and 49.9% with shallow tillage, deep tillage and ridge and furrow treatments respectively over control. Mulching in maize was also quite effective in conserving soil moisture and improving yields. There was a 10.6% increase over control in grain yield with ridge and furrow sowing of maize.

Key words: Moisture conservation, soil, land, maize, nutrient, foothill, rainfed

Introduction

The foothills of the Shivaliks, the submontaneous tract of northwestern India, are about 300 km long and 30 km wide. This region is also known as the *Kandi* area which more or less coincides with the boundaries of the Shivalik range. The major part of this tract is rainfed because of high annual evapotranspiration compared to annual rainfall. There has been a decline in the size of landholdings in this region, which is one of the causes of the poor economic status of farmers here. The challenge therefore lies in improving or raising the productivity of land in this rainfed submontane region. One of the most important parts of this challenge lies in *in situ* conservation of moisture especially in the winter season which may the allay the difficulties arising from the occurrence of droughts in the area (Arora 2006). However, most of the rainstorms received in the summer (kharif) season here are of short duration and high intensity whereas those received in the winter (rabi) season are of low intensity and erratic in distribution. These conditions result in soil erosion due to excess rainfall in the summer monsoon months and soil moisture deficit in the winter months. Therefore, the situation warrants efficient management of both land and rainwater for conserving the *in situ* moisture and utilizing it as per need. The water table lies deep to very deep, and rainfall is the only source of water here. Lack of irrigation facilities and large-scale erosion are the main constraints of this region's agricultural economy. The rainfed areas are mostly underfed from the point of view of application of inputs compared to the outputs obtained (Hadda *et al.* 2000). However, the challenge of improving productivity can be addressed by efficient utilization of nutrients and natural resources. One of the ways is to use nutrients from all possible resources in an integrated manner and maximizing the utilization of applied nutrients by crops (Acharya and Bandopadhyay 2002). Thus, there is a need to enhance crop yields through *in situ* moisture conservation coupled with land, soil and nutrient management practices in this region.

Materials and Methods

A group of 15 farmers were selected in Kokowal-Majari village in Hoshiarpur district (experiment I) in the undulating agricultural subcatchments in the foothills of Punjab. Each of the farmers represented a single unit of land in the village. The maize variety Prakash was sown. The four treatments consisted of *in situ* soil moisture conservation through adopted land and soil management practices. These included shallow tillage, deep tillage and raised-bed sowing and they were compared with farmers' practice. Farmers of the area generally practice sowing conventional (local) crop varieties without following any land management and moisture conservation practices. They mostly cultivate fields along the slope with application of only one-third of the recommended dose of nitrogenous straight fertilizers.

In another experiment (experiment II) at Ballowal Saunkhari village in Nawanshahr districtin Punjab state, the treatments included five modes of straw mulch applications: nonmulched (control; M_o), fully covered plots (M_f), partially covered plots (lower one-third covered; M_p) , strip (M_s) and vertical mulching (M_v). In the strip application, rice straw mulch was applied in 6" x 2" strips constructed in alternative rows, and in vertical mulching rice straw was applied in holes (10 cm diameter and 15 cm deep) made with an auger. Thus there were 6 strips and 17 vertical holes per plot in the strip and vertical mulch treatments respectively. Maize cv Prakash was planted with 45 cm row-to-row and 22.5 cm plant-to-plant spacing. Rainfall was received in 31 rainstorms out of which 12 were erosive (producing runoff). A total of 547 mm of rain was received during the monsoon season, which was much below the normal rainfall for the area.

In experiment III in the foothills of Jammu, the treatments were as follows: T_1 = farmers' practice; T_2 = recommended fertilizer + contour cultivation and sowing of improved variety GS 2 in lines across the slope; $T_3 = T_2$ + 25% N replacement through farmyard manure (FYM); $T_4 = T_3$ + addition of zinc @ 25 kg $ZnSO_4$ ha^{-1} and atrazine as a preemergence weedicide. The farmers' practice included application of FYM (4 t ha^{-1}) before sowing and application of only urea (40 kg ha^{-1}) as top-dressing one month after sowing. A local variety of maize was sown through broadcasting.

The treatments were replicated thrice in a randomized block design (RBD). Periodic soil moisture samples were collected up to a depth of 120 cm before sowing and at different days after sowing (DAS). The crop was harvested at maturity from each treated plot, and the grain were separated and the yields recorded.

Results and Discussion

Tillage and mulching practices in maize

Soil moisture storage at 40 DAS increased 11.7%, 29.8% and 45.3% over the farmers' practice in the shallow tillage, deep tillage and raised-bed sowing treatments respectively. However, it was observed in all the treatments that moisture storage at 40 DAS was greater in the subsurface layers than in the surface (0-15 cm) layers (Table 1). The highest amount of moisture at lower depths (60-90 cm) was observed in the raised bed sowing treatment. This may be due to greater absorption and less evaporation of moisturein that treatment. At 80 DAS, soil moisture storage was higher by 9.3%, 12.7% and 16.8% over the farmers' practice in the shallow-tillage, deep-tillage and raised-bed treatments respectively (Table 1). The increase in soil moisture may be because of better soil management practices and raised-bed sowing.

There was a 10.6% increase in maize grain yield in the raised-bed sowing treatment compared to farmers' practice. In the other treatments, while grain yield was higher over control, it was nonsignificant. Increase in maize grain yield with moisture conservation practices has also been observed by Gaur (2002) in a watershed in Rajasthan state of India.

Mulch material was found to be quite effective in conserving soil moisture. However, its role differed significantly with different modes of application. Periodic observations of the moisture content of surface and subsurface soil in experiment II revealed that all modes of mulch application were effective in conserving moisture at all stages of crop growth. Compared to control at 40 DAS, soil moisture was higher by 3.80% and 1.80% in surface soil and by 4.20% and 2.30% in subsurface soil respectively in wholly covered and partially covered plots (Table 2). The other two modes also helped in conserving soil moisture but the amount of water conserved was relatively small compared to that under the fully covered treatment. Similarly, at 60 DAS, gravimetric soil moisture was higher by 4.85%, 2.30% and 1.05% in the 0-15 cm soil layers and by 4.25%, 2.75% and 1.80% in the 15-30 cm soil layers in the M_f, M_p and M_s modes of mulch application over M_o (Table 2). At all stages of crop growth, moisture storage was greater in the subsurface layers than in the surface soil layer. The greater the surface area covered by the mulch material in a particular mode, the greater was its effectiveness in conserving soil moisture. Thus, among the different modes of mulch application, the area covered

Table 1. Effect of different treatments on moisture storage (at 90 cm depth) and maize yield.

Treatment	Moisture storage at 30 DAS (cm)	Moisture storage at 60 DAS (cm)	Grain yield (kg ha^{-1})
Farmers' practice	10.0	21.2	2270.5
Shallow tillage	11.2	23.2	2344.4
Deep tillage	13.0	23.9	2435.6
Raised bed	14.5	24.7	2470.2
RDF (100%)	10.8	20.5	2525.2
RDF (75 %) + FYM	13.5	24.0	2568.5

Table 2. Effect of different mode of mulch application on soil moisture storage.

Mode of mulch application	Soil depth (cm)					
	0-15 cm			15-30 cm		
	40 DAS[1]	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Nonmulched (M_o)	7.85	7.95	3.45	7.80	9.10	3.85
Vertical (M_v)	8.20	8.50	4.10	8.50	10.00	4.72
Strip (M_s)	8.75	9.00	5.00	9.35	10.90	5.40
Partially covered (M_p)	9.65	10.25	6.00	10.10	11.85	6.40
Wholly covered (M_f)	11.65	12.80	8.20	12.00	13.35	10.15
LSD (P = 0.05)	1.79	1.17	0.74	1.58	1.27	1.75

[1.] DAS: days after sowing.

varied from 98% in the fully covered treatment followed by 33% in the partially covered treatment, 18% in strip mulching, 1.8% in vertical mulched plots to 0% in the nonmulched control.

It was also observed that fully covered plots had 156% higher dry matter as compared to nonmulched plots. Dry matter yield under different modes of straw mulch application, ie, M_p, M_s and M_v was observed to be 99%, 53% and 8% higher respectively than the nonmulched control plots (M_o). The average dry matter yields of maize in the mulched plots were significantly higher than that the nonmulched plot which was because of the favourable influence of mulching on the soil (Weeraratna and Asghur 1990; Gajera *et al.* 1998). Maize grain yield was significantly higher in the wholly covered, partially covered and strip mulching plots as compared to control. Mulch spread on the whole plot increased grain yield by 58.6% over the nonmulched control (Table 3). In the vertical-mulched and control plots grain yield was almost similar.

The mode of straw mulch application significantly influenced maize straw yield too. It was observed that straw yields in the wholly covered, partially covered and strip application plots were significantly higher by 35.05%, 31.39% and 25.3% respectively than that of the nonmulched plots (Table 3). Vertical mulching also returned a higher yield than the control plots but it could not reach the level of significance. Similar increments in maize fodder yields with mulch application have been observed in the rainfed areas of Punjab state in India (Khera and Singh 1998).

Contour cultivation in maize

Soil moisture storage at 45 DAS increased by 15.0%, 25.5% and 30.2% with T_2, T_3 and T_4 treatments respectively over the farmers' practice. However, it was observed that

Table 3. Effect of different modes of mulch application on grain and straw yield of maize.

Mode of mulch application	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Nonmulched (M_o)	2466	4074
Vertical (M_v)	2502	4352
Strip (M_s)	3163	5105
Partially covered (M_p)	3206	5353
Wholly covered (M_f)	3912	5502
LSD *(P = 0.05)*	104	256

moisture storage was more in the subsurface layers at 45 DAS than in the surface (0-15 cm) layers in all the treatments (Table 4). This may be due to greater absorption and less evaporation of moisture in the treatment. At 90 DAS, soil moisture storage increased by 20.3%, 24.4% and 27.8% T_2, T_3 and T_4 treatments respectively over the farmers' practice. This may be because of better land management in the form of contour cultivation across the slopes and FYM application.

There was a 20.3%, 35.6% and 49.4% increase in maize grain yields in the T_2, T_3 and T_4 treatments compared to farmers' practice while maize straw yields were 18.0%, 33.9% and 42.2% higher in these treatments (Table 4). Increases in maize grain yield with improved soil moisture conditions and application of FYM have also been observed by Gaur (2002) in a watershed in Rajasthan and by Arora and Hadda (2003) and Hadda *et al.* (2005) in submontane region of Punjab. Also, application of weedicide has an added effect on maize yield.

Our experiment showed that adoption of improved moisture conservation practices through land and soil management helped in improving maize crop yields in rainfed areas in a gradual but sustainable manner.

Table 4. Effect of contour cultivation on soil moisture storage and yield of maize.

Treatment	Soil moisture (cm/30 cm)		Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
	45 DAS[1]	90 DAS		
T_1 (Farmers' practice)	17.4	21.2	1047	2125
T_2 (RDF + contour cultivation and sowing of improved variety in lines across the slope)	20.0	25.5	1260	2508
T_3 (T_2 + 25% N replacement through FYM)	21.1	26.4	1420	2845
T_4 (T_3 + addition of zinc @ 25 kg $ZnSO_4$ ha^{-1} and atrazine as preemergence weedicide)	21.6	27.1	1564	3022
LSD *(P = 0.05)*	1.20	2.40	114	282

[1.] DAS = days after sowing.

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Baby Corn: A Better Option to Diversify Maize Cultivation in Hill Regions for Higher Income

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Abstract. Maize (Zea mays L.) is the most important rainy-season crop in the northwestern Himalayan region. It is cultivated on 664 000ha, which is 43.2% of the total area under rainy-season cereal crops. Maize production in this region, especially in Himachal Pradesh, is surplus. However, due to the lack of processing industries, the surplus produce is sold at throwaway prices to traders from the neighboring states. In view of the maize production potential of this hill region and the low economic returns from grain maize, cultivation of maize for baby corn offers an alternative option for resource-poor hill farmers. It would allow them to diversify their agriculture without having to get away from what they know the best, ie, maize growing. It would also enable them to access high prices in the national and international markets. The Directorate of Maize Research at the Hill Agricultural Research and Extension Centre of the CSK Himachal Pradesh Krishi Vishvavidyalaya, Bajaura, conducted field experiments from 1993 to 2004 to identify suitable cultivars of baby corn maize and standardize production techniques, eg, population density/planting geometry, fertilizer requirements, appropriate harvesting time and baby corn-based cropping systems. Out of a number of hybrids and open-pollinated varieties evaluated, three, hybrid VL 42 and composites Early Composite, and VL 78, were found suitable. A complete package of baby corn cultivation practices has been generated and recommended to farmers in the region.

Key words: Baby corn, Zea mays, diversification, hill regions

Introduction

Maize is cultivated on 664 000 ha in the northwestern Himalayan region, which is 43.2% of the total area under rainy-season cereal crops. In Himachal Pradesh state of India, it is cultivated on 299 000 ha, producing 695 400 t of grain at an average 2326 kg ha^{-1} (2006-07). About 200 000 t of this is surplus, which is sold at throwaway prices to traders in neighboring states due to the lack of food processing industries in Himachal Pradesh. An interesting recent development has been the cultivation of maize for use as 'baby corn.' This development promises to aid diversification of and value addition to maize cultivation in addition to promoting the growth of the food processing industry. The agronomic requirements of baby corn are similar to grain maize, except suitable varieties, higher plant population per unit area, higher rates of nitrogen fertilizer application and, most importantly, the appropriate time of harvesting. Systematic research work on evaluation of varieties and standardization of baby corn production techniques was initiated by the CSK Himachal Pradesh Krishi Vishvavidyalaya at the Hill Agricultural Research and Extension Centre, Bajaura, under the All-India Coordinated Maize Improvement Project (Directorate of Maize Research) in 1993. Since then, recommendations of cultivation practices have been made to baby corn farmers of the region.

Material and Methods

To develop suitable production technologies for baby corn cultivation, a series of field experiments were conducted at the research farm of the Hill Agricultural Research and Extension Centre in Bajaura, situated at an altitude of 1090 m above mean sea level during the rainy seasons of 1993-2004. More than 40 cultivars were evaluated during this period in a randomized block design (RBD) with 3 replications. The results of 3 selected cultivars are shown in Table 1. Table 2 presents data from on-farm trials conducted in farmers' fields Studies on the effect of tassel removal on baby corn yield were carried out in an RBD with 7 maize cultivars during the rainy seasons of 1995-1997. Experiments on plant spacing/population density were carried out with 6 plant spacing treatments and 2 cultivars in an RBD with 3 replications during 1995-1996. An experiment on nitrogen rates and their schedule of application was conducted with 12 treatment combinations consisting of 3 nitrogen rates and 4 schedules of application in an RBD with 3 replications using the 'Early Composite' cultivar with 40 x 20 cm plant spacing during 1997-1998. Four baby corn-based cropping systems were evaluated from 1996/97 to 1998/99 in an RBD with 4 replications.

The soil of the experimental fields had a sandy loam texture and near-neutral pH. All the experiments were carried out under irrigated conditions with 2-3 irrigations each season as per need. Fertilizers were applied @ 150 kg ha^{-1} N, 26 kg ha^{-1} P and 33 kg ha^{-1} K. A one-third dose of N and whole P and K were applied as basal dressing and the remaining two-thirds N was side-dressed in 2 equal splits each at knee-high (25 DAS) and tassel-emergence (40 DAS) stages of the crop. The average annual rainfall in the experimental area is 919.4 mm, only 44.8% of which is received during the rainy season. Young cobs were handpicked when the silk length was about 2-4 cm. The picking was done every third day. The criteria for marketable yield were 4.5-10 cm length and 0.7-1.7 cm diameter of dehusked cobs having a regular row arrangement (Bar-Zur and Saadi 1990). Green fodder was harvested after completion of cob-picking. To calculate net returns, a selling rate of Rs. 35 kg^{-1} fresh weight for baby corn and Rs. 700 t^{-1} for green fodder were used.

Results and Discussion

Varieties suitable for baby corn

Choosing a suitable maize cultivar is the most critical input in baby corn cultivation. Early maturity, short duration, synchronized flowering and prolificacy are some of the important criteria in the selection of such a cultivar. An early-flowering cultivar can give two or three crops in a maize-growing season in the hills. Short plant height is another desirable character to attain lodging resistance in order to efficiently utilize solar radiation at a high plant density of 120 000-180 000 plants ha^{-1} and thereby obtain a higher baby corn yield per unit area. Similarly, synchronized flowering will require less harvesting time. The composite VL 78, at par with VL 42, gave a significantly higher mean marketable baby corn yield over the cultivar Early Composite(Table 1). This cultivar produced 47% and 9% higher marketable baby corn yield over Early Composite and VL 42 respectively. VL 42 in turn outyielded Early Composite by 35%. The higher yields given by the composite VL 78 and the hybrid VL 42 can be ascribed to their higher number of cobs per plant and cob diameter of dehusked young cobs as compared to Early Composite. Similar results have been reported in the 11th Thai National Corn and Sorghum Reporting Program (Anonymous 1979) and by Thakur (2000). An almost similar performance by these cultivars with respect to marketable baby corn yield was recorded in on-farm trials conducted in farmers' fields at different locations in Kullu and Mandi districts (Table 2) of Himachal Pradesh.

Detasseling

The average yield data of three crop seasons during 1995-1997 showed that tassel removal just after emergence gave a higher marketable baby corn yield than tassel retention because the former resulted in significantly higher cob yield with husk and number of cobs per plant and less discarded baby corn and barrenness (Table 3).

Table 1. Yield and agronomic characteristics of three selected cultivars for baby corn production (mean of 1993-2006).

Cultivar	Marketable baby corn yield (kg ha^{-1})	Cob yield with husk (t ha^{-1})	Fodder yield (t ha^{-1})	Size of de-husked young cob (cm)		No. of cobs plant^{-1}	Plant height (cm)	Days to harvest initiation	Husk (%)	Barren-ness (%)
				Length	Diameter					
VL 42	1602	8.08	26.6	8.3	1.25	2.52	164	47	77.7	4.47
VL 78	1740	8.60	30.4	8.1	1.31	2.86	186	50	80.5	6.06
Early Composite	1182	7.49	38.8	9.4	1.18	1.70	200	54	81.9	10.00
CD *(P = 0.05)*	280	0.51	228	NS	NS	0.40	18	2.4	2.13	1.42

Table 2. Performance (mean of two years) of maize cultivars grown for baby corn purposes in on-farm trials at different locations in Kullu and Mandi districts in Himachal Pradesh, India.

Cultivar	Marketable baby corn yield (kg ha^{-1})		
	Kullu	Mandi	Mean
VL 78 (composite)	1370	1680	1530
VL 42 (hybrid)	1260	1620	1440
Early composite	810	880	850

Plant spacing

The hybrid VL 42, which is a short plant type (average plant height 160 cm), gave the highest marketable baby corn yield at 45 x 20 cm plant spacing (2 plants per hill) having a population density of 175 000 plants ha^{-1} (Table 4). However, a significant increase in yield was obtained up to a population density of 143 000 plants ha^{-1} with a plant spacing of 40 x 35 cm (2 plants per hill). In the case of the open-pollinated cultivar Early Composite, having an average plant height of 200 cm, the maximum baby corn yield was obtained at a plant spacing of 40 x 40 cm (2 plants per hill) with a population density of 125 000 plants ha^{-1}.

Fertilizer nitrogen

Baby corn yield increased with an increase in the rate of nitrogen application (Table 5). However, the increase was not significant beyond 150 kg ha^{-1}. Cob yield with husk increased 17.84% and 6.82% with successive increases in the nitrogen dose. Significantly, the highest green fodder was obtained at a nitrogen rate of 200 kg ha^{-1}. Application of N @ 150 kg ha^{-1}and 200 kg ha^{-1} gave 29.2% and 37.6% higher net return over 100 kg N ha^{-1}, respectively. Motto and Mall (1983) have also reported an increase in prolificacy due to high rates of nitrogen fertilization in baby corn.

Table 3. Effect of tassel removal on baby corn yield (mean data of 1995-97).

Treatment	Cob yield (t ha^{-1})			Fodder yield (t ha^{-1})	Cobs plant^{-1}	Barrenness (%)
	With husk	Without husk				
Without tassel*	8.86	1.48	0.10	33.0	2.14	5.27
With tassel	7.59	1.26	0.16	32.6	1.95	7.81
CD *(P = 0.05)*	0.29	0.07	0.01	NS	0.18	0.73

*Tassel removed just after emergence from leaf sheath.

Table 4. Interaction effect of planting geometry and cultivars on baby corn yield (mean data of 1995-1996).

Planting geometry {cm*/plant population (million ha^{-1})}	Baby corn yield (kg ha^{-1})		
	VL 42	Early composite	Mean
60 x 30 (0.111)	1514	1282	1398
40 x 40 (0.125)	1744	1528	1636
50 x 30 (0.133)	1815	1381	1598
40 x 35 (0.143)	1952	1256	1604
50 x 25 (0.160)	1976	1186	1581
45 x 20 (0.178)	2021	1138	1580
Mean	1837	1295	
CD *(P = 0.05)* Cultivar (C)		47	
Planting Geometry (P)		84	
C x P		119	

Table 5. Yield and net return of baby corn (average data of 2 years) as affected by the rate of nitrogen application.

Nitrogen (kg ha^{-1})	Cob yield with husk (kg ha^{-1})	Marketable baby corn yield (kg ha^{-1})	Discarded baby corn (kg ha^{-1})	Fodder yield (t ha^{-1})	Average net return (Rs. ha^{-1})
100	6132	1174	172	37.8	35236
150	7226	1450	183	39.6	45516
200	7719	1499	191	42.3	48481
CD *(P = 0.05)*	165	47	8	2.0	

Table 6. Yield and net return of baby corn (average data of 2 years) as affected by the rate of nitrogen application.

Schedule of N application	Cob yield with husk (kg ha^{-1})	Marketable baby corn yield (kg ha^{-1})	Discarded baby corn (kg ha^{-1})	Fodder yield (t ha^{-1})	Average net return (Rs. ha^{-1})
1/2 basal + 1/2 at knee-high stage*	6876	1316	190	39.2	40545
1/3 basal + 2/3 at knee-high stage	6923	1354	206	40.0	42506
1/2 basal + 1/4 at knee-high stage + 1/4 at pretasseling stage **	6776	1308	167	39.8	40320
1/3 basal + 1/3 at knee-high stage + 1/3 at pretasseling stage	7446	1517	165	405	48125
CD *(P = 0.05)*	190	55	17	NS	

Table 7. Performance of the baby corn-based cropping system (average data of 1996/97-1998/99).

\Cropping system	Baby corn equivalent yield(t ha^{-1} $annum^{-1}$)				Net return (Rs. ha^{-1} $annum^{-1}$)
	Crop I	Crop II	Crop III	Total	
Baby corn-baby corn-cauliflower	2.13	1.72	1.39	5.24	93211
Baby corn-baby corn-pea	1.98	1.74	1.18	4.90	90626
Baby corn-french bean-wheat	1.78	0.70	0.98	3.46	65842
Baby corn-baby corn-argentine rape	1.94	1.01	0.58	3.56	66555
Maize-wheat	0.83	0.72	-	1.55	32000

Schedule of nitrogen application

Nitrogen application in three equal splits (1/3 basal + 1/3 at knee-height and 1/3 at pretasseling stages) resulted in significantly higher marketable baby corn yield (1517 kg ha^{-1}) and lower discarded baby corn (165 kg ha^{-1}). On the other hand different schedules of nitrogen application could not influence fodder yield significantly (Table 6). The maximum net return (Rs. 48 125 ha^{-1}) was obtained with N applied in three equal splits, which was 13.2%-19.4% higher than that obtained with other schedules of N application..

Baby corn-based cropping systems

The baby corn-baby corn-cauliflower crop sequence resulted in the maximum baby corn equivalent yield (5.24 t ha^{-1} $annum^{-1}$) followed by baby corn-baby corn-pea (4.9 t ha^{-1} $annum^{-1}$). The former crop sequence also gave the highest annual net return of Rs. 93211 ha^{-1} followed by the latter (Rs. 90626 ha^{-1}), whereas the traditional maize-wheat sequence gave the minimum net return of Rs. 32000 ha^{-1} (Table 7). It was observed that the baby corn-french bean-wheat and baby corn-baby corn-argentine rape (*gobhi sarson)* cropping systems resulted in more than double the net return given by the maize-wheat cropping system.

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Influence of Integrated Nutrient Management on QPM Productivity and Soils of Southern Rajasthan

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Abstract. This experiment was conducted at the Rajasthan College of Agriculture, Udaipur, during the rainy seasons of 2003 and 2004 to evaluate and develop an integrated nutrient management package for quality protein maize (QPM) in southern Rajasthan. The experiment included 3 fertilizer levels (75% RDF, 100% RDF and 125% RDF), 3 sources of organic manure (control, FYM and vermicompost) and 2 phosphorus sources (DAP and SSP) with 3 replications in an RBD. The FYM and vermicompost were used as an additional dose over the fertilizer levels. Application of 125% RDF significantly enhanced growth, yield components and yield. It maintained a higher nutrient status in the soil and proved more profitable than 100% RDF and 75% RDF. This treatment also favoured soil health functions compared to other treatments. Integration of either FYM or vermicompost with chemical fertilizers was equally effective with respect to all parameters. However, sources of P did not bring significant variation in any of the parameters.

Key words: Maize, QPM, fertilizer, organic source, yield, soil properties

Introduction

Maize is a staple food for the vast tribal population of southern Rajasthan. It fulfills their food demand but they still suffer from malnutrition due to the lack of a protein source. Therefore, QPM would have great significance for their nutrition security. However, productivity of maize (1.2 t ha^{-1}) in general and of QPM in particular is low because of the inherent low soil fertility in this region (Fig.1). Poor crop management practices tend to further deplete soil nutrient levels. The recommended dose of fertilizer (RDF) for maize is 90 kg N + 40 kg P_2O_5 ha^{-1}. There is no separate recommendation for QPM, a newly introduced crop with a high requirement of fertilizers (DMR 2006). Use of sole chemical fertilizers in the poor and degraded soils of this risk-prone tribal region may be a mistake whereas conjunctive use of organic manure and chemical fertilizers can augment the efficiency of both substances to enhance productivity (Anchal *et al.* 2008). Farmyard manure (FYM) as a source of plant nutrients has been used since ancient times. Now vermicompost is being advocated for use in integrated nutrient management practices (Shroff and Devasthali 1992). Vermicompost, the droppings of earthworms after the intestinal digestion of organic matter, is high in nutritive value. The limited studies done on vermicompost indicate that it increases macrospore space, resulting in improved air-water relation. Application of vermicompost also favorably affects soil pH, microbial population and soil physical and chemical properties (Maheswarappa *et al.* 1999).

Among phosphorus sources, diammonium phosphate (DAP) is water-soluble and efficient, but its higher cost restricts its wider application. Another indigenous water-soluble source of P, single superphosphate (SSP), has proved effective and economically viable for various crops. In addition to phosphorus, SSP contains calcium and sulfur which may be useful in synthesizing certain amino acids and are involved in various metabolic enzymatic processes in plants, which may improve the productivity of QPM. This necessitated the evaluation of these two sources of P for their relative efficiency and economic viability in reference to QPM. Thus, the present investigation was carried out to find out the most appropriate integrated nutrient management option for QPM in southern Rajasthan.

Material and Methods

Our field experiment was conducted during the rainy seasons of 2004 and 2005 at the Instructional Farm of the Rajasthan College of Agriculture, Udaipur, which has a typical subtropical climate. The farm is situated at an altitude of 582 m above mean sea level. The soil is a clay loam, alkaline (pH 8.5) in reaction, medium in available nitrogen (258.4 kg ha^{-1}), available phosphorus (10.5 kg ha-

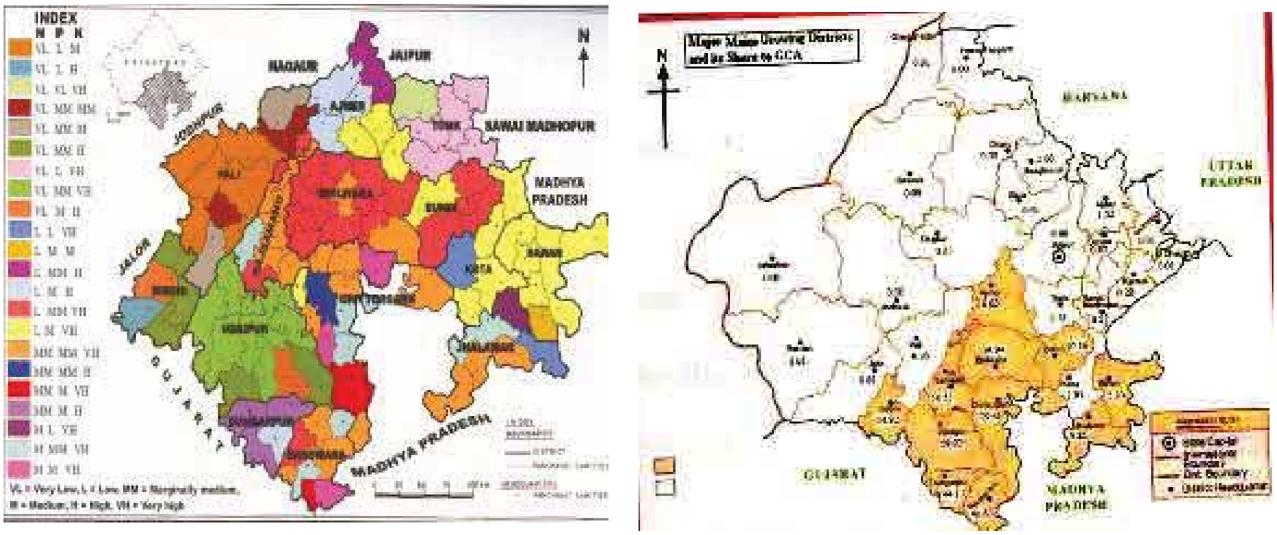

Figure 1. Soil fertility status of the maize-growing area in Rajasthan, India.
N: Low to very low P: Marginally medium to medium K: Medium to very high

^{1}) and available potassium (285.3 kg ha^{-1}). The bulk density and organic carbon content were 1.37% and 0.73% respectively. The experiment consisted of three fertilizers levels (75%, 100% and 125% RDF), three organic sources (no organic manure, FYM 10 t ha^{-1} and vermicompost 5 t ha^{-1}) and two phosphorus sources (DAP and SSP). The 100% RDF was 90 kg N + 40 kg P_2O_5 ha^{-1}. The organic sources were used as an additional dose over the fertilizer treatments. Well-rotted FYM was incorporated in the respective plots 15 days before sowing, whereas vermicompost was incorporated at the time of sowing. The FYM contained 0.50% N, 0.25% P, 0.53% K and the vermicompost 2.00% N, 1.0% P, 1.42% K. A full dose of P and a 1/3rd dose of N were applied at the time of sowing through urea, DAP and SSP as per the treatment. The remaining N was applied in two equal splits at the knee-high and tassel initiation stages. The QPM variety HQPM 1 was used as the test crop. In order to control weeds, preemergence application of atrazine (0.5 kg ha^{-1}) was carried out followed by one hoeing and weeding 25 days after sowing. In both years, the crop was sown in the first fortnight of July with 60 cm x 25 cm spacing and harvested in October. To determine soil properties, soil samples were taken up to 30 cm depth from each experimental plot and subjected to chemical analysis. Standard methods were employed for analysis of the soil samples for nitrogen, phosphorus, bulk density and organic carbon content as well as for computation of the crop growth rate (CGR) and the leaf area index (LAI).

Result and Discussion

Application of 100% RDF significantly improved plant height, LAI, cobs per plant, cob length and test weight over 75% RDF (Table 1). A further increase in the fertilizer level failed to record any statistical significance over the 100% RDF treatment in this respect. However, dry matter at harvest, crop growth rate during 45-75 days after sowing and grain per cob responded significantly up to 125% RDF. At 125% RDF, grain and stover yields improved significantly by 7.60% and 40.71% and 8.62% and 43.23%, respectively, over 100% and 75% RDF. Net returns declined when the fertilizer level was reduced to 75%. Contrarily, a significant increase was obtained by increasing the rate of fertilizer application from 100% to 125% RDF. The highest B:C ratio was recorded under 125% RDF, which was at par with 100% RDF but significantly higher over 75% RDF. After two years of experimentation, application of 125% RDF favorably improved the organic carbon, N and P status of the soil over the 100% and 75% RDF treatments. It, however, reduced bulk density significantly compared to the lower doses of fertilizer application The better nutritional environment due to increased fertilizer application seemed to have promoted plant height and growth of individual leaves by way of active cell division and elongation, which might have increased interception, absorption and utilization of radiant energy, consequently increasing LAI and CGR and thereby overall growth and grain yield. These results support the findings of Singh and Totawat (2002).

Table1. Effect of integrated nutrient management on growth and yield attributes of QPM.

Treatment	Plant height at harvest (cm)	Dry matter (g/plant) at harvest	LAI	CGR 47-75 DAS (g/m^2/day)	Cobs/plant	Grains/cob	Cob length	Test weight
Fertilizer level:								
75%RDF	177.6	150.9	2.64	11.55	1.04	249	14.6	201
100%RDF	200	184.6	2.79	14.2	1.14	288	15.8	210
125%RDF	210.8	201.6	2.9	17.78	1.17	305	16.4	216
CD(P=0.05)	11.12	12.27	0.13	2.06	0.06	15.3	0.81	6.18
Sources of manure								
Control(No manure)	180.5	153.3	2.64	12.22	1.04	254	14.7	198
FYM (10 t/ha)	200.1	185.4	2.81	14.7	1.15	283	15.9	212
Vermicompost (5 t/ha)	207.7	198.4	2.88	17.3	1.16	305	16.1	218
CD(P=0.05	11.12	12.27	0.13	2.06	0.06	15.3	0.81	6.18
Phosphorus source								
DAP	189.6	175.4	2.73	14.46	1.1	279	15.2	206
SSP	202.6	182.5	2.83	15.02	1.14	283	15.9	212
CD(P=0.05	9.08	NS	NS	NS	NS	NS	NS	5.05

RDF = Recommended dose of fertilizer; FYM = Farmyard manure; CGR = Crop growth rate; LAI = Leaf area index.

Application of FYM and vermicompost significantly increased growth, yield attributes and yield of QPM over the treatment without any organic matter. Among these two organic sources, vermicompost significantly improved dry matter, crop growth rate and grain per cob over FYM. However, both sources proved equally efficient in terms of plant height, LAI, cobs per plant, cob length and grain and stover yields. The highest net returns were recorded under FYM, which was at par with vermicompost; and both these sources proved significantly superior to control. The B:C ratio recorded under FYM was significantly higher over vermicompost and the treatment without any organic manure. The better performance of vermicompost might be on account of readily available plant nutrients, growth-enhancing substances and the number of beneficial organisms like N_2-fixing and P-solublizing and cellulose-decomposing organisms (Sultan1997). The low net returns and B:C ratio recorded under vermicompost application were due to its higher cost. It was also observed that in the FYM and vermicompost treatments, bulk density was lower compared to the treatment without organic manure. However, they improved the organic carbon, N and P status of the soil. The low bulk density seen under FYM and vermicompost application could be ascribed to better soil aggregation and aeration brought about by organic amendments by adding various humic fractions (Kadalli *et al.* 2000), whereas improvement in the organic carbon, N and P status of soil might be on account of direct addition of organic matter through FYM and vermicompost and recycling of organic material in the form of crop residue like roots, leaf fall and stumps (Gupta *et al.* 2000).

Except plant height and test weight, most of the growth and yield attributes and yield were at par under both two sources of phosphorus tested in the experiment. This seems to be due to the complete water solubility of both sources (Havlin *et al.* 2003).

It is concluded that application of 125% RDF through urea SSP or DAP along with 10 t ha^{-1} of FYM or 5 t ha^{-1} of vermicompost forms an ideal module of integrated nutrient management for higher productivity of QPM in southern Rajasthan.

Table 2. Effect of integrated nutrient management on soil properties, yield and economics QPM.

Treatment	BD (%)	OC (%)	Available N(kg/ha)	Available P(kg/ha)	Grain yield (Kg/ha)	Stover yield (kg/ha)	Net returns (Rs/ha)	B:C ratio
Fertilizer level:								
75%RDF	1.36	0.79	272.6	19.17	3058	5518	23615	1.87
100%RDF	1.3	0.84	291.5	19.39	3999	7273	34352	2.66
125%RDF	1.25	0.86	297.1	20.17	4303	7900	37600	2.83
CD(P=0.05)	0.009	0.007	0.783	0.114	245	549	2882	0.221
Sources of manure								
Control(No manure)	11.35	0.74	287.4	18.57	2940	5479	24636	2.38
FYM (10 t/ha)	1.2	0.89	289.7	19.87	4131	7462	35531	2.67
Vermicompost (5 t/ha)	1.3	0.86	290.8	20.29	4289	7751	35400	2.31
CD(P=0.05	0.009	0.007	0.783	0.114	245	549	2882	0.221
Phosphorus source								
DAP	1.3	0.85	286.3	19.54	3695	6722	30773	2.37
SSP	1.31	0.81	287.7	19.61	3879	7072	32938	2.54
CD(P=0.05	NS	NS	NS	NS	NS	NS	NS	NS

DAP = Diammonium phosphate; SSP = Single superpPhosphate; BD =Bulk density; OC = Organic carbon.

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Response of Promising Maize Cultivars to Different Nitrogen Levels in Winter

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Abstract. Maize can play a vital role in improving the economic status of marginal farmers in Nepal. In the lowland plains of Nepal, wheat farming is gradually being replaced by winter maize because of the latter's higher yield potential and higher demand for feed and foodgrain purposes. This experiment attempted to find out the optimum dose of nitrogen for two pipeline maize genotypes under winter conditions. A field experiment was conducted at the National Maize Research Program, Rampur, Chitwan, Nepal during the winter cycle (September to March) in two consecutive years. The experiment was laid out in a two-factor factorial randomized complete block design with four replications. Two maize varieties, ZM 621 and Arun 4 were tested at eight nitrogen levels (0, 30, 60, 90, 120, 150, 180 and 210 kg ha^{-1}). The research revealed that increasing nitrogen levels from 0 to 180 kg ha^{-1} increased the grain yield of both cultivars but the differences in grain yields from 90 kg N ha^{-1} to 180 kg N ha^{-1} were nonsignificant. Application of 30, 60, 90, 120, 150, 180 and 210 kg ha^{-1} nitrogen increased grain yield by 19%, 27%, 30%, 41%, 54%, 59%, and 49% over control, respectively. Yield-attributing characters like number of harvested cobs per unit area, number of grain per cob, and 1000-grain weight also increased with increasing nitrogen levels. Maize cultivar ZM 621 (5,735 kg ha^{-1}) yielded higher than Arun 4 (4,988 kg ha^{-1}). Net returns increased with increasing N levels and the maximum net return (2.58) was found at 180 kg ha^{-1} N. The benefit: cost ratio was also highest at this level of N. There was nonsignificant interaction between maize cultivars and nitrogen levels for all the tested parameters, suggesting that both cultivars' responded similarly to different rates of N fertilizer.

Key words: Cultivars, experiment, marginal, promising, nitrogen, potentiality

Introduction

Maize is the second most important staple food crop after rice in Nepal in terms of area and production. It is grown on 851 000 ha with an average yield of 2.037 t ha^{-1}, and contributes about 6.87% to the country's agricultural gross domestic product (ABPSD 2006). Maize makes a major contribution to food security in the hills whilst in the accessible areas it is gradually becoming a commercial commodity due to increasing poultry production and other industries (Pathik 2001). The average annual growth rate for maize yield in Nepal has been 1.05% (Poudyal and Ransom 2001). On the other hand, demand for maize is estimated to grow 6-8% per annum over the next two decades because of the increased food demand in the hills as well as the demand for livestock feed in the accessible areas as a result of the growing demand for milk, meat and meat products (Pathik 2001). In recent years, the increased use of high-yielding crop varieties in intensive cropping systems has led to a substantially higher demand for nutrients. Locally available sources of nutrients, mainly farmyard manures (FYM), compost and biologically fixed nitrogen (N), have not been sufficient to meet this demand. Depletion of organic matter content is at the centre of the overall problem of soil fertility decline. Nitrogen is the major plant nutrient, and unfortunately this element is very low in Nepalese soils (Tripathi and Shrestha 2001). Most of the research conducted by the National Maize Research Program (NMRP) in Nepal are for determining the yield performance of maize varieties is flat (120 kg ha^{-1} N). It has not addressed all the growing conditions, varieties, domains and economic status of farmers. Fertilizer recommendations must balance the risk posed by overapplication as well as underapplication to the profitability of farmers. So, there is need for research that addresses the interests of both small-scale as well as commercial maize farmers in Nepal. The need is further justified by observations that the bulk of maize in the country is produced by smallholder farmers on the one hand and that maize farming in the plains and irrigated areas has become more commercial where high input supply is suitable for higher yield. Therefore, it is necessary to identify the yield performance of promising genotypes at different levels of N fertilizer. This study aims at identifying a more efficient, economic and representative N recommendation system for maize in winter. The objectives of this research were:

- To identify the optimum N dose for ZM 621 and Arun 4 under winter conditions; and
- To determine the effects of different levels of N on growth, yield and yield attributes of maize under winter conditions.

Materials and Methods

The field experiment was conducted at NMRP's research farm in Rampur, Chitwan, which is situated in central Nepal at $27^0 37'$ N latitude and $84^0 25'$ E longitude at an elevation of 228 m above sea level. This region has a subtropical hot humid climate with a minimum temperature of 5-10^0 C in January and a maximum of 34-38^{0}C in April/May. Soil analysis data showed that the total N and organic matter at a medium level whereas available P_2O_5 and K_20 levels were high. The soil was moderately acidic in reaction. The experiment was laid out as a two-factor factorial randomized complete block design (RCBD) with four replications. The treatments were two varieties of maize as one factor and eight different doses of nitrogen as another. The row-to-row distance was maintained at 75 cm and the plant-to-plant spacing at 25 cm. All plots received 60 kg ha^{-1} phosphorus as single superphosphate and 40 kg ha^{-1} potash as muriate of potash at sowing. Every plot except the 0 kg ha^{-1} N treatment received half a dose of N as urea at sowing. The other half a dose of N was side-dressed with urea at 30 days after sowing (DAS) and at the silking stage, respectively. Two seeds of maize in a hill were sown with a planter at 5 cm depth but only one plant was kept per hill by thinning the extra plants at 21 DAS. Weeding was done manually at 21 DAS and earthing up at 45 DAS. Other cultural practices were followed as per the recommendation for improved maize cultivation.

Vegetative parameters like the number of green leaves per plant, leaf area index (LAI), plant and ear height, male and female flowering, physiological maturity, lodging percentage, barren plants per plot, and yield-attributing characters like the number of harvested ears, ear length and circumference, kernels per cob, and 1000-grain weight were evaluated. Similarly, stover yield, above-ground dry matter production, harvest index and grain:stover ratio, grain nitrogen uptake and nitrogen-use efficiency (agronomic efficiency, apparent N recovery percentage and physiological efficiency) were also calculated during the study. Economic analysis of the benefit:cost ratio, net income, and percentage increment in net income over control were also calculated.

Results and Discussion

There was a significant difference between the two cultivars (Table 1) in respect of the number of kernels per cob, ZM 621 producing significantly more (392.9) than Arun 4 (363.8). The number of grain of a cultivar depends upon the potential number of reproductive ovules, which is established early in plant development and fixed at the 5th leaf stage of crop growth (Below 1996). Similarly, the growth duration of a cultivar might cause variation in the number of grain per cob. Adhikari *et al.* (2004) and Gurung *et al.* (2000) observed fewer grain per cob in early-maturing cultivars than full-season ones. The number of kernels per cob differed significantly due to the different N levels. Increasing N levels increased the number of kernels per cob, which was highest (410.0) at 180 kg ha^{-1} N. It was significantly higher than the number obtained at 0-90 kg ha^{-1} N levels but statistically similar with all other N levels.

Table 1. Response of maize cultivars as influenced by different N levels, National Maize Research Program, Rampur, Chitwan, Nepal, winter 2005/06.

Treatment	Grain yield (kg ha^{-1})	Dry matter (kg ha^{-1})	Grain N uptake kg ha^{-1}	Number of kernel ear^{-1}	1000-grain weight (g)
Cultivars					
ZM 621	5735a	11290.8a	108.02a	392.9a	326.2a
Arun 4	4988b	10147.5b	94.32b	363.8b	312.9b
Nitrogen level					
0 kg ha^{-1}	3975d	7703c	75.92e	331.2d	268.3d
30 kg ha^{-1}	4739cd	9436bc	87.72de	359.2cd	304.4c
60 kg ha^{-1}	5036bcd	9937b	92.98cde	372.4bc	311.3c
90 kg ha^{-1}	5179abc	10130b	97.42bcd	378.2bc	316.1bc
120 kg ha^{-1}	5620abc	11240ab	106.1abcd	379.3abc	341.3a
150 kg ha^{-1}	6115ab	12280a	117.7ab	400.9ab	346.8a
180 kg ha^{-1}	6301a	12700a	119.3a	410.0a	328.5abc
210 kg ha^{-1}	5925ab	12320a	112.3abc	395.4ab	339.6ab
LSD	1020	1801.00	19.17	28.54	22.47
SE (±)	357.9	632.33	6.73	10.02	7.89
CV (%)	18.88	16.69	18.81	7.49	6.98

Treatment means followed by common letter/letters within that column are not significantly different within cultivars and N levels respectively, by DMRT at 5% level of significance.

Figure 1. Leaf area index of two maize cultivars at different stages of growth.

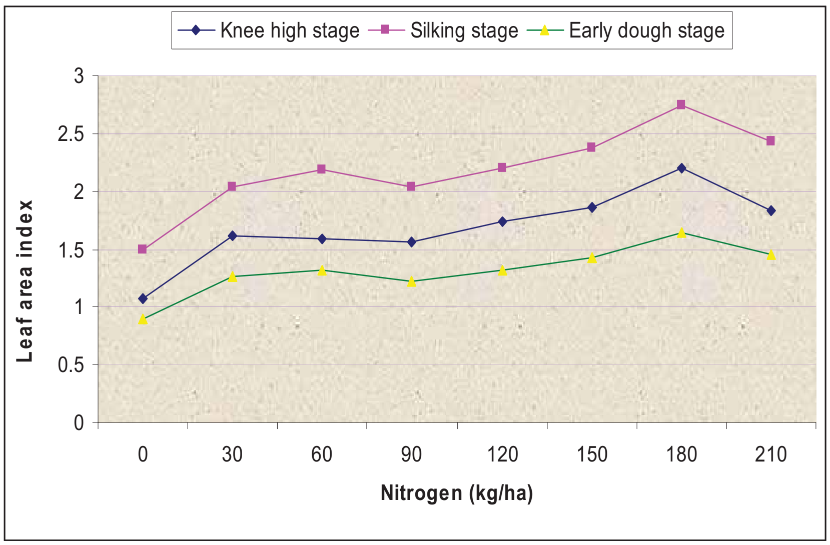

Figure 2. Influence of leaf area index on maize due to different nitrogen levels during winter.

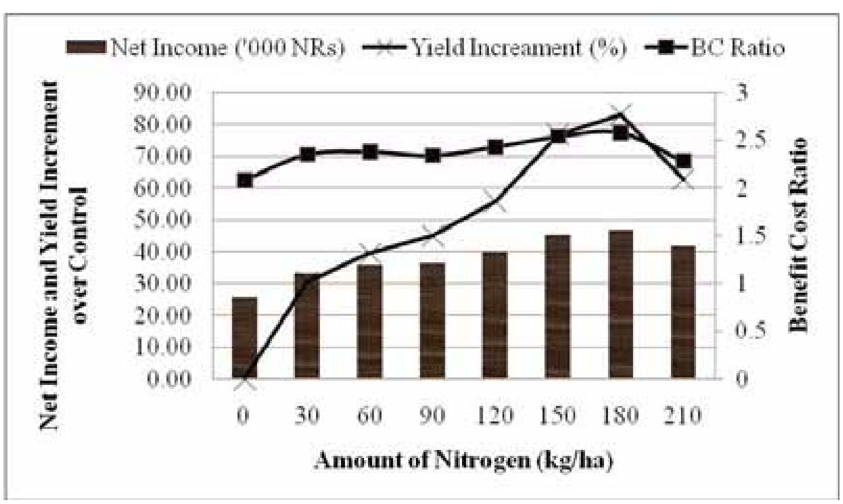

Figure 3. Influence of nitrogen levels on maize net economic return, benefit:cost ratio and yield increment.

The cultivar significantly influenced grain yield. The variety ZM 621 produced a significantly higher grain yield (5735 kg ha^{-1}) than Arun 4 (4988 kg ha^{-1}). It is a full-season maize cultivar with a maturity period of 143 days whereas Arun 4 is an early-maturing cultivar with a maturity period of 120 days. It has been reported that full-season cultivars yield more than early-season cultivars because they get more time for photosynthesis and use more resources than early cultivars. N levels significantly influenced grain yield. The highest grain yield (6301 kg ha^{-1}) obtained at 180 kg ha^{-1} N was statistically higher than the yield obtained at 0, 30 and 60 kg ha^{-1} N but not significantly different from yields at 90, 120, 150, 180 and 210 kg ha^{-1} N.

In general, grain yield increased with increasing N level. This was in agreement with results reported by Tripathi and Pathak (1999) and Kamprath *et al.* (1982). Greenwood (1976) reported that N can influence photosynthesis by affecting leaf area itself or through changes in the photosynthetic rate per unit leaf area (NAR). Grain yield increases with increase in LAI only up to a point. When there is more LAI, there is more photosynthesis, more assimilates and more grain yield. Kamprath *et al.* (1982) stated that increase in maize yield with N application is largely due to an increase in the number of ears, the total dry matter distributed to the grain and an increase in the average ear weight. Greenwood (1976) reported that photosynthesis provides most of the increase in crop dry weight as well as the metabolic energy for crop development.

There was a significant difference between the varieties in respect of the above-ground dry matter, ZM 621 producing more (11 290.8 kg ha^{-1}) than Arun 4 (10 147.1 kg ha^{-1}). Yoshida (1972) reported that accumulated dry weight bears a close relationship with maximum LAI. LAI for ZM 621 was higher than for Arun 4.

Nitrogen levels significantly influenced the above-ground dry matter accumulation. Dry matter increased with increasing nitrogen levels up to 180 kg ha^{-1} and declined at 210 kg ha^{-1}. Grain yield and stover yield also followed the same trend, indicating that dry matter is directly related to grain yield and stover yield. As with grain yield and stover yield, there was a good correlation of yield-attributing characters and high dry matter accumulation at 180 kg N ha^{-1}; it declined at 210 kg N ha^{-1} because of a decrease in photosynthesis. This is in agreement with Greenwood (1976): nitrogen can influence photosynthesis through affecting leaf area itself or through changes in the photosynthetic rate per unit leaf area (NAR). Grain yield increases with increase in maximum LAI only up to a point. He further reported that photosynthesis provides most of the increase in crop dry weight as well as the metabolic energy for crop development. There was no significant difference between the two varieties as regards their interaction with nitrogen levels for above-ground dry matter production.

There was a significant difference between the cultivars in relation to N uptake by grain. ZM 621 (108.02 kg ha^{-1}) absorbed significantly more N than Arun 4 (94.32 kg ha^{-1}). N uptake also differed significantly due to the different N levels. It was highest (119 kg ha^{-1}) at 180 kg ha^{-1} N. It was statistically similar to N uptake at 120, 150 and 210 kg ha^{-1} N but significantly higher than N uptake at all other N levels. Similarly, N uptake at 150 kg ha^{-1} N was significantly higher than N uptake at 0-60 kg ha^{-1} N levels but statistically at par with 90, 120, and 210 kg ha^{-1} N.

Net income was higher for ZM 621 (42,060) than Arun 4 (34,149), as were grain yield and stover yield. Therefore, net return was higher for ZM 621. The highest (NRs 46 880 ha^{-1}) net benefit was observed at 180 kg ha^{-1} N, followed by 150 kg ha^{-1} N (NRs 45 290 ha^{-1}) and the lowest (NRs 25 590 ha^{-1}) at 0 kg ha^{-1} N. Based on average data, the highest (2.58) benefit:cost ratio (B/C) was observed at 180 kg ha^{-1} N, followed by (2.54) at 150 kg ha^{-1} N and the lowest (2.08) at 0 kg ha^{-1} N. In general, the B/C was found to increase with increasing N levels up to 180 kg ha^{-1} N. The maximum (83.20%) increment in net income over control was observed at 180 kg ha^{-1} N followed by 150 (76.98%) and 210 kg ha^{-1} (62.95%) N. An average of 30.52%, 39.55%, 42.28%, and 55.80% increment in net income was obtained over control with the application of 30, 60, 90, and 120 kg ha^{-1} N, respectively.

Conclusion

Grain yield of maize was found to increase with increasing nitrogen levels in this experiment. The highest yield was obtained at 180 kg N ha^{-1}. Application of 30, 60, 90, 120, 150, 180, and 210 kg N ha^{-1} increased grain yield by 19, 27, 30, 41, 54, 59, and 49% over control, respectively. Yield-attributing characters like the number of harvested ears per unit area, number of grain per cob, and 1000-grain weight, above-ground dry matter production and net economic return also increased with increasing nitrogen levels. There was no significant interaction between the two maize cultivars and nitrogen levels for all the tested parameters.

Acknowledgement

We are thankful to the Hill Maize Research Project for funding our research. We express our appreciation to Mr. K Adhikari, Coordinator; Mr. Diwakar Sharma, Senior Scientist; Mr. B. Pandey, Technical Officer; Mr. N. Dhakal and Mr. H. Bohra, Technicians of NMRP, Rampur, Nepal, for their contribution in their respective fields.

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Accelerating Adoption of Suitable Cultural Practices of Maize to Minimize the Yield Gap and Increase Farmers' Income in Karo, North Sumatra

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Abstract. The district of Karo, located in a hilly region characterized by high rainfall intensity, is a major maize-producing area in North Sumatra. Farmers here practise a maize-maize cropping system. Productivity is low at about 7-8 t ha^{-1}. Site-specific nutrient management (SSNM) was introduced to farmers of this region, with a recommendation of 160 kg ha^{-1} N, 72 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O. This study was conducted in Tigabinanga subdistrict of Karo district at a site located 600-700 m above mean sea level during August-December 2007. The study also disseminated a new high-yielding variety of maize together with recommendations for planting density and the number of seeds per hole. The purpose of this participatory experiment was to identify suitable technologies and practices that can increase productivity and labor efficiency. The results indicated that 15 farmers applied SSNM recommendations besides 47 farmer's cooperator, cause labor limitation and the same planting date with the whole farmers. The study found that SSNM technology can increase production by about 11.49% and farmers' income by 17.13%.

Key words: Maize, SSNM recommendations, labor, increased farmers' income

Introduction

Increase in maize production is possible through expansion of cultivable area and/or by intensifying cultivation practices. Expansion of area under maize is difficult because of the limited land available. Intensification of cultivation through measures such as balanced use of fertilizers to supply appropriate and adequate nutrients to the crop, therefore, offers a better opportunity of increasing production and enhancing farmers' income.

Site-specific nutrient management (SSNM) is an approach toward delivering nutrients to crops in dosages appropriate for the season and the location. This approach was developed for irrigated rice farming in Asia (Doberman *et al*. 2002; Witt *et al*. 2002) and has since been disseminated in Indonesia (Samijan *et al*. 2003). Adoption of SSNM for paddy has been shown to have increased the efficiency of fertilization of N, P and K. This approach is being applied to develop site-specific recommendations for maize too (Doberman *et al*. 2003a).

An SSNM study of maize done in Tigabinanga subdistrict in North Sumatra during 2004-2007 obtained a site-specific recommendation for fertilization of maize of 160 kg ha^{-1} N, 72 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O with plant spacing of 70 x 20 cm (1 seed per hole) and 70 x 40 cm (2 seeds per hole) (Akmal *et al*. 2007). These recommendations were then disseminated to farmers by various means including conducting farmers' meetings. It was left to the farmers whether or not to adopt any or all of these technologies. They were given freedom to choose the technology they were capable of executing.

Methodology

Studies of the impact of a technology are generally of two types: (1) ex-post impact studies assess the effects of a technology that has already been adopted and applied by the farmer; and (2) ex-ante impact studies deal with technology that has not yet been adopted but is showing good enough promise. At this activity will be focused at study (ex-ante impact). Approach which applied in this study is (1) before and after (ex-ante vs ex-post) and (2) adopter vs non-adopter (with before and after) adopter vs non-adopter (with vs without).

Our study evaluated the recommendations obtained from an SSNM study on maize done in Tigabinanga subdistrict in the previous year. Our purpose was to promote the use of SSNM for maize with the aim of increasing production and raising farmers' income. The study was carried out at five sites, Perbesi, Pertumbuken, Gunung, Simolap and Kutabangun, in Tigabinanga subdistrict, a dryland farming region located 600-700 m above mean sea level during August-December 2007.

Table1. Farmers' adoption of site-specific nutrient management recommendations on fertilization ofmaize at five sites in Tigabinanga subdistrict, North Sumatra.

Date	Location	Σ of farmers who attended the meeting	Σ of farmers who accepted the FPE	Σ of farmers who implemented the FPE
07/08/2007	Perbesi	16	10	1
07/08/2007	Pertumbuken	15	6	1
08/08/2007	Gunung	22	15	6
08/08/2007	Simolap	25	8	3
08/08/2007	Kutabangun	14	8	4
	Total	92	47	15

Results and Discussion

The results of the study are presented as follows:

Farmers' meeting

A meeting of farmers was conducted over two days (7-8 Aug 2007) to assess the adoption of of SSNM recommendations on fertilization of maize in Tigabinanga subdistrict (Table 1).

The meeting was attended by a total of 92 farmers in the five study sites. The number of farmers who had agreed to adopt the fertilization recommendations was 47, but only 15 actually had begun to practise them.. The following were the constraints to the adoption of this technology: (1) farmers were not yet convinced about the working of these recommendations and there was a lack of infrastructure and an information network that facilitates spread of awareness among farmers; (2) the absence of an agriculture technology dissemination system and the low level of knowledge of farmers makes it difficult for them to access the benefits of new technology; (3) in general, resource-poor farmers are risk-averse because change of technology often means additional requirement of labor; and (4) change of technology also often adds toproduction costs, which, given the high marketing and transport costs and the low market prices for produce, makes the farmer wary of investing in new technology. As a result of these constraints, farmer enthusiasm for adopting new technology is less.

Agronomic findings

Agronomic survey. The cropping system prevalent in Tigabinanga is maize-maize with use of hybrid varieties. Application of fertilizers is generally in abundance, reaching 1 t ha^{-1} with use of urea going up to 500 kg ha^{-1}, SP36 200 kg ha^{-1}, KCl 100 kg ha^{-1} and Ponska 200 kg ha^{-1}. Weed control is usually done by spraying herbicide (gliposat). Farmers in Tigabinanga prefer to burn the crop residue in theirfields to prepare the land for cultivation and remove any pests and diseases (Table 2).

Table 2. Maize farmers' practices in Tigabinanga subdistrict, North Sumatra, Indonesia, 2007.

Parameter	Farmers' practice
Cropping system	Maize-maize
General soil fertility	Fertile
Rainfall pattern	WS: Oct-DecDS: June-July
Crop calendar	Feb/March-June/July Aug/Sep-Dec/Jan
Fertilizer rates	N = 46-184 kg ha^{-1}, P_2O_5 = 18-144 kg ha^{-1} K_2O = 30-90 kg ha^{-1}
Plant spacing (cm)	70 x 40, 75 x 40, 80 x 40, 2 seeds hole^{-1}
Plant density	65,000-75,000 plants ha^{-1}
Crop management	
Manure	Generally no manure application
Variety	Hybrid
Water management	Rain-fed, short dry sessions
Crop residue	Burn
Weed control	Herbicide (gliposat) and manual
Best yield	12 t ha^{-1}

The abundant use of fertilizers and the imprecise method and time of application point to the need for site-specific nutrient management of maize in this region. SSNM is an effort to supply nutrients to the crop in accurate doses appropriate for the particular location. In keeping with the approach of prescription farming, SSNM considers the nutritional requirement of that particular plant/variety, the soil conditions, and also the season and intensity of solar radiation (Makarim 2000). Therefore nutrients are delivered more efficiently to the crop in accordance with its requirements.

Study of fertilization of maize, 2004-2006. To derive SSNM recommendations for N, P and K fertilization of maize in this location, we first assessed the impact of omission of N, P and K separately. Separate maize subplots were cultivated without either N, P or K fertilizer but with optimal crop management. The relevant nutrient accrued to the

Table 3. Results of a study of SSNM nutrient requirements for maize in Tigabinanga, North Sumatra, 2004-2006.

NPK/SSNM ± ICM yield (t ha^{-1})	10.2 ± 1.4	Yield target (t ha^{-1})	11.0
N-limited yield (t ha^{-1})	6.2 ± 2.3		
P-limited yield (t ha^{-1})	8.9 ± 1.2		
K-limited yield (t ha^{-1})	9.2 ± 1.3		
Yield response to N (t ha^{-1})	3.8 ± 1.9	Expected yield response to N (t ha^{-1})	4.0
Yield response to P (t ha^{-1})	1.0 ± 0.7	Expected yield response to P (t ha^{-1})	1.5
Yield response to K (t ha^{-1})	0.8 ± 0.7	Expected yield response to K (t ha^{-1})	1.0
AEN (kg grain kg^{-1} N)	17.5 ± 9.6	Expected AEN (kg grain kg^{-1} N)	25.0
AEP (kg grain kg^{-1} P)	28.3 ± 17.7	Expected AEP (kg grain kg^{-1} P)	40.0
AEK (kg grain kg^{-1} K)	6.1 ± 4.8	Expected AEK (kg grain kg^{-1} K)	12.0
		Fertilizer N (kg ha^{-1})	160
		Fertilizer P_2O_5 (kg ha^{-1})	72
		Fertilizer K_2O (kg ha^{-1})	90

crop from the soil, inclusion from water and fixation from air but not from applied fertilizer. The yield thereby obtained indicated the ability of the local soil to supply the required nutrients. From the agronomic efficiency figures thus obtained, we derived the SSNM requirements of N, P and K for maize in this region..

The yield achieved by the treatment NPK/SSNM±ICM in 2004 approached 11 t ha^{-1}, the targeted yield. The yield response to N, P and K amounted to 4.0 t ha^{-1}, 1.5 t ha^{-1} and 1.0 t ha^{-1} respectively (Table 3). The expected agronomic efficiency of N (kg of grain per kg of N) was 25 kg kg^{-1}; that of P equalled 40 kg kg^{-1} and of K 12 kg kg^{-1}. From this study the following recommendations for site-specific nutrient management of maize in Tigabinanga were obtained: 160 kg ha^{-1} N, 72 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O (Table 4).

Recommendations for site-specific nutrient management of maize. The SSNM recommendations have been disseminated in the location during 2004-2006 (Table 4).

At activity of socialization site-specific nutrient management of maize, BPTP North Sumatra make rules in execution of specific fertilizations recommendations of locations for farmers (a) Farmer determine technology type which will be applied by farm each farmers; (b) Farmer using fertilization technology type is obliged apply foliage color schema in the application of fertilization of III; (c) Farmer make note of execution of maize technology evaluation like: technological type, date of planting, date of fertilization (I, II and III) and date of crop, (d) no aid in the form of supporting facilities and expense of execution of evaluation and available in the form of technological adjacent and foliage leaf color chart; (e) given opportunity of training for maize technology evaluation participant farmer concerning usage of foliage color schema which will be submitted by researcher; (f) At the (time) of crop, researcher permit for taking result data; (g) accompany is technological at least 5 times; rill that is at the (time) of planting, fertilization of I II and III and harvest. (Team BPTP, SUMUT, 2007)

Table 4. SSNM recommendation for maize in North Sumatra.

Parameter	SSNM recommendation
(A) Plant spacing	70 x 20 cm
(B) Number of seeds $hole^{-1}$	1 seed $hole^{-1}$
Fertilizer rates	(C) Urea 350 kg ha^{-1}
	(D) SP36 200 kg ha^{-1}
	(E) KCl 150 kg ha^{-1}
Fertilizer splits	(F) 7 DAP
	Urea 100 kg ha^{-1}
	SP36 200 kg ha^{-1}
	KCl 150 kg ha^{-1}
	(G) 30 DAP (urea 125 kg ha^{-1})
	(H) 45-50 DAP (based on LCC)
	(I) VT (based on LCC)

Production

Folowing the dissemination of SSNM fertilization recommendations for maize in five locations, we assessed their impact on production in Aug-Dec 2008 (Table 5).

The average production achieved by farmers who used farmers' fertilizer practice (FFP) was 8278 t ha^{-1} whereas those who adopted SSNM recommendations averaged 9353 t ha^{-1}, an increment of 11.49%. This thing happened because the application of fertilization by the way of SSNM 3 application times; rill by the way of dibber and then cover, differ from way of FFP the application of fertilization counted 2 times; rill by the way of put down by is crop side without closed so that happened runoff and evaporation. The constraints to farmers adopting SSNM technology were: (1) Rainfall uncertainty which makes it difficult to

Table 5. Maize production at five sites in North Sumatra.

Site	Average yield (kg ha^{-1})		
	PHSL	FFP cooperator	FFP noncooperator
Perbesi	10,122	8141	-
Pertumbuken	8521	8056	-
Gunung	8826	8000	-
Simolap	9708	8879	-
Kutabangun	9586	8313	-
Average	9.53	8278	-
% to FFP Co	11.49	-	-

Table 6. Economics of maize cropping (per ha) under SSNM recommendations and farmers' fertilization practices (FFP) in North Sumatra, 2007.

Parameter	FFP (Rp.)	SSNM (Rp.)
Land rent	500000	500000
Tractor	550000	550000
Planting	200000	200000
Herbicide on plant	75000	75000
Weed clearance I	100000	160000
Weed clearance II	100000	160000
Fertilization I	-	200000
Fertilization II	160000	200000
Fertilization III	160000	160000
Harvest cost (@ Rp.75000 t^{-1})	620850	701475
Transportation cost (@ Rp.70000 t^{-1})	579460	654710
Processing (@ Rp. 75 kg^{-1})	620850	701475
Dolomite labor cost	100000	100000
Total UHL	3766160	4362660
Urea (kg)	500	350
SP36 (kg)	250	200
KCl (kg)	150	150
Urea cost kg^{-1}	1500	1500
SP36 cost kg^{-1}	1800	1800
KCl cost kg^{-1}	3000	3000
Total fertilizer cost (Rp. ha^{-1})	1650000	1335000
Total seed cost (Rp. ha^{-1})	880000	792000
Dolomite 500 kg (1 sack = 50 kg)	120000	120000
Herbicide	255000	180000
Sack	321000	375000
Total material	3226000	2802000
Yield (t ha^{-1})	8.278	9.353
Maize price (Rp. kg^{-1})	2000	2000
Gross output (Rp. ha^{-1})	16556000	18706000
Total cost	6992160	7164660
Income	9563840	11541340
R:C ratio	2.37	2.61
B:C ratio	1.37	1.61
Increas in income (%)		17.13

apply fertilizer three times; according to fomentation; (2) difficulty in getting labor because of the planting schedule, crop and fertilization do together; (3) Rare of urea and if was have to be bought by tandem with other fertilizer (4) Lack of capital farmer so that have to borrow to warehouse maize or fertilizer shop with interest money large 5-10% per month; (5) farmers' habit of using abundant fertilizers; (6) Far the apart settlement of resident to hilly agriculture location so that many farmers only rely on labor in cultivations, fertilization, operation of HPT and harvest so that less observation (7) Habit of farmer in receiving seed, fertilizer, free herbicide so that difficulty in socialization without there is aid.

Because limitation of the farmer so that for planting season here in after BPTP North Sumatra give solution that is introducing appliance plant and fertilization for overcoming faced by problems is farmer.

Farmers' income

The benefit:cost ratio of farmers' fertilizer practice (FFP) treatment was 1.37 while that of the SSNM treatment was 1.61. The FFP treatment earned farmers an income of Rp. 9563.840 while the SSNM treatment fetched Rp. 11 541.340, an increase of 17.13%. this thing is caused by treatment of SSNM cost money compared higher labor of way of farmer, where farmers sweeping of weed apply herbicide which will influence continuity of life of soil microorganism and health of area of whereas treatment of SSNM apply way of manual this thing is for the sake of soil fertility and area. (Table 6).

Conclusions

1. SSNM recommendation for fertilization of maize in Tigabinanga subdistrict: 160 kg ha^{-1} N, 72 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O.
2. 31.9% of farmers followed the fertilizer recommendation.
3. Production under SSNM recommendations was9,353 t ha^{-1} while production under farmers' practices was 8,278 t ha^{-1}, an increase of 11.49%.
4. Farmers' income increased 17.13% from Rp. 9,563.840 to Rp. 11,541.340
5. Due to labor constraints, farmers did not follow all the recommended practices.
6. Introduction of seed planters can solve difficulties of labor.
7. There is need for fertilizer applicators and seed planters to minimize labor costs.

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Development of rapid screening technique for improving nitrogen use efficiency of corn under normal and drought environments

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Abstract. The damage to corn production due to drought caused by the El Niño episodes that hit the Philippines from 1990 to 1992 was estimated to be as much as PhP 3.2 billion. Large losses can be avoided by developing corn varieties with the ability to produce relatively high yields under water-limited conditions. To produce good yield, varieties must also be able to absorb and utilize nutrients efficiently specially nitrogen. Experiments were conducted using different levels of nitrogen fertilizer (0, 60 and 120 kg N ha^{-1}) and water stress (irrigated and non-irrigated). Corn seedlings were grown for 3 to 4 weeks in trays using sand culture in the greenhouse. Field experiments were conducted in Laguna and Ilocos Norte using selected corn genotypes to measure the grain yield and validate results of greenhouse experiments. Genotypes with seedlings that have higher shoot length, shoot weight, root length, root weight, total N-uptake and total dry matter yield after drought treatment were also found to give better grain yields and to have higher nitrogen use efficiency potential when grown under intermediate water stress in the field. These genotypes were identified to be CML 8, P6-1-3, (P6-1-3 x CML 337), (CML 8 x CBR/PDC 2-5-2-4-5) for white corn and SMCE, (95-6 x Pi 23), (CLOO331 x Pi 23) for yellow corn. A protocol for rapid greenhouse screening at seedling stage is presented while the performance of drought tolerant F1 hybrids will be validated on-farm before recommendation for variety release.

Keywords: corn genotypes, water stress, nitrogen use efficiency, nitrogen, drought, diallel cross

Introduction

In Phillipine agriculture, the government's two major thrusts are food security and global competitiveness. As an initial step towards this goal, the Department of Agriculture identified key production areas for selected crops and launched a program to sustain self-sufficiency in rice and corn with a target national production of at least 12 million metric tons and 7 million metric tons, respectively. However, in regions where farmers depend largely on rainfall as source of water or in areas without supplemental irrigation, yield reduction may range from 10 to 75% (Logrono and Lothrop, 1996) and yield may be nil under severe water-deficit stress in corn. The El Nino episodes that hit the country particularly in 1990 and 1991 brought crop damage worth P3.2 B and P2.1 B, respectively (DA Report, 1994). The total corn losses due to drought in 1992 amounted to P2.1 billion affecting about 281,349 hectares of corn farms.

The normal rainfall in the Phillipines is in the range of 1,000 – 5,000 mm and drought occurs when rainfall in an area is erratic or falls below 1000 mm or when there is almost no rain during the normal growing season. As water becomes available, water deficit stress is relieved and other factors limiting plant growth assume greater role such as soil nutrient availability/utilization. Nitrogen is the fourth most abundant nutrient in plants and it is an essential component of proteins, nucleic acids, hormones chlorophyll, primary and secondary metabolites. In general, plants obtain the bulk of their nitrogen from the soil in either nitrate or ammonium form however, the supply of nitrogen in the soil is limited (Power, 1990) and plants must compete with soil microorganisms for nitrogen. As a result nitrogen is often a limiting nutrient for plants in both natural and agricultural ecosystems.

The occurrence of water and nitrogen stress may increase, due partly to global changes, partly to the displacement of corn to more difficult production environments by high value crops and partly to the decline in soil organic matter which may result in reduction in soil fertility and water holding capacity. At the micro level, fertility and water availability vary greatly in many farmer's fields. This means that a single variety must be able to withstand a wide range of drought stress and nitrogen availability – a condition that is more pronounced in the tropics than in higher input fields of the temperate areas. In order to maximize grain yield under condition of nonlimiting and limiting water supply, it is equally important to select corn genotypes with high nitrogen efficiency under such environment.

The general objective of the project is to increase corn production in the Philippines (by 10%) through selection of genotypes with high nitrogen efficiency under normal and water limiting conditions. The specific objectives are to develop a rapid screening technique for high nitrogen use efficiency under normal and drought environment for corn; to verify and correlate the performance of identified genotypes under greenhouse and field conditions; to identify physiological traits associated with high N-efficiency under limiting and non-limiting water conditions and to initiate development of drought-tolerant, N-efficient and high-yielding corn breeding lines/population

Materials and Methods

Greenhouse Screening

Experiments were conducted at IPB greenhouse using sand culture technique to evaluate the nitrogen use efficiency (NUE) and productive efficiency of applied nitrogen (PENA) of white and yellow inbreds and diallele crosses. The water regimes were designated as irrigated and limiting water. The nitrogen levels include a control (no added N), 60 kg N/ha and 120 kg N/ha. Phosphorus and potassium fertilizers in the form of solophos (0-18-0) and muriate of potash (0-0-60) were added at the rate of 120 kg/ha. The entries were grown at field capacity for both water regimes. To effect the drought treatment, water was withheld 7 days after seedling emergence.

A split-split-plot design in RCB with two replications was used with water regime as the main plot, N level as the subplot and variety as the sub-subplot. Each main plot used styrofor trays containing 17 kg sand previously analyzed for nutrient composition and water holding capacity. Seeds were drilled in 35 cm rows to serve as sub-subplots. Twelve entries were initially evaluated per set-up but as the project progressed more entries were screened per set-up. Nutrient requirements were added at sowing time. Equal volumes of water were used to irrigate the different treatments at planting to allow seed germination and seedling establishment. One week after germination, irrigation was withheld in the drought and for recovery treatments. Visual ratings were taken and soil moisture status were monitored. Plant sampling for N analysis commenced when visible differences were observed among the entries (leaf rolling). The entries were also evaluated for rate of recovery after rewatering the plants. Plant samples were collected when differences to rewatering among entries became visible. Dry matter accumulation (shoot and root dry weights), shoot and root length and nitrogen contents of the plants were determined. N-efficient genotypes are entries with the ability to produce high total dry matter yield, high plant N-accumulation and rapid root and shoot growth.

Field Trials

Field verification and validation of results obtained from sand culture experiments were conducted at Laguna and Ilocos Norte using the following levels of N: no addition of N (control), 70 kg/ha and 140 kg/ha, under water limiting and normal water regimes. Phosphorus and potassium were applied in all plots at the rate of 120 kg/ha each. The recommended cultural management for corn was adopted except for N-fertilization. The experiment was laid out using RCBD in split-split plot with water regime as main plot, N level as the sub-plot and variety as sub-sub-plot. Five to eight potentially drought tolerant and susceptible white and yellow corn genotypes were tested separately. To effect the drought treatment, water was withheld one month after planting and rewatering to field capacity was done at grain-filling stage. The sample plots or harvest area consisted of the three inner rows from five-row plots which served as the basic experimental unit. Soil samples for moisture and N analyses were collected.

Samples for N analysis, dry matter accumulation, grain yield and its components were collected at physiological maturity. N-efficient (NUE) genotypes and those genotypes with high productive efficiency of N added (PENA) were identified. Comparison between the morphological and physiological traits of plants grown in the greenhouse and their yield performance in the field was done. This is to determine whether genotype response in sand culture will be similar to genotype response under field conditions. Moreover, responses in the seedling stage were compared with responses of same genotypes at maturity. The ability of the plant to recover rapidly from stress is an important drought tolerance mechanism. The entries were also evaluated visually for rate of recovery after rewatering the plants.

Results and Discussions

Initial Soil Characteristics

The sand used in the greenhouse experiment had strongly acidic reaction (pH 6.90), very low nitrogen content (0.009 %), high phosphorus (25 ppm) and high potassium

(0.50 cmol(+)/kg) levels. The Cervantes clay loam soil used in the field experiments in Piddig, Ilocos Norte had moderately acidic reaction (pH 5.95), medium nitrogen content (0.155 %), high amount of available P (16.75 ppm) and high K (0.65 cmol(+)/kg) content. The Alipit clay soil used in the field experiments at Bay, Laguna had moderately acidic reaction (pH 5.90), low nitrogen content (0.116%), high amount of available P (18.47 ppm) and very high K (1.70 cmol(+)/kg) content. To mask the possible effect that will be caused by the problems of P and K availability to the plants in the experiment, 120 kgs each of these two nutrient elements were added to the soil at planting.

Greenhouse results

Greenhouse experiments involving white and yellow corn inbreds and diallele crosses were conducted at IPB greenhouse to determine the plant parameters that could be used in the development of a rapid screening technique that will limit the number of corn entries to be tested in the field for grain yield determination and verification trial under water limiting conditions.

Based on the data obtained from the experiments, the parameters that showed significant differences across treatments imposed (water regime, nitrogen levels and varieties) were shoot length, shoot weight, root length, root weight, total N-uptake and total dry matter weight. The soil moisture content after drought imposition based on oven dry weight is 3.74 % while the moisture content of irrigated sand is 9.34 %. The moisture content of sand at field capacity is 6-12 % while at permanent wilting point is 2-6 % (Hansen et al., 1980).

Field verification trial under normal and water limiting conditions

Promising drought tolerant white and yellow corn entries which showed good performance in the greenhouse experiments and corn genotypes susceptible to water stress were tested under field condition during the dry cropping season at Ilocos Norte and Laguna to determine whether they will produce good or similar results to the tray experiments. One month after drought imposition, soil sampling was done to determine the amount of soil moisture in the field. Based on the determination made on plots under drought, the moisture content decreased from 29.09% (5 weeks after planting) to 19.31% (9 WAP) which coincided with the grain filling stage. The moisture content for a clay loam soil at field capacity is 23-31% while for permanent wilting point is 11-15% (Hansen et al.,1980). At this moisture level, the plants were under moisture stress. The corn plants undergo water stress prior to the flowering stage which is the most crucial time for the plant to produce grains. After this stage, the corn plants were irrigated for the recovery period.

Among the white corn inbreds and parentals tested, P6-1-3 and CML 8 produced the highest absolute yield at 140 kg N/ha under drought stress (Table 1). Their grain yields ranged from 2.79 to 3.53 t/ha with 26 – 33% corresponding yield reduction due to water stress. CML 377 and Lagkitan gave the lowest yield at 1.44 and 1.58 t/ha, respectively, equivalent to yield reduction of 58 – 63%. On the other hand, higher grain yield levels were shown by the white diallel crosses developed for stress when compared to their respective parentals. At 140 kg/ha N fertilization, the diallel crosses with higher yields were (CML 8) x (CBR x PDC2-5-2-4-5) and (P6-1-3 x CML 377) with grain yields of 3.17 and 3.04 t/ha, respectively. In general, yield increased with increasing levels of nitrogen in most entries. The same results were observed in the experiments conducted in Laguna.

For the yellow corn entries tested, the highest yielder among the inbreds was SMCE 9-18 with a yield of 2.57 tons per hectare at high N fertilization under water limiting condition or a yield decrease of 30.91% while the entry with the lowest yield is CL00331 with yield reduction of 49.41%. Three diallel crosses of yellow corn were also developed. These crosses produced higher grain yields than the parent materials. CLOO331 x Pi 23 recorded the highest yield of 3.72 t/ha at high N rate and drought

Table 1. Grain yield (t/ha) of selected white and yellow corn entries under irrigated and drought conditions at 140 kg N/ha.

White Corn	Grain Yield(t/ha)	
	Normal	Drought
CML8	4.19	2.79
P6-1-3	4.77	3.53
CML 151-32-1-4-2	3.43	2.69
P6-1-3 x CML337	5.50	3.04
(PK2 x Var1-12-2-1-1) x (CML337)	5.53	2.93
CML8 x (CBR x PDC2-5-2-4-5)	5.73	3.17
CML 337	3.91	1.58
Lagkitan	3.82	1.58
Yellow Corn		
SMCE	3.72	2.57
TUPI S3-5-18 (TUPI 1YL7)	3.79	2.25
IPB Var 1	5.42	2.28
IPB Var 7	5.03	2.37
(Pi17) x (Pi23)	5.99	2.60
(95-6) x (Pi23)	6.29	3.17
(CLOO331) x (Pi23)	6.41	3.72
CLOO331	1.70	0.86

Table 2. Productive efficiency of nitrogen applied (PENA) of selected corn entries under irrigated and drought conditions at different N levels.

White Corn	Productive Efficiency of Nitrogen Applied (PENA) (kg grain yield/kg N applied)			
	70 kg N/ha		140 kg N/ha	
	Control	Drought	Control	Drought
P26-4-1	7.57	10.86	3.57	6.71
P6-1-3	10.14	3.43	8.64	13.14
CML 151-32-1-4-2	5.57	3.14	2.07	9.14
(P6-1-3) x (CML337)	19.00	24.86	8.29	8.79
(PK2 x Var1-12-2-2-1-1) x (CML 337)	12.71	8.14	8.21	8.43
(CML8) x (CBR x PDC2-5-2-4-5)	13.43	16.00	11.79	10.66
Lagkitan	9.71	4.00	5.07	1.36
Yellow Corn				
95-6	11.71	5.43	3.71	2.07
TUPI S35-1B (TUPI 1YL7)	7.71	7.43	2.71	6.50
(95-6) x (Pi23)	19.86	5.57	13.79	8.93
(CLOO331) x (P123)	20.43	5.43	13.86	12.36
CLOO331	5.71	2.29	1.71	0.71

condition followed by 95-6 x Pi 23 with 3.17 t/ha. The reduction in yield ranged from 42 to 50 %. It was noted that there is higher yield reduction in yellow corn compared to white corn due to the fact that the yellow corn materials showed higher yield potential under irrigated condition.

In testing for the correlation of grain yield obtained from the field with that of the parameters used in the greenhouse for white corn, correlation coefficients of greater than 80% were obtained for the parameters like shoot length, shoot weight, root length, root weight, total dry matter weight, shoot N and total N-uptake under water stressed condition. The test for correlation with yellow corn, however, showed correlation coefficients of less than 50% obtained for root weight and root length while the rest of the parameters had greater than 80% correlation coefficients.

Table 2 shows the N-use efficiency of white corn entries under irrigated and drought conditions. P6-1-3 which had a highest yield under water limiting condition gave a productive efficiency of N applied (PENA) conversion of 13.14 kg grain yield per kg of N applied under the fertilization rate of 140 kg N/ha while for the diallel cross, (CML 8) x (CBR x PDC2-5-2-4-5) was also noted to have a PENA equivalent to 10.66 kg grain per kg of added N fertilizer. At lower rate of N application (70 kg/ha), the entries with high PENA and high NUE under drought condition were: (P6-1-3 x CML 337), (CML 8) x (CBR x PDC2-5-2-4-5) and (CML8 x P6-1-3). This shows that these entries can thrive and produce good yield under water limiting condition and low N fertilization.

Among the yellow corn entries, Tupi 1YL7, which is one of the highest yielder under drought condition, was also observed to have high PENA at low and high N fertilization. Compared with the yellow diallel crosses, CLOO331 x Pi 23 was the best under drought conditions in converting applied N into grain yield at 12.36 kg grain per kg N applied when fertilization rate was 140 kg N/ha.

For the nitrogen use efficiency of the white and yellow corn tested in the field, high PENA values were shown at fertilization rates of 70 and 140 kg N/ha. This suggests that some corn entries can make use of the available native or indigenous N in the soil and applied fertilizer N better than others and this could result to lower N inputs, less production cost and higher returns/benefits to the farmers.

These results showed the validity of the protocol or methodology that was developed for the greenhouse studies. The entries that were identified in the greenhouse to be tolerant or susceptible to water stress and with high N-use efficiencies showed similar responses or results in the field. Thus, rapid screening for the selection of drought tolerant corn genotypes can be conducted during seedling stage under greenhouse condition.

Conclusions

The different objectives of this project were answered by conducting greenhouse and field experiments involving yellow and white corn genotypes and several developed diallel crosses. The greenhouse experiments were able to determine the parameters that could be used for the development of rapid screening technique for the selection of genotypes with strong potential for high N-use efficiency under water limiting condition. Plant parameters such as shoot length, shoot weight, root length, root weight, total N-uptake and total dry matter weight were found to significantly affect the growth of corn genotypes under water stress condition. This was verified by the field experiment which showed that the identified potentially tolerant and susceptible entries and those with high N-use efficiency under water limiting condition also corresponds to the best and least performing corn genotypes respectively, in the greenhouse experiment. Based on greenhouse and field experiments, the corn genotypes which possessed potential drought tolerance and high N-use efficiency were P6-1-3 and (CML 8) x (CBR x PDC2-5-2-4-5) for white corn and (CLOO331 x Pi 23) and (95-6 x Pi23) for yellow corn. The generation of diallel crosses that was initiated and results obtained showed good promise in the development of these materials in terms of higher grain yield and high N-use efficiency under water limiting conditions.

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Effects of row spacing and densities on grain yields of five maize hybrids in three cropping seasons in Ha Tay province, Vietnam

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Abstract. Within 3 cropping seasons (Spring 2006, Spring and Autumn 2007) experiments of 5 different maize hybrids (LVN4, LVN184, LVN99, LVN45 and LVN10) on maturity, plant stature, lodging tolerance, major diseases and insects resistance were conducted in Ha Tay province (latitude - 20Ú40'N; Longitude: 105Ú47E; Elevation: 7.2 m) under 3 row spacing (50, 70 and 90 cm) and 4 densities (50, 60, 70 and 80 thousand plants/ha).The results showed that: 1) At the same density, the higher yield was obtained at the narrower and lower at wider row spacing for all hybrids and densities; 2) Four of five hybrids produced the highest yields at the 80 thousand plants/ha and row spacing 50cm (50 x 25cm) while LVN10 gave the highest yield at 70 thousand plants/ha and row spacing 50cm(50x28cm); 3) Efficiency of plant population increased when narrowing row to row distance from 90 to 50cm. Yield difference between 80 thousand plants/ha and 50 thousand plants/ha at row spacing 50cm was 1,623 kg (23%); at row spacing 70cm was 721 kg (10.8%) and at row spacing 90cm was 623 kg (9.9%) respectively. 4) The higher densities the more apparent efficiency of narrower row spacing, at the 50 thousand plants/ha and row spacing 50cm yields increased by 6.0% and 11.9% in comparison with row spacing 70cm and 90cm; Similarly, the increment was 8.8% and 17.2% at the 60 thousand plants/ha ; 11.4 % and 18.5% at the 70 thousand plants/ha and highest of 17.8% and 25.4% at the 80 thousand plants/ha. When the densities reached from 70 thousand plants/ha to 80 thousand plants/ha, yield increased by 536 kg (6.6%) at row spacing 50cm, by 70 kg (1%) at row spacing 70cm and by 56 kg (0.8%) at row spacing 90cm

Keyword : Hybrid maize, row spacing, density, grain yield, effects, cropping seasons

Introduction

Density and row spacing are the subjects having occupied many researches in maize cultivation. Some researchers have studied row spacing from over 30cm to over 200cm and the density from 5 to 240 thousand plants/ha (Barbieri et al, 2000; Ming et al, 2005; Phan, 2007; Phan and Hai, 2007; Tollenaar, 1992; William et al 2002). Almost all of the studies showed that at the same density, yield in the narrower row spacing was higher than that in the larger one. This finding explained that when maize was planted at narrower row spacing, the distance between plants was more evenly distributed; therefore it helped to minimize competition on light, nutrition and other growing elements (Sprague and Dudley, 1988).

In Vietnam atleast one study on plant density has been conducted on several Open Pollinated Varieties and hybrids but no specific research has carried out on row spacing (Ngo Huu Tinh, 1987). The common row spacing is 70-75 cm in accordance with the recommendation of CIMMYT. The density ranges from 43-60 thousand plants/ha depending on growth duration, variety morphologies and ecological regions of cropping seasons (Anon, 2006; Ngo Huu Tinh, 1987). In reality the farmer grows maize in lower density than recommendation in many areas. The experiment was in the program of seeking solutions to improve yield and enhance the production efficiency of maize in Vietnam.

Materials and method

Five different maize hybrids: LVN4, LVN184, LVN99, LVN10 and LVN45 were sown in 3 seasons, Spring 2006, Spring and Autumn 2007. LVN184 was typical of short plants and erect leaves; LVN99 was small but quite strong stalk and sparse leaves; LVN4 and LVN45 were not very strong stalk with large leaf angles; LVN10 was stout, high plants and quite sparse leaves. In terms of growth duration, these above maize hybrids were early or medium with the exception of LVN10, the late one. Beside 4 common densities: 50, 60, 70 and 80 thousand plants/ha applied for all 5 hybrids, an additional one, 90 thousand plants/ha was also applied for LVN99 (2 cropping seasons) and LVN184 (3 cropping seasons); Furthermore, although the even row spacing of experiment were 50, 70 and 90cm, the additional row spacing of 40cm was also applied on LVN4, LVN10 and LVN45 at the density of 70 thousand plants/ha (40 x 35cm) in the Spring and Autumn 2007. At the density of 50 thousand plants/ha, fertilizers for 1 hectare were 160kg N, 90 kg P_2O_5, 90 kg K_2O; these increased by 10% when the density increased by 10 thousand plants. The experiments were in form of random completed block design with 3 replications, 4 rows per one.

Results and discussion

Of all 3 cropping seasons, all hybrids produced the highest yields at the row spacing of 50cm and lowest at 90cm. For the experiments adding the row spacing of 40cm, the advantage on yield of this row spacing was highest. The early hybrids with short plants produced the highest yields at the density of 80 thousand plants/ha at spacing of 50 x 25cm. The only late and high plant hybrid, LVN-10,

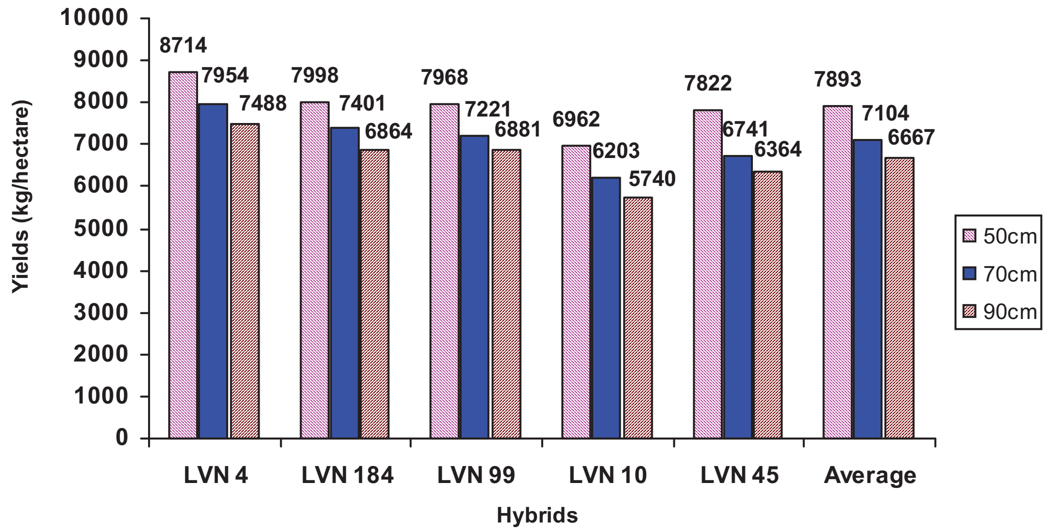

Figure 1. Yields of 5 hybrids at different row spacing

Table 1. Average yields of 5 maize hybrids in 3 cropping seasons at different densities and row spacing

Density of 10^3 plants/ha	Spacing (cm)	Yield of the maize hybrids (kg/ha)					
		LVN 4	LVN 184	LVN 99	LVN 10	LVN 45	Aver row spacing
5	40 x 50	7933	7185	6958	6156	7068	7060
	28.5 x 70	7590	6708	6755	5983	6276	6662
	22.2 x 90	7130	6457	6515	5474	5977	6311
	Ave. dens.	7551	67,83	6743	5871	6440	**6678**
6	33 x 50	8442	7820	7806	6645	7693	7681
	23.5 x 70	8115	6973	7301	6164	67,37	7058
	18.3 x 90	7110	6751	6768	5754	6350	6546
	Ave. dens.	7889	7181	7291	6188	6927	**7095**
7	28 x 50	8950	8043	8115	**7856**	7769	8147
	20 x 70	8226	7934	7099	6323	6982	7313
	15,3 x 90	7758	7052	7095	6081	6403	6878
	Ave. dens.	8311	7676	7436	6753	7051	**7446**
8	**25 x 50**	**9531**	**8942**	**8992**	7192	**8759**	**8683**
	17.8 x 70	7884	7988	7730	6344	6968	7383
	14 x 90	7953	7194	7145	5652	6726	6934
	Ave. dens.	8456	8041	7956	6396	7484	**7667**
9	22 x 50		8614	8480*			
	15.7 x 70		7718	7420*			
	12.2 x 90		6930	7024*			
	Ave. dens.		**7754**	**7641**			

* Average yields in 2 cropping seasons

Table 2. Average yields of 5 hybrids in 3 cropping seasons at 4 densities and 3 row spacing

Row spacing	50 cm			70 cm			90 cm		
Density of 10^3 plants/ha	Yield (kg)	±/lower density (kg)	±/lower density (%)	Yield (kg)	±/lower density (kg)	±/lower density (%)	Yield (kg)	±/lower density (kg)	±/lower density (%)
5	7060	-	-	6662	-	-	6311	-	-
6	7681	621	6.2	7058	396	5.9	6546	236	3.7
7	8147	466	6.1	7313	255	3.6	6878	332	5.1
8	8683	536	6.6	7383	70	1.0	6934	56	0.8
8-5		1623	23.0		721	10.8		623	9.9

Table 3.Impact of row spacing on average yields of 5 hybrids in 3 seasons at 4 densities

Row spacing	50 cm			70 cm			90 cm		
Density of 10^3 plants/ha	Yield (kg)	±/lower density (kg)	±/lower density (%)	Yield (kg)	±/lower density (kg)	±/lower density (%)	Yield (kg)	±/lower density (kg)	±/lower density (%)
5	7060	-	-	6662	-	-	6311	-	-
6	7681	621	6.2	7058	396	5.9	6546	236	3.7
7	8147	466	6.1	7313	255	3.6	6878	332	5.1
8	8683	536	6.6	7383	70	1.0	6934	56	0.8
8-5		1623	23.0		721	10.8		623	9.9
5	7060	398	6.0	749	11.9	6662	351	5.6	6311
6	7681	623	8.8	1135	17.3	7058	512	7.8	6546
7	8147	834	11.4	1269	18.5	7313	435	6.3	6878
8	8683	1300	17.8	1749	25.4	7383	449	6.5	6934
Average	7892	788	11.1	1225	18.4	7104	437	6.6	6667

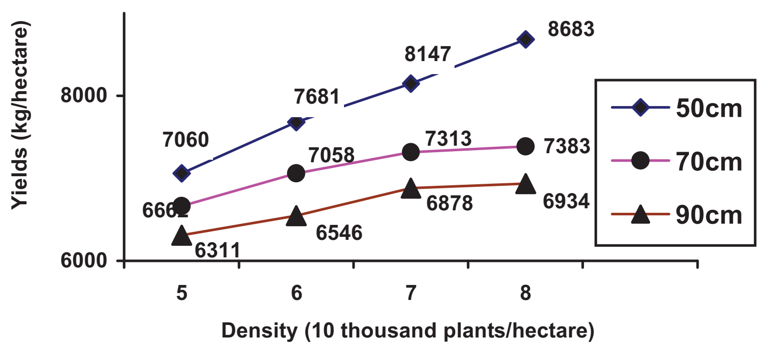

Figure 2. Yields at the same density but 3 different row spacing

produced the highest yield at the density of 70 thousand plants/ha but at the spacing of 50 x 28cm or 40 x 35cm

All hybrids produced the highest yields at row spacing of 50cm and the lowest at 90cm

By the same density, yields of the maize hybrids were clearly different at 3 row spacing

Figure 3. Average yields of 5 hybrids at different row spacing and densities

The 5 hybrids produced highest yields at row spacing of 50cm, and lowest at 90cm at every density.

At density of 50 thousand plants/ha, yield at the row spacing of 50cm was 6.0% and 11.9% higher than that of 70cm and 90cm, respectively; at density of 60 thousand plants/ha, the figure was 8.8% and 17.3%; at density of 70 thousand plants/ha, the figure was 11.4% and 18.5%; at density of 80 thousand plants/ha, the difference was greatest, 17.8% and 25.4%, respectively.

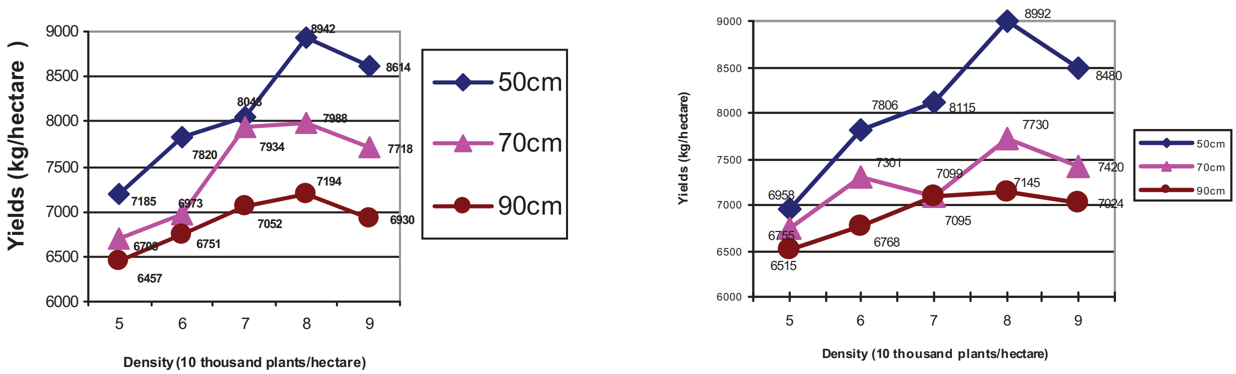

Figure 4a,b. Yields of LVN184 and LVN 99 at different row spacing and densities Early hybrids LVN184 with short plants, erect leaves(Figure 4a) and LVN99 with thin stalk, sparse leaves (Figuge 4b) produced highest yield at the density of 80 thousand plants/hectare at row spacing 50cm.

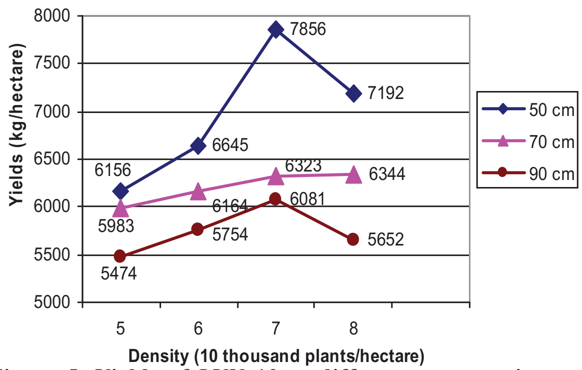

Figure 5. Yields of LVN 10 at different row spacing and densities

There were also the significant differences in yields of these 5 hybrids at different row spacing. Yields at row spacing of 70cm were quite higher than those at 90cm but significantly lower than those at 50cm, the gaps of yields between row spacing of 70cm and 90cm were 5.6%, 7.8%, 6.3% and 6.5% at density of 50, 60, 70 and 80 thousand plants/ha, respectively.

Results of the experiments were also in parallel with those of the other researches having been implemented since 2005 such as the project *Site-Specific Nutrient Management for Maize* by the International Plant Nutrition Institute IPNI), Vietnam Soils and Fertilizers Institute and some other institutes (Pham et al 2007).

- Narrowing the row spacing was the highly effective method to increase maize density and productivity.
- By the same density at all checked densities, narrower row spacing produced higher yields for all hybrids.
- All experimented hybrids at all densities produced highest yields at row spacing of 50cm (or 40cm), followed by 70cm, and lowest at 90cm.
- By the exception of LVN-10, the late and high plant hybrid which produced highest yield at density of 70 thousand plants/ha at spacing of 50 x 28cm or 40 x 35cm, the remaining ones including short plants and erect leaves or thin stalk and sparse leaves produced the highest yields at density of 80 thousand plants/ha at spacing of 50 x 25cm.
- The advantage of narrowing row spacing was clearer when the density was relatively high.
- There were no evident of the distinguish on growth duration, plant morphologies, abilities of lodging tolerance, pest and diseases resistance, rate of fruit or fruitless plants among the experiment partners.
- The experiment results were also in parallel with those of many researches announced inside and outside the country, especially they were suitable with the recommendations of the IPNI

Conclusion

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Nitrogen assimilation at different growth stages of maize (*Zea mays* L.)

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Abstract. Assimilation of nitrogen in plants depends upon the availability of inorganic form of nitrogen in soil. Again uptake of N by different parts of a growing plant in a particular time is not only controlled by the plant type itself but also by the microenvironment of rhizosphere which influences different N- transformation processes in soil. The present investigation was, therefore, conducted to make a relationship of changes in different forms of inorganic and organic N in alluvial, limed and corresponding unlimed soils. With the uptake of N by different parts of maize plant in which judicious application of nitrogenous fertilizer can be made during a growing season of the crop. Results reveal that irrespective of cropping and N-addition, in general, the amount of inorganic and organic forms of N decreased with increase in the period of investigation. Furthermore, in general, the amount of different forms including hydrolysable NH4+ and amino acid- N were maintained in higher amount in cropped over the uncropped system suggesting that presence of plant influences the micro-environment of rhizosphere soil. The congenial atmosphere created in rhizosphere soil in presence of maize leads to the release of higher amount of hydrolysable organic N in cropped situation. As the organic and inorganic form of N in soil are interconvertible within themselves, the utilization of inorganic N by maize crop is replenished by different fractions of organic N released through mineralization. Liming an acid soil favours the N mineralization and thus comparatively higher amount of different forms of N are accumulated in limed over the corresponding unlimed soil. Again, irrespective of liming, in general, the assimilation of N is more in cropped over the uncropped system. Assimilation of N in maize plant is more in vegetative parts than the root portion. This trend of results is observed in all the soils under investigation. However, N uptake is of higher order by maize cropped in limed over the unlimed system. Results of the investigation thus pointed out that there is a relationship between the changes in different forms of N in soil with the uptake pattern of N by the maize. However, concluding evidences can only be drawn using N^{15} tracer studies.

Keywords: Nitrogen assimilation, Organic and Inorganic fractions, N Uptake, etc.

Introduction

Nitrogen is one of the major nutrients required by plants for growth and metabolic activities, mostly taken up by plants. Mineralization of nitrogen in soil varies due to various factors like soil characteristics, cropping, liming, different management practices etc. Presence of plant changes the microenvironment in the soil. When the plants are growing, during respiration the roots are excreting exudates which are beneficial for microbial growth and their activities. Liming soils of acidic reaction has been reported to increase the production of mineral nitrogen and decrease the amount of soil organic matter (Saha and Mukhopahdyay 1983 a and b). When acid soils are limed, a portion of soil organic matter becomes more susceptible to mineralization but after this portion has decomposed, mineralization returns to its original level. With the age of crop the uptake of nitrogen from the soil increases upto reproductive phase and then shows a decreasing trend. At reproductive phase extensive internal mobilisation of nitrogen occurs from vegetative parts to fruits and seeds till the crop are harvested at maturity. A considerable variation occurs between and within plant species in pattern of uptake, assimilation and distribution of N within plants of different types and age.

Materials and Methods

The present investigation was conducted to study the effect of cropping and N fertilization on changes in different fractions of inorganic and organic N in soil cropped with maize plant. Assimilation of nitrogen at different parts at different stages of crop growth was also monitored to make a relationship between the pattern of release of different forms of N in soil and its uptake by different portions of plant at different growth stages.

The soils (0-15 cm depth) used in the present investigation were composite samples of cultivated fields and were collected from the experimental farm of B.C.K.V. at Chakdah in the district of Nadia and Patrasayer village in the district of Bankura, West Bengal, India. Some of the relevant physical and chemical characteristics of the soils are presented in Table 1.

The liming material equivalent to lime requirement was added to the soil uniformly and allowed to react with soil mass for 3 month period with repeated wetting followed by air drying. The effect of liming was studied in presence and absence of added inorganic N on the so prepared limed soil. The soils were air dried and passed through 5 mm sieve. Green house experiments were conducted with 2 kg air dried soil in each earthern pot. Two seeds of maize (var. A-de Cuba) were sown in each pot. Phosphorus and potassium were applied at 50 kg P_2O_5 and 50 kg K_2O/ha in the form of single super phosphate and murate of potash respectively to each pot as basal application. Altogether three sets were maintained for three soils namely alluvial, unlimed acid soil and limed acid soil. For each soil three

Table 1. Physical and Chemical Properties of the soils used for the investigation

	Physical and Chemical Properties	Soils used			Method adopted
		Alluvial	Acidic		
			Unlimed	Limed	
1.	pH (1:2.5)	7.15	4.5	6.87	Glass electrode pH meter
2.	Mechanical seperates				Hydrometer method
	Sand (%)	21.90	59.94	58.4	
	Silt (%)	34.0	15	20.0	
	Clay (%)	44.0	25.6	21.6	
3.	Textural class	Clay	Sandy clay loam	Sandy clay loam	
4.	EC (dsm-1)	0.35	0.21	0.31	Conductivity meter
5.	Water holding capacity (%)	49.53	35.66	39.2	Keen Rackzowski method (Piper, 1942)
6.	Oxidisable Organic Carbon (%)	1.27	0.58	0.605	Walkley and Black (1934)
7.	Cation Exchange Capacity (cmol (P+) kg–1)	2.448	1.44	0.3	Schollen berger and Simon (1945)
8.	Exchangable NH_4+N (mg kg–1)	50.54	45.22	77.14	Bremner and Keeney (1996)
9.	Soluble NO^-_3 - N (mg kg–1)	23.94	66.5	50.54	"
10.	Available N	74.48	111.72	127.68	"
11.	Bulk density (g cc–1)	1.21	1.4	1.686	Keen Rackzew
12.	P.D. (g cc–1)	2.3	2.23	2.49	Ski method (Piper, 1942)
13.	Pore space (%)	54.29	45.66	50.768	"
14.	Available K_2O(kg ha–1)	335.5	517	478.5	Jackson (1973)
15.	Available P_2O_5 (kg ha–1)	68.7	71.56	217.55	Jackson (1973)
16.	Fixed NH^+_4 - N (mg kg–1)	806.4	470.4	537.6	Silva and Bremner (1996)
17.	Exchangeable Ca (mol kg–1)	13.05	2.55	3.4	Sparks *et al.* (1996)
18.	Exchangeable Mg (c mol kg–1)	0	0.35	0.15	,,
19.	Organic fractions of N				Bremner (1996)
i)	Hydrolysable organic NH^+_4-N (mg kg–1)	186.6	182.2	221.6	
ii)	Hexosamine - N (mg kg–1)	16.8	28.3	28.6	
iii)	Amino acid - N (mg kg–1)	192.0	173.8	173.8	
iv)	Total hydrolysable organic N (mg kg–1)	727.1	602.9	594.1	
v)	Serine + Threonine	121.3	119.0	110.8	
vi)	Unidentified hydrolysable organic N (mg kg–1)	331.7	218.6	170.1	
vii)	Total non-hydrolysable organic N (mg kg–1)	892.8	296.2	297.02	
20.	Total N (mg kg–1)	1694.4	1010.8	1018.8	Bremner (1996)
21.	C : N ratio	7.49 : 1	5.7 : 1	5.9 : 1	
22.	Nomenclature	Haplaquents	Haplustalfs	Haplustalfs	USDA (1975) according to USDA system

doses of inorganic N were applied viz., zero dose, 70 mg N kg^{-1} and 100 mg N kg^{-1} as basal application through ammonium sulphate. Destructive sampling procedure was followed for collection of soil and plant sample on the 0th, 30th, 60th, and 90th day after sowing. Similarly, another set was maintained for all the uncropped soils. All the treatments were replicated three times.

Soil and plant samples were collected from both the uncropped and cropped pots on the 0th, 30th, 60th, and 90th day of the experiment and analysed in the laboratory. The data of inorganic and organic N as well as total N in plant at each growth stage as per treatment were analysed statistically for analysis of variance as well as critical difference was calculated at 5% level of probability to test the significance of means for the treatment difference.

Results and Discussion

The results of the effect of cropping on changes in the amount of different forms of inorganic and organic N in an alluvial, limed and acid soils as well as N-uptake by different parts of maize plant at different growth stages are presented in Tables 2 to 9.

Data in Table 2 reveals that irrespective of cropping and N-fertilization, the amount of available N decreased with increase in the period of investigation. The decrease is more pronounced in the inorganic N added systems. Furthermore, the amount of decrease of available N is comparatively more in soil cropped with maize than the uncropped system. This observation is true for both the untreated and N-treated systems. However, irrespective of stages of sampling, comparatively higher amount of available N is accumulated in cropped over the uncropped system. This result is found in both in presence and absence of inorganic N. Data further shows that the order of accumulation of available N is more in cropped soil which was not fertilized than the soil fertilized with inorganic N. This is perhaps due to the priming effect of inorganic N.

Perusal of the data in Table 3 reveal that irrespective of cropping and N-fertilization the amount of available N decreased in unlimed whereas, in general, increased in limed soil upto 60th day of the investigation. Results further pointed one that recovery percentage of the higher doses of added inorganic N on 0-day is more in unlimed than the limed. This is perhaps due to immobilization of added N by the microorganisms as well as loss through denitrification process under limed situation (Goffman *et al.*, 1987). At the later stage of investigation (90th day), irrespective of cropping and N-fertilization, in general, the amount of available N is more in limed than the corresponding unlimed soil. Again, in general, comparatively higher amount of available N is accumulated in cropped over the uncropped

Table 2. Effect of cropping and inorganic N on changes in the amount (mgkg-1) of available N in an alluvial soil (Mean of three replicates)

Cropping	N-Fertilization	DAS						
		0	30		60		90	
			X	Y	X	Y	X	Y
Without	N0	124.46	83.19	-41.27	78.34	-46.12	78.25	-46.21
	N70	169.26	106.74	-62.52	87.46	-81.796	84.25	-85.006
	Effect of N (+/-)	+44.8	+23.55		+17.12		+6	
	N100	220.48	123.54	-96.94	104.67	-115.81	94.53	-125.95
	Effect of N (+/-)	+96.02	+40.35		+26.33		+16.28	
With	N0	124.46	90.30	-34.16	96.55	-27.91	94.84	-29.62
	N70	169.26	106.05	-63.21	101.11	-68.15	96.34	-72.919
	Effect of N (+/-)	+44.8	+15.75		+4.56		+1.5	
	N100	220.48	129.25	-91.23	107.22	-113.26	106.77	-113.71
	Effect of N (+/-)	+96.02	+38.95		+10.67		+11.93	
CRD (P=0.05)	N	71.62	23.22		NS		NS	
	C	NS	NS		NS		13.73	
	N x C	NS	NS		NS		NS	

N0=Zero dose of N, N70=N added at 70 ppm, N100=N added at 100 ppm, X =Amount in mgkg-1,
Y=Increase (+)/decrease (-) over zero day, DAS= days after sowing, N= Doses of nitrogen, C=Cropping, NS=Non significant

Table 3. Effect of cropping and inorganic N on changes in the amount (mg kg-1) of available N limed and corresponding unlimed soil (Mean of three replicates)

Soil	Cropping	N-Fertilization	DAS 0	30	60	90
Unlimed						
		N0	146.95	109.44	82.67	112.84
	Without	N70	207.79	160.10	101.41	105.80
		N100	237.88	139.85	105.42	99.73
		N0	146.95	90.89	73.79	109.70
	With	N70	207.79	151.11	93.81	98.83
		N100	237.88	140.16	95.15	106.43
Limed						
		N0	146.99	77.68	89.80	92.66
	Without	N70	213.50	77.69	91.74	92.66
		N100	230.02	89.08	97.12	93.30
	N0	146.99	72.39	77.69	111.65	
	With	N70	213.50	117.01	90.80	94.86
		N100	230.02	79.62	127.30	119.37
		N Dose	14.69	11.96	15.53	NS
		Crop	NS	NS	NS	7.07
		Lime	NS	9.76	NS	7.07
CRD (P = 0.05)	N x C	NS	NS	NS	12.25	
		N x L	NS	16.92	NS	NS
		C x L	NS	NS	NS	10.01
		N x C x L	NS	NS	NS	NS

Abbreviations are same as in Table 2 and L= Lime

Table 4. Effect of cropping and inorganic N on changes in the amount (mgkg-1) of total hydrolysable organic N in an alluvial soil (Mean of three replicates)

Cropping	N-Fertilization	DAS 0	30	60	90
	N0	727.10	727.10	789.04	881.04
Without	N70	789.04	673.80	762.50	939.04
	N100	789.04	638.40	638.40	962.40
	N0	727.10	789.04	727.10	823.8
With	N70	789.04	789.04	717.26	857.1
	N100	789.04	824.60	700.00	889.6
	N Dose	NS	NS	NS	19.60
CRD (P=0.05)	Crop	NS	90.89	NS	16.0
	N x C	NS	NS	NS	NS

Abbreviations are same as in Table 2.

system. The explanation furnished earlier in this regard is equally applicable here as well.

Changes in the amount of total hydrolysable organic N in soil due to cropping of maize and N fertilization are presented in Table 4. Results reveal that irrespective of N-fertilization, the amount of total hydrolysable organic N decreased in uncropped but increased in cropped soil upto 30^{th} day of the investigation. Again, in absence of crop, the amount of total hydrolysable organic N found to increase with increase in time upto last stage of the experiment. This is true both for untreated and N-treated systems. On the other hand, in presence of maize crop, the amount tended to decrease on 60^{th} day but thereafter showed an increasing trend upto the last stage of the investigation. This trend of results is found both in presence and absence of N-fertilizer. The decrease in the amount of total hydrolysable organic N on 30th day in the uncropped soil particularly treated with N-fertilizer is perhaps due to conversion of organic to inorganic N through mineralization process (Saha, 1997).

Data in Table 5 reveals that irrespective of liming, cropping and N-fertilization, the amount of total hydrolysable organic N increased upto 30th day and then showed a decreasing trend upto last stage of the investigation. The increase in total hydrolysable organic N at early stage of the experiment is perhaps due to immobilization of available N by the microorganisms under favourable environment particularly under N-added systems (Mukhopadhyay *et al.*, 1985). Results further show that comparatively higher amount of total hydrolysable organic N is accumulated in the N-treated over the untreated system. This trend of results is found throughout the experiment both in presence and absence of liming and cropping. Obviously this is the effect of added inorganic N. It is interesting to note that irrespective of cropping and N-addition as well as stages of crop growth, the amount of total hydrolysable organic N decreased in limed over the unlimed soil. This is perhaps due to creation of favourable environment under limed situation which encourages mineralization of organic N and loss of N from the soil system through mineralization (Saha and Mukhopadhyay, 1985b). However, in general, the amount of total hydrolysable organic N is more in cropped over the uncropped system. This trend of results is true both for unlimed and limed soils. It is note worthy to mention that irrespective of liming, cropping and N-addition, although the amount of total hydrolysable organic N decreased or increased over the whole period of investigation but in general, the amount remains more or less same at the start and at the end of the experiment.

Changes in the amount of total N in soil in presence and absence of cropping and N-addition are presented in Table 6 and 7. Perusal of the data in Table 6 reveal that irrespective of N-addition, the amount of total N tended to decrease upto 30th day in the uncropped and upto 60th day in the cropped system and then both the systems show an increasing trend of total N upto the last stage of the investigation. Data further reveal that irrespective of cropping and stages of sampling, in general, comparatively higher order of total N was recorded in soil with increase in the dose of addition of inorganic N. However, with time the uptake of N by growing maize crop increased (Table 8) showing a comparatively lower amount of total N in cropped than the uncropped systems.

Data in Table 7 reveal that irrespective of N-addition, in general, comparatively lower amount of total N was

Table 5. Effect of cropping and inorganic N on changes in the amount (mg kg-1) of total hydrolysable organic N in limed and corresponding unlimed soil (Mean of three replicates)

Soil	Cropping	N-Fertilization	DAS 0	DAS 30	DAS 60	DAS 90
Unlimed						
	Without	N0	602.90	629.50	691.60	602.90
		N70	670.40	771.40	770.30	670.60
		N100	686.20	780.30	780.30	742.10
	With	N0	602.90	691.60	638.40	558.60
		N70	670.40	780.30	689.80	652.70
		N100	686.20	851.20	780.30	652.70
		N0	594.10	594.16	602.90	558.60
Limed						
	Without	N70	602.90	638.40	656.13	602.90
		N100	629.50	686.20	686.20	677.70
	With	N0	594.10	691.60	670.40	672.70
		N70	602.90	770.30	770.30	742.10
		N100	629.50	780.20	770.90	770.90
	CRD (P = 0.05)	N Dose	NS	59.92	38.90	10.87
		Crop	NS	48.93	NS	8.87
		Lime	NS	48.93	NS	8.87
		N x C	NS	NS	NS	15.37
		N x L	NS	NS	NS	NS
		C x L	NS	NS	44.92	12.55
		N x C x L	NS	NS	NS	NS

Abbreviations are same as in Table 3.

Table 6. Effect of cropping and inorganic N on changes in the amount (mgkg-1) of total nitrogen in an alluvial soil (Mean of three replicates)

Cropping	N-fertilization	DAS			
		0	30	60	90
	N0	1141.42	865.4	907.14	1059.75
Without	N70	1216.02	885.14	983.46	1092.25
	N100	1235.68	885.14	978.27	1102.53
	N0	1141.42	885.57	865.40	983.46
With	N70	1216.02	1031.1	885.57	1037.14
	N100	1235.68	1172.52	907.14	1092.25
	N Dose	NS	43.47	NS	47.65
CRD (P=0.05)	Crop	NS	35.49	46.64	38.91
	N x C	NS	61.48	NS	NS

Abbreviations are same as in Table 2.

Table 7. Effect of cropping and inorganic N on changes in the amount (mg kg-1) of total N in limed and corresponding unlimed soil (Mean of three replicates)

Soil	Cropping	N-Fertilization	DAS			
			0	30	60	90
Unlimed						
		N0	1009.88	948.64	912.99	772.84
	Without	N70	1065.26	948.64	940.61	857.4
		N100	1102.86	965.42	1011.82	903.09
		N0	1009.88	1009.88	867.12	715.28
	With	N70	1065.26	1011.82	912.99	779.35
		N100	1102.86	1067.41	1067.41	879.79
Limed						
		N0	1012.28	715.28	734.72	833.94
	Without	N70	1076.62	837.40	837.40	864.66
		N100	1104.00	864.66	895.10	918.42
		N0	1012.28	837.40	833.94	772.84
	With	N70	1076.62	895.10	879.79	837.40
		N100	1104.00	915.42	939.62	965.42
		N Dose	21.099	36.976	12.383	6.968
		Crop	NS	30.190	10.110	5.689
		Lime	NS	30.190	10.110	5.689
	CRD (P = 0.05)	N x C	NS	NS	17.512	9.854
		N x L	NS	NS	17.512	9.854
		C x L	NS	NS	14.298	8.046
		N x C x L	NS	NS	24.765	13.936

Abbreviations are same as in Table 3.

accumulated in cropped unlimed soil, whereas, in general, a completely reverse trend of results was observed under limed situation. Cropping in limed soil encourages maintaining comparatively higher amount of total N in cropped over the uncropped system. It is interesting to note that N-addition increased the total N content in soil. Irrespective of liming about 90 per cent added inorganic N was recovered on 0 day of the experiment. This is observed in both the doses of N-addition.

Nitrogen percentage and N-uptake by different parts of maize plant with time in N-fertilized and unfertilized soils are presented in Table 8. Data in Table 8 reveal that irrespective of stages of sampling, N percentage and N-uptake by maize plant in alluvial are of higher order in the N-added over the unadded system. N-addition boosted the vegetative growth of the plant and in turn showed a higher percentage, as well as uptake of N by plant treated with inorganic N fertilizer. Data further show that assimilation of total N is more in above ground vegetation than the underground root portion of plant. This trend of results is true for both the untreated and N-treated systems. Irrespective of treatments, N-percentage as well as N-uptake per plant decreased with the period of investigation. This is an expected trend of results as was found earlier by Saha and Mukhopadhyay (1986b). Again, within above ground parts N percentage and N-uptake is more in the top portion of plant which has not received any inorganic N fertilizer from outside. However, in presence of inorganic N, the N-percentage is more in the mid-portion of plant. This trend of results is observed under both the doses of N-fertilizer treated systems (Table 8) throughout the investigation. Certainly inorganic N-addition has changed the uptake mechanism of N by different parts of the above ground maize plant cultivated in an alluvial soil. It may be mentioned here that the decrease in available N corroborate to the increase in N-uptake with time but it is very difficult to mention that which fraction of inorganic or organic N contributed most particularly when all the forms showing an increase and/or decrease over the whole period of investigation. However, the exact contribution of the fraction concerned to the uptake of N by maize crop may be determined using N^{15} as a tracer tool.

Results of the percentage of N in different parts of maize plant with period of investigation in presence and absence of liming and N-addition reveal that irrespective of liming and N-addition, the percentage of nitrogen in roots increased with period and then showed a decreasing trend at the last stage of the investigation. This is an expected trend of results. On the 60th day, the proliferation of roots is at its maximum level and higher amount of N is taken up showing a net effect of increase in N percentage at the mid stage of the experiment. It is interesting to note that in general, the percentage of N in roots decreased with increase in the dose of inorganic N addition in both the unlimed and limed soil. N-addition favours microbial growth and so called priming effect (Alexander, 1978) is responsible for taking up more N by plant roots in untreated over the N -treated systems.

Data further reveal that irrespective of liming and N-addition, the nitrogen percentage in both the middle and top portion of the maize plant decreased with the advancement of experiment. This is also an expected trend of results. The vegetative parts of the maize plant takes up more N at the early stage of the experiment because this N is used up for protein synthesis within plants. But with time the requirement of N by plant decreased, showing a decreasing trend of N percentage in plant. Similar observation was also reported earlier by (Saha and Mukhopadhyay, 1986b). Liming did influence greatly the N percentage in different parts of the plant.

Results further reveal that irrespective of liming and N-addition, in general, the dry matter yield of different parts of plant increased with the period of investigation. This is an expected trend of results. With time the vegetative growth of plants increased and in turn dry matter yield

Table 8. Changes in N percentage, dry matter yield and N uptake by different parts of maize plant-1 in presence and absence of inorganic nitrogenous fertilizer in an alluvial soil (Mean of three replicates)

N-Fert	Different parts of plant	DAS								
		30			60			90		
		A	B	C	A	B	C	A	B	C
	X	1.53	50.00	76.5	1.46	42.50	62.1	1.27	50.00	63.50
N0	Y	3.42	38.30	130.98	1.66	50.00	83.0	1.31	70.00	91.90
	Z	3.50	50.00	175.0	2.36	110.00	258.8	1.63	172.50	279.80
	X	1.66	50.00	83.00	1.08	70.00	77.35	0.64	77.50	48.90
N70	Y	2.75	43.30	119.14	1.70	72.50	123.30	1.63	182.50	297.50
	Z	1.64	61.60	101.02	1.53	182.50	278.80	1.47	300.00	404.00
	X	1.69	55.00	92.95	1.21	50.00	60.50	0.89	55.00	48.95
N100	Y	2.88	43.30	124.70	2.09	62.50	130.60	1.77	150.00	265.50
	Z	2.48	61.60	152.77	1.53	107.50	164.70	1.34	265.00	353.20
	P	0.023	2.259	4.936	0.033	17.798	34.723	0.024	26.960	30.751
CRD (0.05)	N	0.023	2.259	4.936	0.033	17.798	NS	0.024	26.960	3.751
	P x N	0.039	3.912	8.549	0.057	30.827	60.143	0.041	46.696	53.262

A=N per cent, B=Dry matter yield (mg) plant-^{1}, C=Uptake (mg), N0=N added at 0 ppm, N70=N added at 70 ppm, N100=N added at 100 ppm, X=Root portion, Y= Mid portion and Z=Top portion.

was also found to increase. Comparatively higher dry matter yield of different parts of plant was produced by maize cropped in limed soil particularly in untreated and lower dose of N-treated system. Liming increases the N-mineralization rate (Saha and Mukhopadhyay, 1985a) and maintain comparatively higher amount of nitrogen in available form which may be taken up by the growing maize crop. The interesting observation is that at higher dose of N-addition, in general, comparatively lower amount of dry matter was produced in limed over the unlimed soil. Higher dose of N-addition has suppressed the effect of liming particularly at the earlier stages of the experiment. If the experiment is continued for longer period of time then effect of liming can be observed in later growth stage of the maize crop.

Changes in uptake of nitrogen by different parts of maize plant in presence and absence of liming and N-fertilization was also studied. Uptake of N by plant parts is the product of respective nitrogen percentage and dry matter yield. Results of N percentage and dry matter yield showed an expected trend of results and therefore the results of N-uptake also showed similar trend of results. Assimilation of total N by different parts of maize plant increased with the period of investigation. Liming causes higher amount of N-uptake by plant. Assimilation of nitrogen is more in vegetative parts than the under ground portion which showed an expected trend of results.

It is very difficult to find out that which fractions of inorganic and/or organic N had contributed most to the N-uptake by maize crop without using N^{15} tagged nitrogenous fertilizer.

Summary and conclusion

The effect of cropping on changes in different forms of inorganic and organic N as well as assimilation of total N by different parts of maize plants were investigated in an alluvial, unlimed and the corresponding limed acid soil in presence and absence of added inorganic nitrogenous fertilizer. Resuslts obtained in the course of investigation are pointwise summarised below.

1. Irrespective of cropping and N fertilization, the amount of available N in alluvial soil decreased with increase in the period of investigation. The decrease is more pronounced in the inorganic N added systems. Again, the amount of decrease of available N is comparatively more in soil cropped with maize than the uncropped system. Irrespective of cropping and N fertilization, the amount of available N decreased in unlimed whereas, in general, increased in limed soil upto 60 day of the investigation.
2. The amount of total hydrolysable organic N decreased in uncropped but increased in cropped alluvial soil upto 30th day of the investigation. The increase in total hydrolysable organic N with time is the result of presence of higher number of micro-organisms whose cells are proteinaceous in nature. Irrespective of liming, cropping and N- fertilization, the amount of total hydrolysable organic N increased upto 30 day and then showed a decreasing trend upto the last stage of the investigation. Comparatively higher amount of total hydrolysable organic N in accumulated in the N treated over the untreated system. This trend of results is found throughout the experiment both in presence and absence of liming and cropping.
3. Irrespective of N addition, the amount of total N in alluvial soil tended to decrease upto the 30th day in uncropped and upt the 60th day in cropped system and then both the systems an increasing trend of total N upto the last stage of the investigation. Higher amount of accumulation of total N at earlier stage of the experiment under cropped situation is due to the presence of higher number of micro-organisms in cropped over the uncropped system. Comparatively lower amount of total N was accumulated in cropped unlimed soil, whereas in general, a completely reverse trend of results was observed under limed situation. Cropping in limed soil encourages to maintain completely higher amount of total N in cropped over the uncropped system.
4. Irrespective of stages of sampling, N percentage and N uptake by maize cropped in alluvial soil are of higher order in the N added over the untreated system. Assimilation of total N is more in aboveground vegetative than the undergound root portion of the crop. This trend of results is true for both the N treated and untreated systems. In N added treatment, the N percentage is more in the mid portion of the plant. In general, the percentage of N in roots decreased with the increase in the dose of N addition in both the limed and unlimed soil. Irrespective of liming and N addition, the N percentage in both middle and top portion of the maize plant decreased with the advancement of the experiment. Irrespective of liming and N addition, in general, the dry matter yield of different parts of maize plant increased with the period of investigation. This is an expected trend of result. Assimilation of total N by different parts of maize plant increased with the period of investigation. Liming causes high amount of N uptake by plant.

Assimilation of N is more in vegetative parts than the underground portion. This is also an expected trend of result.

Acknowledgement

Authors are grateful to Bidhan Chandra Krishi Viswavidyalaya for availing laboratory facilities during the course of the study. We also acknowledge ARS, Pakhribas for providing facilities to prepare the paper. Support from HMRP for presenting the paper at Indonesia is highly acknowledged. We thank Dr. Ferrera and Dr T.P. Tiwari for thier day to day inspiration.

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Verification and validation of intercrops in double plants maize per hill system in the eastern middle hills of Nepal

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Abstract. A set of experiments were conducted for the verification and validation of system based technology in order to increase the productivity and households' income of the small holder women farmers under maize based system of the mid hills (1100-1600 m) in the eastern Nepal during 2003-2006. Two independent research activities were executed including soyabean and ginger intercrops under double plants maize per hill (100x50 cm) system (DPS). Along with biological data, feedbacks from farmers were also collected. Yield data revealed significant differences of maize spacing (P<0.05) and soyabean varieties (P<0.01). The yield in DPS of maize was higher (4334 kg/ha) and the performance of soyabean varieties Seti and Chaing Maw 60-63 was better under the same system. The combined analysis of ginger (rhizome) yield over locations showed a significant difference (P<0.05), however interaction with locations and treatments were non-significant. Sole ginger followed by ginger planted under maize DPS yielded higher in both the years. Maize yield was at par and/or better among the treatments. The combined analysis for the years (2004 & 2006) showed the highest yield from DPS (maize+ginger) (17.0 & 23.4 t/ha) followed by sole cropping ginger (15.1 & 18.6 t/ha) respectively. The economic analysis for the years 2004 and 2006 revealed the highest profit from DPS of maize+ginger was Rs.86250/ha (US$ 1224)& Rs. 80942/ha (US$ 1156) followed by sole ginger Rs.78500/ha (US$ 1114) & Rs. 53656/ha(US$ 767) respectively while compared with sole maize (Rs.158/ ha or US$ 2.2/ha) which wasn't profitable. The results clearly indicated that soyabean intercropping under DPS of maize performed better without lowering the maize yield. Similarly, on-farm income can be easily increased with the intervention of ginger in the maize-based system.

Introduction

Maize is a crop grown under varied agro-climatic conditions and the problems associated with its production are varied. As maize is mixed or intercropped with legumes, ginger or relayed millet, variety suitable for those situations as well as for sole cropping is required for the hills. In additions plant population, spacing and intercrops plant ratio are also important for maximizing the benefit to farmers. Ginger is grown in large area of Ilam district as an intercrop with maize. Farmers in Bhojpur, Dhankuta, Terhathum and other districts with similar biophysical conditions are also growing ginger as cash crop. As a shade loving crop, the farmers from Ilam districts are intercropping ginger with maize. However, in the other areas it has been grown either as sole or inter-cropped with maize or both.

Similarly, maize+soyabean is one of the major cropping patterns after maize/millet system in the eastern hills of Nepal. As the recommended varieties as well as farmers' local are not suitable for intercropping with improved maize varieties, identification of suitable varieties for intercropping under maize + soyabean system is needed.

Recently, DPS is found equally good with SPS (75 x 25 cm row to row and plant to plant) with ginger, soyabean, millet and other intercrops (Katuwal 2002). This technology is suitable for intercrops, which provides more space for the intercrops, efficient utilization of fertilizers use in top-dressing for maize crop and it reduces labour requirements due to easy intercultural operations. For this, it is necessary to assess the DPS in maize that may give good maize production.

For the technology development from the intercropping trials, the disadvantaged particularly women and small to medium farmers will be benefited with suitable intercrops such as ginger with maize. Similarly, the health of women and children and the poor people will be improved by the use of soyabean a good source of protein.

This report covers the results of the varietal combination for maize+soyabean and maize+ginger intercropping under double maize plants per hill system in the Mid Eastern hills of Nepal conducted at the station and outreach research sites during 2003-2006. The study was undertaken to disseminate and verify the maize + ginger and maize + soyabean intercropping technologies under double plants maize/hill system with high production potential for mid hills through demonstration and verification for wider adaptation in the farmers' fields.

Materials and Method

The experiments were executed in mid hills (1100-1700 m) at the research station and different outreach research (OR) sites of the eastern hills from 2003 to 2006. The on-station trials were conducted in a split plot arrangement and replicated three times. Diamond trials and the promising technologies obtained from on station research were demonstrated at the Fikkal, Belhara and Fakchamara OR sites in different years (2004-2006).

Maize cultivar Manakamana-3 was used in the trials. Five soyabean varieties with local check were tested in on-station trials whereas the promising Seti soyabean and popular local ginger were included in maize+soyabean and maize+ginger intercropping studies at the OR sites. Site and farmers were selected considering the gender, caste and disadvantaged groups of the OR sites. Maize was sown following two systems i.e. Double plants maize per hill system (DPS) and single plant maize per hill system (SPS) with the spacing of 100cmx50cm vs farmers' practice (FP) respectively. Plot size allocated to each treatment was 9 m^2 for on-station trials whereas 50m^2 plots were used and replicated six to times (farmer as replication) in diamond trials and demonstration plots at farmers' fields. Fertilizers 60:30:30 $NP_2O_5K_2O$ kg/ha and farm yard manure (FYM) 10 t/ha (farmers' practice) was applied for the maize crop. FYM rate was up to 30 t/ha for ginger. Two weeding were done and soyabean was seeded during the first weeding of maize.

Observations such as plant stand, plant height, maturity and yields were recorded. Along with the cost of cultivation, price of the produce and feedbacks from farmers were also collected. Statistical analysis for the yield and important parameters and economic analysis of the technologies was performed.

Results and Discussion

Maize + soyabean inter-cropping in double plants/ hill system of maize, 2003-2004

Maize yield obtained from both DPS and SPS systems were at par statistically. The performance of soyabean varieties grown in DPS maize was found significantly ($P<0.05$) better than SPS during 2003. The differences among the soyabean varieties and intercropping interaction effect between soysbean varieties x maize spacing was also found significant ($P<0.01$). Though there was poor yield performance of the soyabean varieties, the highest seed yield was obtained from Seti planted under DPS of maize. The low seed yield was produced from Chaing Maw 60-63 (276 kg/ha) followed by Local (291 kg/ha) planted under DPS of maize, which was also better than the varieties seeded under SPS in 2003 (Table 1). The other parameters i.e. plant stand, plant height and maturity duration were also observed and recorded but most of them were not significant statistically. This year's result also suggests repeating the experiment with the change of soyabean varieties for further confirmation.

Maize yield was significantly ($P<0.05$) better in DPS system. From the second year's (2004) study found

Table 1. Yield performance of maize+soyabean inter-cropping SPS Vs DPS

Variety	2003				2004			
	Maize		Soya		Maize		Soya	
	SPS	DPS	SPS	DPS	SPS	DPS	SPS	DPS
Seti	4855	4108	281	504	1912	3452	298	446
Chaing Maw 60-63	4571	4623	213	276	2176	4173	195	266
Ransom	4716	4992	250	294	4149	4738	209	210
GC 82234. 22C	4844	4995	226	294	3632	4931	149	190
Local	4062	5026	200	291	3446	4377	156	244
Mean	4610	4749	234	332	3069	4334	201	271
F-test: Var.	NS		*		NS		**	
Spacing	NS		**		*		**	
Int.	NS		**		NS		NS	
LSD: Var.	-		34.4		-		66.6	
Spacing	-		42.1		1259.8		42.1	
Int.	-		63.6		-		-	
CV%	12.3		12.9		24.4		23.2	

Note: DPS= double plants per hill system (100 x 50 cm) and SPS= Single plant per hill system (100 x 25 cm).

significant differences of soyabean varieties (P<0.01) and maize spacing (P<.05) between DPS with the spacing of 100 x 50 cm and SPS at a spacing of 100 x 25 cm for the spacing of both maize and soyabean yields. The yield produced by DPS of maize was higher (4334 kg/ha (Table 1).Plant height of both crops showed significant effect at P<0.05 and P<.01 levels between double plants in maize and soyabean crops respectively. The plants of maize were taller in height in DPS whereas the plant height of soyabean was taller in SPS. The other parameters measured such as plant stand and maturity were found non-significant (P>0.05) differences among the treatments.

Verification and Demonstration of Maize + Soyabean in DPS of Maize, 2005

As the experiment was implemented for the verification and validation of maize + soyabean system-based technology so as to increase the productivity and households' income of the small-scale women farmers under maze-based system of the mid hills, maize yield was found very impressive in both sites (Figure 1).

The maize yield was obtained more from Belhara site. It was mainly due to lower altitude (1100-1200m) of the mid hill and less intercropping competition due to less vegetative growth without lodging of soyabean at Belhara. The plants were taller and the problem of lodging in soyabean was observed at Fakchamara causing low yield of soyabean. The performance of soyabean crop was better at Belhara site (Figure 1). There was no variation in maturity duration among the farmers' fields because the variety used of both crops were also same in the demonstration; the crops maize and soyabean were harvested during August and October respectively. There was some Problem of *Cercospora* spp. disease in soysbean in the demonstrations.

Maize+soyabean diamond trials under double plants per hill systems, 2006

Analysis of variance on grain yield of maize showed non-significant differences whereas the yield of soyabean was significant (*P*<0.01) statistically among the treatments. The better yield was obtained from DPS maize+Seti soya (649 kg/ha) followed by DPS maize+local soya (533 kg/ha) (Table 2).

Maize+ginger diamond trials under double plants per hill systems, 2003-2004

Statistical analysis for individual location showed non-significant results for both maize and ginger yields. Combined analysis over locations, however, showed a significant result (P<0.05) in rhizome yield, however interaction with the locations and treatments were found non-significant. Sole cropping ginger produced the highest rhizomes yield (16.1t/ha) over the locations, followed by ginger planted under double plants maize per hill systems (15.0 t/ha) in 2003 (Table 3).

Similarly, the same treatments i.e. sole cropping ginger (15.1 t/ha) and DPS (12.3 t/ha) gave higher rhizome yield in

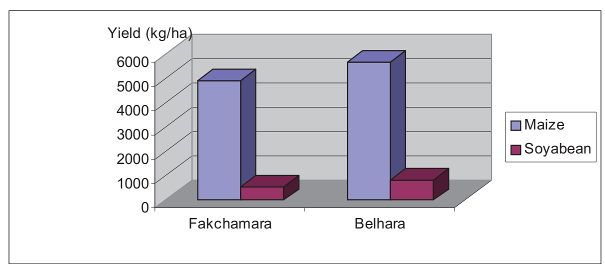

Figure 1. Performance of maize+soyabean intercropping in DPS system, 2005

Table 2. Results of maize+soyabean intercropping diamond trial, combined over locations

Treatment	Grain yield kg/ha (maize)		Seed yield kg/ha (Soyabean)	
	Fakchamara	Belhara	Fakchamara	Belhara
DPS maize+Seti soya	4623	5157	538	649
FPS maize+Seti soya	4582	4674	315	316
DPS maize+local soya	4543	4742	372	533
FPS maize+ local soya	3839	4515	389	378
F-test: Trt.	NS		**	
Int.	NS		NS	
LSD: Trt.	-		92	
Int.	-		-	
CV%	27		23	
Mean	4584		436	

Table 3. Yield performance of maize+ginger diamond trial, combined over locations

Treatment	2003			2004		
	Maize	Ginger	Combined	Maize	Ginger	Combined
DPS maize (100x50cm) +ginger	4.8	15.0	19.8	4.7	12.3	17.0
Farmers' practice (maize+ginger)	3.8	12.6	16.3	4.3	10.1	14.4
Sole maize (farmers' practice)	5.1	-	5.1	4.8	-	4.8
Sole ginger (farmers' practice)	-	16.1	16.1	-	15.1	15.1
F-test	NS	*	-	NS	**	*
LSD	-	1.3	-	-	2.0	3.5
CV%	29.7	26.3	-	36.1	15.0	18.9

Note: DPS= double plants per hill system (100 x 50 cm).

Table 4. Average yield, Land Equivalent Ratio (LER) and Net Benefit (NB)

Treatment	Yield t/ha	LER	Net Benefit (Rs.)
DPS maize (100x50cm) +ginger (FP)	18.4	1.83	86250
Farmers' practice (maize+ginger)	15.4	1.56	14634
Sole maize (farmers' practice)	5.0	0	158
Sole ginger (farmers' practice)	15.6	0	78500

2004. There were non-significant (P< .05) yield differences of maize among the treatments in both years. It was found significant (P< .05) difference in combined yield of maize + ginger. The higher yield was obtained from DPS (17.0 t/ha) followed by sole cropping ginger (15.1 t/ha) and farmers' practice (14.4 t/ha).

The higher LER was found from the double plants per hill system of maize (100x50cm) + ginger (FP). Likewise, the net benefit also obtained more from the DPS (Rs.86250/ha) followed by Sole ginger (Rs.78500/ha) in the experiment (Table 4). Sole maize was not profitable (Rs.158/ha) while considering the cost of cultivation i.e. the total cost that vary (TCV) in the system at mid hills.

Verification and Demonstration of Maize+ginger in DPS of Maize, 2005

The experiment was implemented for the verification, validation and dissemination of maize+ginger intercropping technology so as to increase the productivity and households' income of the small-scale women farmers under maze-based system of the mid hills. The highest ginger and maize yield were produced at Fikkal when compared to Belhara site. It was due to the higher compost (30 t/ha) application and timely rain at Fikkal and in contrast only 20 t/ha compost was applied and was drought problem during tasseling stage of maize and emergence and rhizome development stage of ginger at Belhara (Figure 2).

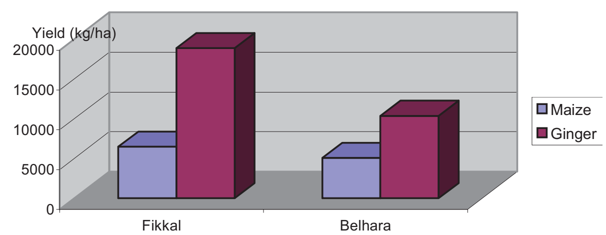

Figure 2. Yield performance of maize+ginger inter-cropping under double maize plants/hill system, 2005.

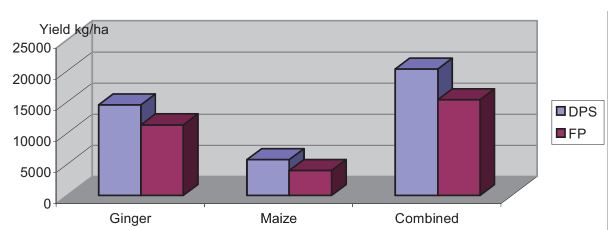

Figure 3. Yield comparison between DPS and FP under inter-cropping in maize-based system, 2005.

There was no variation of maturity duration among the farmers' fields because the variety used for both crops were same. Maize and ginger were harvested during August and November respectively. No differences were observed in plant stand, plant height and other yield attributes.

Both crops produced higher yields in DPS and the yield increased by 28%, 42% and 32% in ginger, maize and combined respectively. DPS gave return of US$ 1223/ha whereas FP gave US$ 208 (Figure 3.)

Table 5. Yield (t/ha) of maize + ginger inter-cropping trial, combined over locations

Treatment	Maize	Mother rhizome	Main rhizome	Total rhizome	Maize+ginger
DPS maize (100x50cm) +ginger (FP)	6.3	3.8	13.3	17.1	23.4
Farmers' practice (maize+ginger)	5.9	3.4	9.3	12.7	18.6
Sole maize (farmers' practice)	6.8	-	-	-	6.8
Sole ginger (farmers' practice)	-	3.5	15.1	18.6	18.6
F-test	NS	NS	**	**	-
LSD	-	-	2.6	2.6	-
CV%	15.6	13.2	24.9	19.4	-

The net benefit obtained was more from the DPS (Rs. 80942/ha) followed by sole ginger (Rs.53656/ha) in the experiment.

Table 6. Average yield (t/ha), TCV (Rs./ha) and Net Benefit (Rs./ha), 2006

Treatment	Total Yield t/ha	Gross Benefit Rs./ha	Total Cost that Vary (TCV) Rs./ha	Net Benefit Rs./ha
DPS maize (100x50cm) +ginger (FP)	23.4	207942	127000	80942
Farmers' practice (maize+ginger)	18.6	162977	130500	32477
Sole maize (farmers' practice)	6.8	47950	19500	28450
Sole ginger (farmers' practice)	18.6	176656	123000	53656

Maize+ginger diamond trials under double plants per hill systems, 2006

Statistical analysis combined over locations showed a significant result ($P<0.05$) in rhizome yields. Sole ginger produced the highest rhizomes yield (18.6 t/ha) over the locations followed by ginger planted under double plants maize per hill systems (17.1 t/ha). There were non-significant yield differences of maize and mother rhizome among the treatments. The higher total yield was obtained from DPS (23.4 t/ha) followed by sole ginger and farmers' practice (18.6 t/ha) (Table 5).

Farmers' reaction

The participating as well as neighbor farmers highly impressed with these activities. DPS of maize for maize+ginger system were liked by the women farmers from both sites. Similarly, they preferred DPS for intercropping soyabean and other inter crops but were concerned about low yield of soyabean variety in the trials. They were ready to follow the DPS system in maize+ginger and other maize based systems in the mid hills.

Conclusion

Results indicated that intercropping soyabean varieties under double plants per hill system (DPS) of maize could gave better yield advantage without loosing the yield of maize. Though the yield was low, the soyabean varieties Seti and Chaing Maw 60-63 were identified as superior variety under DPS system of maize. The effect of the verifications and demonstrations have clearly indicated that farmers income can be easily increased with the intervention of ginger in maize-based system by following DPS of maize (100x50 cm). It can be concluded that DPS is more profitable than the other systems. Maize yield was also better which is very important for the food security of the farmers.The maize+ginger intercropping technology with DPS of maize technology increases the production and households' income of the small holder women farmers under maize based system of the mid hills (1100-1600 m) of eastern Nepal.

Acknowledgements

We are very grateful to Dr. DB Gurung, Coordinator, the National Maize Research Program, Rampur for the technical support. HMRP Collaborators and participating farmers deserve due thanks for their coordination and assistance in carrying out this research.

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Optimum nitrogen fertilizer rate of elite drought tolerant hybrid corn

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Abstract. To find out a optimum rate of nitrogen fertilizer for drought tolerant hybrid corn, the experiment was established at Nakhonsawan Field Crops Research Center on brown forest and renzina soil during 2007 growing season. The experiment consisting of 3 hybrids as main plot and 4 nitrogen level (0 62.5 125.0 187.5 kg N/ha) as sub plots in split plot design with 3 replications. In brown forest soil hybrids did not differ significantly with respect to yield however, corn yields were significantly increased with gradual increase in N applications and the corn yield was 4119,5612,6162 and 6275 kg/ha respectively at 0,62.5,125.0 and 187.5 kg N/ha. The best response of NS 2 was observed at 125.0 kg N/ha, whereas NSX042022 and NSX 042029 responded best at 187.5 kg N/ha. There was significant interaction between hybrid and N fertilizer. Highest benefit (573 USD/ha) was recorded with NS 2 at N 62.5 kg N/ha. Similarly at renzina soil hybrid did not produced significant yield variation however, gradual increase in N fertilizers produced significantly higher yields and corn yield was 3462, 4694, 5294 and 5381 kg/ha respectively with 0 62.5 125.0 and 187.5 kg/ha. Interactive study suggest that NS 2 and NSX 042029 responded best with 187.5 kg N/ha whereas, NSX 042022 response was best with 125.0 kg N/ha. Highest benefit (160 USD/ha) was recorded wit NS 2 with N 0 kg N/ha.

Key words: corn, hybrid, drought, nitrogen, response, benefit

Introduction

Nowadays farmer use high rate of chemical fertilizer for hybrid corn production and chemical fertilizer is expensive. The objective of the experiment is to find out a optimum rate of nitrogen fertilizer of drought tolerant hybrid corn and nitrogen response of elite corn hybrid. In a central region of Thailand corn planting area is 278,720 ha in Nakhonsawan province corn planting area is 86,560 ha (Office of Agricultural and Development Region 5 Chinat, 2004). Department of Agricultural recommend in black soil if available P> 10 ppm apply urea 156 kg/ha, if available P< 10 ppm apply 20-20-0 rate 250 kg/ha at planting and 46-0-0 rate 62.5 kg/ha at 3 weeks after planting (DOA,2002).

Materials and Methods

We use three hybrid corn NSX 042022 NSX 042029 and NS 2 chemical fertilizer are 15-15-15 and 46-0-0 and herbicide is alachlor is to be use . Design is split plot with 3 replications, main plot is 3 varieties of corn , sub plot is rate of nitrogen fertilizer.

Results and Discussion

Brown forest soil

Plant per area. Number plant per area is not significantly different between variety and number plant per area is not significantly different between rate of nitrogen, number plant per area is between 53,037 to 54,150 plant/ha (Table 1) it is no interaction between variety and rate of nitrogen.

Ear per area. Number ear per area is not significantly different between variety, number ear is 52,056 to 54,944 ear /ha. (Table 1) Number ear is not significantly different between rate of nitrogen and number ear is 52,519 to 54,150 ear/ha (Table 1) it is no interaction between variety and rate of nitrogen.

Plant fresh weight. NSX 042029 gave the highest plant fresh weight 8,731 kg/ha that higher than 2 varieties, NSX 2 and NSX 042022 gave plant fresh weight 6,756 kg/ha and 6,731 kg/ha respectively (Table1). Plant fresh weight is not significantly different between rate of nitrogen and plant fresh weight is 7,262 to 7,512 kg/ha (Table 1) and no interaction between variety and rate of nitrogen.

Table 1. Effects of variety and rate of fertilizer to number of plant , ear, plant fresh weight, and corn yield on brown forest soil, 2007.

Treatment	No.plant/ha	No.ear/ha	Plant fresh wt. (kg/ha)	Yield (kg/ha)	Plant height (cm.)
NS 2	52,887	54,944	6,756 b	5,787	188 b
NSX 042022	52,887	53,600	6,731 b	5,456	209 a
NSX 042029	53,225	52,056	8,731 a	5,337	214 a
F-test A	NS	NS	*	NS	*
N 10 kg/rai	53,037	53,925	7,494	5,612 b	204 a
N 20 kg/rai	53,556	53,556	7,262	6,162 a	205 a
N 30 kg/rai	54,150	54,150	7,344	6,275 a	206 a
F-test B	NS	NS	NS	**	*
F-test AB	NS	NS	NS	*	*
CV(%)	1.28	3.10	10.08	5.22	1.53

Table 2. Effects of variety and rate of fertilizer to number of plant, ear, plant fresh weight, and corn yield on renzina soil, 2007

Treatment	No.plant/ha	No.ear/ha	Plant fresh wt. (kg/ha)	Yield (kg/ha)	Plant height (cm.)
NSX 042022	51,056	51,781	7,562	5,000	149 b
NSX 042029	52,225	53,444	6,969	4,444	159 b
NS 2	52,500	50,944	9,275	4,687	177 a
F-test A	NS	NS	NS	NS	*
N 0 kg/rai	51,337	50,000 b	6,481 b	3,462 c	156
N 10 kg/rai	51,856	52,225 a	7,950 a	4,694 b	163
N 20 kg/rai	52,300	53,187 a	8,725 a	5,294 a	163
N 30 kg/rai	52,225	52,819 a	8,556 a	5,381 a	163
F-test B	NS	*	*	*	NS
F-test AB	NS	NS	NS	NS	NS
CV(%)	1.69	2.66	12.86	12.59	5.71

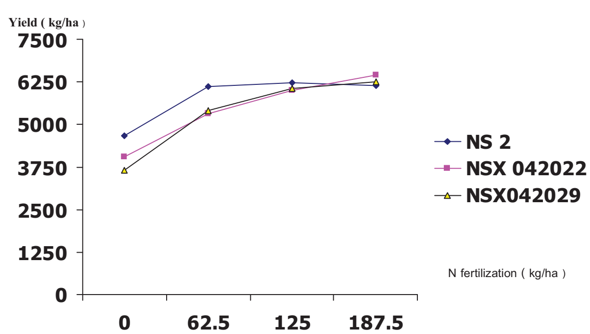

Figure 1 Effect of rate of N fertilization to yield on brown forest soil NSFCRC 2007

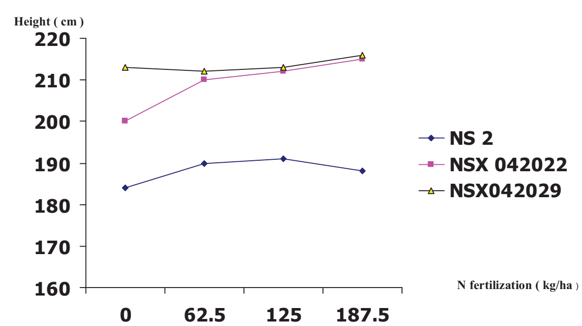

Figure 2 Effect of rate of N fertilization to height at harvest on brown forest soil NSFCRC 2007

Plant height. Variety gave plant height significantly different, NS 2 gave plant height lowest 188 cm. (Table 1) rates of nitrogen gave plant height significantly different, no nitrogen fertilizer gave the lowest plant height 199 cm, (Table 1). There is interaction between variety and rate of nitrogen, NSX 042029 with nitrogen 187.5 kg/ha gave the highest plant height 216 cm (figure 2).

Yield. In brown forest soil we found that it is not different between varieties on yield, corn yield were significantly increased when nitrogen fertilizers were applied. Nitrogen fertilizers 0 62.5 125.0 and 187.5 kg N/ha gave yield 4,119 5,612 6,162 and 6,275 kg/ha respectively (Table 1). NS 2 response at 125.0 kg N/ha. NSX 042022 and NSX 042029 response at 187.5 kg N/ha. Interaction show between variety and fertilizer. NS 2 with 62.5 kg N/ha gave highest benefit 573 USD/ha. (Table3).

Renzina soil

Plant per area. Number plant per area is not significantly different between variety, number plant is 51,056 to 52,500 plant/ha (Table 2). Number plant is not significantly different between rate of nitrogen , number plant is 51,337 to 52,300 plant/ha. (Table 2) and no interaction between variety and rate of nitrogen.

Ear per area. Number ear per area is not significantly different between variety, number ear is 50,944 to 53,444 ear /ha (Table 2) . There are significantly different between rate of nitrogen, no nitrogen gave the lowest ear 50,000 ear/ha . Number ear is not different between rate of nitrogen , number ear is 52,225 to 53,187 ear/ha (Table 2) and no interaction between variety and rate of nitrogen.

Plant fresh weight. Plant fresh weight is not different between variety, plant fresh weight is 6,969 to 9,275 kg /ha (Table 2). Plant fresh weight is different between rate of nitrogen, no nitrogen gave the lowest plant fresh weight 6,481 kg/ha. Plant fresh weight is not different between rate of nitrogen, plant fresh weight is 7,950 to 8,725 kg/ha (Table 2) .

Plant height. Plant height is significantly different between variety, NSX 042022, NSX 042029 and NS 2 gave corn height 149, 159 and 177 cm. respectively (Table 2) Corn height is not different between rate of nitrogen, corn height is 156 to 163 cm. (Table 2) and no interaction between variety and rate of nitrogen.

Yield. In renzina soil we found that no different as yield between variety, corn yield were significantly increased when nitrogen fertilizer were apply, fertilization 0 62.5 125.0 and 187.5 kg N/ha gave yield 3,462 4,694 5,294 and 5,381 kg/ha respectively (Table 2). NS 2 and NSX 042029 response at 187.5 kg N/ha and NSX 042022 response at 125.0 kg N/ha. NS 2 with N 0 kg N/ha gave the highest benefit 160 USD/ha. (Table 4).

Table 3. Yield, return, cost, benefit and cost per metric ton of corn on brown forest soil

Variety	N kg/ha	Yield kg/ha	Return (USD/ha)	Cost (USD/ha)	Benefit (USD/ha)	Cost (USD/ton)
NS 2	0	4,618	891	428	463	91.4
	62.5	6,100	1,162	589	573	96.5
	125	6,237	1,188	665	523	103.5
	187.5	6,131	1,168	701	467	114.3
NSX 042022	0	4,037	769	428	341	106.0
	62.5	5,312	1,012	589	423	110.8
	125.0	6,006	1,144	645	499	107.3
	187.5	6,456	1,230	701	529	108.6
NSX 042029	0	3,644	694	428	266	117.5
	62.5	5,412	1,031	589	442	108.9
	125.0	6,056	1,154	645	509	106.7
	187.5	6,244	1,189	701	488	112.4

Table 4. Yield, return, cost, benefit and cost per metric ton of corn on renzina soil.

Variety	N kg/ha	yield kg/ha	return (USD/ha)	cost (USD/ha)	benefit (USD/ha)	cost (USD/ton)
NS 2	0	3,047	588	428	160	141
	62.5	3,697	713	598	115	159
	125.0	4,123	795	665	130	161
	187.5	4,199	810	701	109	167
NSX 042022	0	2,305	445	428	17	186
	62.5	3,497	675	589	86	168
	125.0	3,805	734	645	89	169
	187.5	3,786	730	701	29	185
NSX 042029	0	2,477	478	428	50	173
	62.5	3,422	660	589	71	172
	125.0	4,041	780	645	135	160
	187.5	4,178	806	701	105	168

Conclusion and Recommendation

The experiment by average corn response at 125.0 kg N/ha, in brown forest soil it is not different between varieties on yield, corn yield were significantly increased when nitrogen fertilizer were applied NSX 042022 and NS 2 response at 125.0 kg N/ha NSX 042029 response at 187.5 kg N/ha. The recommendation is applied125-187 kg N/ha depend on varieties that gave high return and high benefit. In renzina soil it is not different on yield between variety , corn yield were significantly increased when nitrogen fertilizer were applied , fertilizer 0 62.5 125.0 and 187.5 kg N/ha gave yield 3,462 4,694 5,294 and 5,381 kg/ha respectively. NS 2 and NSX 042029 response at 187.5 kg N/ha and NSX 042022 response at 125.0 kg N/ha . NS 2 with N 0 kg N/ha gave the highest benefit 160 USD/ha. The recommendation is applied 0-187.5 kg N/ha depend on varieties that gave high return and high benefit.

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Enhancing maize productivity in post-rice environments in Thailand

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Abstract. Maize is considered to be a suitable upland crop to replace the second rice due to its less water consumption than the rice. However, yield of maize is often limited by crop management and commercial hybrid applications. Thus, development of appropriate technologies for maize production in paddy fields was urgently needed to increase its yield at various farmers' fields of 3 districts (20 farmers) in Phitsanulok province, Thailand during December 2002 and June 2005. The farmer participatory research covered site selection, socio-economic survey, hybrid trial and crop management including on the job training and field day. The integrated technology for improving yield should be done according to the following steps: A uniform area should be selected, with well-drained soils such as loam, sandy loam or clay loam. The best time for planting is November. After harvesting rice, the land was plowed, harrowed and a fertilized with 15-15-15 or 16-16-8 at the rate of 312 kg/ha. The Hybrid seeds namely NK 48 should be sown with a two-row planter at a spacing of 0.70 x 0.20 m spacing with 1 pl/hill (71,425 pl/ha) under residual soil moisture. Urea (46-0-0) at the rate of 156 kg/ha was recommended to be applied twice, a 94 kg/ha at three to four weeks after sowing and the soil was hilling up, the remaining (62 kg/ha) was applied again at flowering. The maize crop must be given its first irrigation water immediately after the first top-dressing. Furrow irrigation was needed two to three times during the growing season. This technology had been then transferred to farmers by ways of farmer participatory research at various farmers' fields of Phitsanulok province. Through this way, the farmers could produce an average grain yield of 80 % over the other farmers' yield (5.0 t/ha).

Introduction

In recent years, total maize production area in Thailand has decreased gradually, although domestic consumption of maize, mainly for feed industry, has increased rapidly. Erratic distribution and low amount of rainfalls together with competition from other cash crops i.e. sugarcane and cassava, are probably the most important physical constraints to rainfed maize production. Thus, it can be forecast that in the near future the total maize production will not meet domestic demand (Boonpradub and Kraokaw, 2002).

The paddy field during dry season is one of the new land types available for maize cultivation. In addition, maize could be considered as a more suitable crop to replace the second rice crop. This is probably due to less water consumption and more economic return than the second rice crop. Dry season maize crop can help to break the population dynamics of rice pests particularly brown planthopper and golden snail and to increase the amount of maize produced to cover the domestic demand. However, the yield of dry season maize is often limited by inappropriate soil, water, fertilizer management including commercial hybrid. In addition, the costs of maize production in dry season are still relatively high. The total production cost consist mainly labor costs and material costs (Ekasingh et al., 2004). Boonpradub and Kraokaw (2002) reported that labor costs compose of mostly land preparation (18 % of total cost). In terms of material costs, the largest item is fertilizer cost (15 % of total cost). Thus, development of appropriate technologies for maize production in paddy fields is urgently needed to increase its yield. These will be transferred to farmers by ways of farmer participatory research at various farmers' fields.

Materials and Methods

The farmers' field experiments consisted of 3 activities were as follows:

A survey on socio-economic factors of farmers

A survey on socio-economic factors of 162 maize grower in dry season were done at Wat Bot district, Phitsanulok province in 2002/2003. A questionnaire and interview was used as a tool for the study.

Study on the productivity of commercial maize hybrids grown after rice

A series of field studies composed of the researches on yield trials, crop management and on-farm trials were conducted for three years (2003– 2005) on paddy soil at the farmers' field of Wat Bot district, Phitsanulok, Thailand

(16° 97' N latitude, 100° 32 E' longitude) during dry season (December to April). The soil textures; namely loam at 0.20 m depth had a pH of about 4.52, organic matter about 1.1 %, available P about 10 mg/kg and Exch. K about 84 mg/kg.

The first experiment as a yield trial was conducted at farmers' fields of 4 districts namely Wat Bot, Muang, Nakhon Thai and Noen Maprang in Phitsanulok province during the dry season of 2002 and 2005. A randomized complete block design with 4 replications was used. Fifty commercial maize hybrids consisted of 11 commercial maize hybrids from a private sector and 4 maize hybrids (one hybrid from Kasetsart University and three hybrids from the Department of Agriculture) from a public sector were evaluated.

An on-farm trial was conducted at a farmers' field Wat Bot district during the dry season of 2003/2004. Maize cv. BIG 949 was grown after rice under different nitrogen in terms of rate and timing of applications and plant density. A randomized complete block design with 4 replications was used.

The transfer of appropriate integrated technology to farmers

The transfer of appropriate integrated technology to farmers through the farmers' field school was done to learn. Farmers were trained to practice from step by step in a field test and in demonstration plots until they could do by themselves and could transfer this appropriate technology to their neighbors. Thirty farmers from 4 districts enrolled to the school where was located at a farm in Muang district, Phitsanulok province during dry season in 2004/2005. Farmers were trained and assigned the appropriate technology to 20 participants of 3 districts to do each field test. This technology was consisted of 4 steps: 1) At planting 2) At cultivating 3) At flowering and 4) At maturity. Finally, the selected farmers could gain experiences which could transfer the appropriate technology to others. Finally, farmers were joined who attended field days, field trip and farm visit held in four districts at Phitsanulok.

Results and Discussion

A survey on socio-economic factors of farmers

A survey on socio-economic factors of farmers found that maize growers were mainly male with a range of age 41-60 years and finished primary education. Main labor was households. They have experience of growing maize for at least 3-5 years. Their major source of fund was the Bank of Agriculture and Cooperatives. Maize growers mainly used lowland areas with sandy loam texture under moderate soil fertility. High yielding single cross maize hybrids from private seed companies were planted in December. The land was plowed and harrowed. They used a two-row planter and used seed at the rate of 18 - 21 kg/ha without thinning. All maize growers applied chemical fertilizers twice during the growing season i.e. basal fertilizer (16-20-0) and top dressing with urea. They irrigated the crop using natural water source through furrow irrigation method about three times during the growing season. Weed, insect and disease were not controlled. Ears were harvested when they turned to light yellow color and sold them to local merchants. Problem found from this study was mainly high production cost particularly fertilizer cost. Ekasingh et al. (2001) also reported that more than 80 % of the costs in maize production was variable costs, 50 % being labor costs and 30 % was material costs. In terms of material costs, the largest item was fertilizer cost and seed costs.

Study the productivity of maize hybrids grown after rice

Series of field studies were conducted to study the productivity of maize hybrids grown after rice under the conventional tillage. It was found that the suitable commercial maize hybrids were DK979, PIO.30Y87, BIG949, NK48, DK959, NK31, KSX4452, CP989, PIO30D55, PIO30N11, CP9774, PAC984 and NS2 (Table 1). Boonpradub

Table 1. Mean grain yield (t/ha) of commercial maize hybrids grown after rice at various farmers' fields of Phitsanulok province during dry season of 2003-2005.

Entry no.	Entry name	Grain yield (t/ha)	Relative to check (%)
1	DK979	8.07	137
2	PIONEER30Y87	8.05	136
3	DK949	8.00	135
4	NK48	7.96	135
5	DK959	7.79	132
6	NK31	7.67	130
7	KSX4452	7.60	129
8	CP989	7.48	127
9	PIONEER30D55	7.45	126
10	PIONEER30N11	7.41	125
11	CP9774	7.38	125
12	PACIFIC984	7.36	124
13	NS2	7.14	121
14	NSX012002	6.73	114
15	NSX982013 (check)	5.91	100
	MEAN	7.47	
	LSD (0.05)	1.01	
	CV.(%)	6.3	

Table 2. Agronomic characteristics of maize hybrid cv. BIG 949 grown after rice under different nitrogen and plant density at Wat Bot district of Phitsanulok in 2003/2004.

Treatment	Grain yield (t/ha)	Grain number per ear	100 grain weight (g)	Plant Height (cm)	Ear Height (cm)
T1**	5.06 d*	538 cd	20.6 d	158 e	77 d
T2	7.91 a	644 a	26.1 ab	184 bc	90 bc
T3	6.99 bc	598 b	25.1 bc	205 a	113 a
T4	7.69 ab	621 ab	25.9 ab	194 b	99 b
T5	4.74 d	513 d	19.9 d	145 e	67 e
T6	6.79 c	560 c	24.1 c	171 d	80 d
T7	7.31 abc	651 a	26.9 a	184 bc	90 bc
T8	6.75 c	639 a	27.2 a	183 c	88 c
F-test	**	**	**	**	**
CV(%)	8.2	4.0	4.6	4.1	6.3

* Values in a column followed by the same letter are not significantly different by using DMRT at $p < 0.05$.
** T1 = plant density at 76,925 plants / ha (65 x 20 cm. 1 plant/hill) + 125 kg.N/ha applied at sowing,1 and 2 months after sowing
T2 = plant density at 71,425 plants / ha (70 x 20 cm. 1 plant/hill) + 125 kg.N/ha applied at sowing, 1 and 2 months after sowing
T3 = plant density at 76,925 plants / ha (65 x 20 cm. 1 plant/hill) + 188 kg.N/ha applied at sowing, 1 and 2 months after sowing
T4 = plant density at 71,425 plants / ha (70 x 20 cm. 1 plant/hill) + 188 kg.N/ha applied at sowing, 1 and 2 months after sowing
T5 = plant density at 76,925 plants / ha (65 x 20 cm. 1 plant/hill) + 125 kg.N/ha applied at sowing and 1 months after sowing
T6 = plant density at 71,425 plants / ha (70 x 20 cm. 1 plant/hill) + 125 kg.N/ha applied at sowing and 1 months after sowing
T7 = plant density at 76,925 plants / ha (65 x 20 cm. 1 plant/hill) + 188 kg.N/ha applied at sowing and 1 months after sowing
T8 = plant density at 71,425 plants / ha (70 x 20 cm. 1 plant/hill) + 188 kg.N/ha applied at sowing and 1 months after sowing

and Kraokaw (2002) also evaluated the twenty-one maize hybrids in paddy field. They found that Pioneer 3012, Cargill 727 and Pacific 700 provided an average of 15 to 26 % increase in kernel yields over NS 72 (check).

Maize seeds should be planted at 0.70 x 0.20 m spacing with 1 plant/hill (71,425 plants/ha) and applied N at the rate of 125 kg./ha about 3 times namely at sowing, 1 and 2 months after sowing (Table 2). Boonpradub et al. (1998) had been studied on maize grown after rice under different plant densities found that mean yield increased consistently with increase in plant density from 53,333 plants ha^{-1} to 80,000 plants ha^{-1} Using urea as a N source at the rate of 125 kg N /ha was recommended to be applied three times i.e. 40% of dosage at planting, 35% at 3-4 weeks after planting (WAP) and the remaining (25%) at and 7-8 WAP to improve maize productivity Boonpradub and Kraokaw (2002) found that the kernel yield of maize with 187.5 kg N ha^{-1} application was not significantly superior to that of 125 kg N ha^{-1} .

The integrated technology for improving yield should be done according to the following steps: A uniform area should be selected, with well-drained soils such as loam, sandy loam or clay loam. The best time for planting is November. After harvesting rice, the land was plowed, harrowed and a fertilized with 15-15-15 or 16-16-8 at the rate of 312 kg/ha. The Hybrid seeds namely NK 48 should be sown with a two-row planter at a spacing of 0.70x0.20 m spacing with 1 pl/hill (71,425 pl/ha) under residual soil moisture. Urea (46-0-0) at the rate of 156 kg/ha was recommended to be applied twice, a 94 kg/ha at three to four weeks after sowing and the soil was hilling up, the remaining (62 kg/ha) was applied again at flowering. The maize crop must be given its first irrigation water immediately after the first top-dressing. Furrow irrigation was needed two to three times during the growing season. Boonpradub and Kraokaw (2002) also recommended practices maize grown after rice as the following steps: The best planting time for maize after rice was from November to December. After harvesting rice the land was plowed, harrowed and fertilized with 50-62.5-0 kg of $N\text{-}P_2O_5\text{-}K_2O$ ha^{-1}. Hybrid seeds mainly from single cross hybrids were planted with 0.75 x 0.20 m spacing with 1 plant hill^{-1} (66,666 plants ha^{-1}). Three weeks after planting, urea (46-0-0) at the rate of 156 kg ha^{-1} was applied as a top-dressing fertilizer. To increase maize production in the paddy field, it was necessary to provide adequate water as applied by furrow-irrigation for its growth.

The transfer of appropriate integrated technology to farmers

The integrated technology provided objective recommendations and methods use to improve the yields, grain quality and profitability of maize growing. This technology increasing a yield cover ten steps: 1) site selection in paddy fields 3-6 months before planting. 2) selection of farmer participants at 1-3 months before planting 3) preparation of variety and production inputs at 3-4 weeks before planting 4) land preparation at 1-2 weeks before planting. 5) management at planting time 6) management of seedling stage 1-2 weeks after planting (WAP). 7) management of vegetative stage at 3-6 WAP. 8) management of flowering stage at 7-8 WAP. 9) management of grain filling stage at 9-14 WAP. 10) management of maturity at 15-16 WAP.

This technology had been then transferred to farmers by ways of farmer participatory research at various farmers' fields of Phitsanulok province. Through this way, the farmers could produce an average grain yield of 80 % over the other farmers' yield (5.0 t/ha) (Table 3). 1,500 farmers were joined who attended field trip and farm visit held in four districts at Phitsanulok.

Table 3. Mean grain yield at 15% MC of maize grown after rice at farmer fields of various districts in Phitsanulok province during 2004/2005.

District (Sites)	Mean grain yield (t/ha)
Wat Bot (5)*	8.3
Muang (5)	10.6
Nakorn Thai (10)	8.2
Mean	9.0

* No. of farmer plots

Conclusions

In conclusion, the integrated crop management technology for maize after the second rice is further developed by the project, which is composed of the research on yield trials, crop management and on-farm trials. The integrated technology for improving yield should be done according to the following steps: A uniform area should be selected, with well-drained soils such as loam, sandy loam or clay loam. The best time for planting is November. After harvesting rice, the land was plowed, harrowed and a fertilized with 15-15-15 or 16-16-8 at the rate of 312 kg/ha. The Hybrid seeds namely NK 48 should be sown with a two-row planter at a spacing of 0.70x0.20 m spacing with 1 pl/hill (71,425 pl/ha) under residual soil moisture. Urea (46-0-0) at the rate of 156 kg/ha was recommended to be applied twice, a 94 kg/ha at three to four weeks after sowing and the soil was hilling up, the remaining (62 kg/ha) was applied again at flowering. The maize crop must be given its first irrigation water immediately after the first top-dressing. Furrow irrigation was needed two to three times during the growing season.

This integrated technology increasing a yield cover ten steps had been transferred to farmers by ways of farmer participatory research at various farmers' fields of Phitsanulok province. Application of the technology recommended, the participated farmers could produce an average grain yield of 9.0 t/ha or 80 % over the other farmers' yield (5 t/ha).

Acknowledgements

The authors express their sincere thank and appreciation to the Thailand Research Fund (TRF) for their financial support of this project.

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Farm-level assessment of the corn postproduction systems in the Philippines

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Abstract. In the Philippines, corn ranks second to rice in importance. About 600,000 farming households rely on corn as a major source of livelihood and these farmers are among the poorest of the group involved in the agricultural sector. Thus, every opportunity to augment corn farming income is extremely important. The study assessed the technical and financial performance of the different corn postproduction systems and identified the problems and constraints of the farmers with the end results of formulating policies and research agenda to improve efficiency. The study mainly used survey involving 300 farmers from the three highest corn-producing provinces of the Philippines. The post production system adopted by the majority of the corn farmers is the longest system that is composed of manual harvesting, piling, shelling, in-field hauling, sun drying and marketing sun-dried shelled grains. It has the highest in terms of quantity and quality losses. However, this system provides the farmers with greater opportunities to increase income by doing value-adding on-farm activities. The shortest postproduction system which involves harvesting and selling husked ear corn had the least quantity loss. However, buying ear corn is practiced only in limited corn-producing provinces. The study also identified the postproduction problems and the implications if problems remained unresolved. Moreover, specific recommendations were forwarded.

Keywords: Postproduction systems, yellow corn, quantity losses, aflatoxin, sun drying, shelling

Introduction

There is a continuing rise in the demand for corn both as food and animal feed in the Philippines. Unfortunately, this demand for corn has not been sufficiently supplied by the local production, both in terms of quantity and quality of corn grains. Due to the rainfed conditions of most of the corn growing areas, the production of corn is extremely vulnerable to severe fluctuations in production levels. Under these conditions, the application of appropriate postharvest technologies to preserve the volume that has been produced has become very important to insure the sustainability of operations of the industries dependent on corn as a raw material.

Maranan, Paz and Rapusas (1996) reported that the corn postharvest loss from harvesting to storage range from 3.7 to 25 percent (average of 12.7 percent), with drying operations (4.6 percent) contributing to the bulk of the total quantity loss. Quality loss due to deterioration in the absence of mechanical dryers has not been accounted in this loss figure which is presumed to be substantial especially in areas where sun drying cannot be done effectively.

The project identified and assessed the technical and financial performances of the major postproduction systems and evaluated the problems and constraints encountered by the corn farmers in their postharvest operations.

Methodology

The study employed a cross-sectional survey following a four-stage purposive sampling involving 300 farmer-respondents. The study also used secondary data from government and non-government organizations involved in the production and postproduction operations of corn as well as key informant interviews of officers and/or representatives of concerned units and organizations.

The top three yellow corn-producing regions in terms of the total quantity of corn harvested, were identified using a six-year data (2001 to 2006). From each of these top producing regions, the highest corn-producing province was selected and the five highest corn-producing municipalities were purposively selected from each province. The sampling area was narrowed down to the four highest corn-producing barangays in each municipality, two barangays represented interior areas while the other two represented exterior areas. Five farmer-respondents were randomly selected from each of the barangays. Exterior barangays were those located near road networks while interior barangays were generally located in far-flung areas with no established road networks.

Major post-production systems

More than one half of the respondents practice the longest postproduction sequence of harvesting, piling (either husked or unhusked), shelling, drying and selling dried shelled grains which extends up to eight to nine days while almost one third adopt a shorter postproductin sequence of harvesting, shelling, drying and selling dried shelled grains that follow a shorter period of four to five days. The shortest postproduction system of harvesting and selling husked ear corn is adopted by less than ten percent of the respondents. This shortest postproduction system requires only one to two days to finish if hauling facilities are readily available.

Generally, piling is done by the farmers to accumulate sufficient volume of corn in a location where mobile shellers could do the shelling operation or accumulate enough volume of ear corn to bring to the nearest stationary shellers. The length of piling is largely influenced by the availability of shelling machine (dehusker-sheller or sheller alone) servicing the farmers.

The results further showed the following: (a) hauling is done two to three times from harvesting to marketing; (b) farmers used two major types of shelling facilities, namely; shellers and dehusker-shellers; (c) almost all of the farmers carry out drying operations by sun drying using drying pavements or underlays; (d) temporary storing before marketing is practiced by very few farmers and (e) almost all of the farmers sell shelled corn while only a few have practiced selling wet ear corn.

Table 1. Comparison of quantity losses in corn passing through the three major postproduction systems, 2007.

Operations	Quantity loss, % of total harvest (dry-weight basis at 14% moisture content)[a]		
	Post-production system A (PPS A)	Post-production system B (PPS B)	Post-production system C (PPS C)
Harvesting	1.05	1.05	1.05
Piling	Trace	-	-
Shelling	0.52	0.52	-
Drying	4.54	4.54	-
Storage	0.51	0.51	-
Hauling/marketing	0.56	0.56	0.56
Total	7.18	7.18	1.61

[a] Salvador et al., 2007.

Technical and financial performance of postproduction systems

Quantity losses. As the longest with relatively more operations, the typical postproduction system (PPS A), had the highest quantitative loss (Table 1). PPS B which differed from PPS A only in terms of piling operation had the same level of quantitative loss of 7.18 percent of the total potential harvest, dry-weight basis. The shortest PPS of selling ear corn had the lowest quantity loss of approximately 1.61 percent of the total potential harvest. Under this PPS, the lesser operations undertaken by the farmers shielded the exposure of corn to quantity and quality loss-influencing factors. On the part of the farmers, the sources of losses are confined to harvesting and hauling operations only. After harvesting, ear corn is either directly hauled or piled in the field waiting for transport vehicle to haul the harvest directly to buyers of ear corn. Under this system, all other operations such as shelling, drying and storage are transferred to the trader-buyers.

Quality losses. Aflatoxin level (expressed in ppb) which was used as the measure of quality of corn was found to be influenced more by the weather condition during the postproduction operations. A postproduction system adopted during the relatively wet condition yielded corn with unacceptable level of aflatoxin because sundrying could not be done effectively (Table 2). A similar postproduction system adopted during the relatively drier harvest season yielded corn with acceptable aflatoxin level. Generally, there was an increasing trend of aflatoxin from harvesting to drying. It is in the drying operation that aflatoxin-producing microorganisms proliferate causing an increase in the aflatoxin level. These results affirm earlier findings that the optimum moisture content of grains where microorganism-producing aflatoxin proliferate is at 17 percent to 20 percent which is generally occurring during the drying operations. Hence, prompt drying operations is very important to maintain corn quality.

Table 2. Levels of aflatoxin at different postproduction operations under two postproduction systems, 2007.

Operations	PPS A/B*		PPS C **
	Dry months	Wet months	
Harvesting	Not detected	8.3	Not available
After shelling	2.1	25.2	-
After drying	4.0	82.4	5.07

* The data are averages of six farmers with two replications per farmer.
** Procurement data of trading center buying fresh ear corn; data was derived from 50 batches of procurement from March-August 2007.

Table 3. Comparison of postproduction systems showing the incremental value of the different operations and the resulting farmer's profit, CY 2006-2007 (1USD = PhP47)

Operation	Postproduction systems: PPS A and PPS B, Scenario 1	PPS A and PPS B, Scenario 2	PPS C, Scenario 1	PPS C, Scenario 2
	Unit price (PhP/kg dried shelled corn)	Unit price (PhP/kg dried shelled corn)	Unit price (PhP/kg fresh ear corn)	Unit price (PhP/kg fresh ear corn)
1. Production cost	4.71	4.71	2.51	2.51
a. interest of loan	0.05	0.74	0.03	0.39
b. total value	4.76	5.45	2.54	2.90
2. Postproduction cost				
a. harvesting	0.61	0.61	0.33	0.33
b. shelling	0.31	0.31	-	-
c. hauling (1)	0.23	0.23	-	-
d. drying	0.45	0.45	-	-
e. hauling (2)	0.23	0.23	0.25	0.25
f. total value	1.83	1.83	0.58	0.58
4. Buying price (first-level buyer)				
a. wet season	7.61	7.61	3.80	3.80
b. dry season	9.58	9.58	4.80	4.80
5. Farmer's profit				
a. wet season	1.02	0.35	0.68	0.32
b. dry season	2.99	2.30	1.68	1.32
6. Farmer's profit per hectare per cropping (all labor are hired)				
a. wet season	3,305	1,134	3,665	1,725
b. dry season	9,691	7,454	9,055	7,115
7. Farmer's profit per hectare per cropping (including value of labor provided by the farming household)				
a. wet season	4,256	2,085	3,665	1,725
b. dry season	10,642	8,405	9,055	7,115

Scenario 1: farmers have own money to finance production (0.5% interest rate/mo)
Scenario 2: farmers get loans from private individuals like traders (7% interest rate/mo)
Dry shelled corn/ha = 3,241kg (after deducting losses from harvesting to market + shrinkage due to drying)
Fresh ear corn/ha = 5,390 kg (after deducting losses in harvesting and hauling)

Financial performance. During the dry season harvest, farmers under the longer PPS (PPS A and PPS B) get higher income/ha-cropping than PPS C (Table 3). Although the profit from the longer PPS is still higher, the difference is minimal implying that farmers will have better income by selling freshly-harvested ear corn than selling dry shelled corn during the rainy season when sun drying can not be done effectively. This result can partly explain why the practice of selling freshly harvested ear corn has been earlier adopted in corn growing areas where harvest of corn falls during rainy season.

Similarly, self-financed farmers get higher income of PhP0.67 to PhP0.69/kg of dry shelled corn or PhP0.36/kg of fresh ear corn than those whose production inputs were taken from private individuals at a mean interest rate of 7%/month. This underscores the need for a sustainable credit support to improve corn profitability of almost one half of the small-scale corn farmers.

Financial performance of alternative drying practice. Selling dry shelled corn will give better financial benefits for the farmers than selling fresh ear corn if sun drying shelled corn can be done effectively in two to three days. In areas where there are buyers of ear corn and where sun drying is not possible within three days, farmers will be better off selling their harvest as fresh ear corn. On the other hand, selling mechanically-dried shelled corn will be advantageous, although with minimal profit, only if there is no market for both fresh ear corn and wet shelled corn and when sun drying can not be done in four days.

Problems of Corn Farmers

The most frequently-reported postharvest problem was still drying. Drying facilities were not sufficient especially during peak harvest periods and when harvest falls during rainy season when the demand for drying facilities is also at peak. If the problem of prompt drying remained unsolved, the quality of corn available in the market is far from being improved.

Next to drying, the most frequently-mentioned problem pertains to harvesting which include the lack of and inefficiency of laborers during peak harvest, bad weather conditions during harvest periods and pilferage. Moreover, sizeable number of farmers mentioned problems on shelling which were related on the limited number of units available to service the shelling requirements during the peak of harvest season as well as the inefficiency of old machines that were still used. The limited number of shellers is the major reason for the prolonged piling of corn in the field. The problem of limited labor during harvest and the limited shelling facilities will have direct influence on the resulting qualities of corn, which in turn, have an immediate effect on the profitability of farmers due to discounted price of poor quality corn.

Recommendations

To address the issue of inadequate dryers in areas where harvesting generally falls during the dry season, the following are recommended: (a) continue the provision of drying pavements close to the farmers' areas with the local government unit (LGU–barangay level) and/or farmers' groups as direct technology users and operators following cost sharing arrangement; (b) provision of loans through government-controlled lending institutions at minimal interest rate to individuals or group of adjacent farmers to construct pavements within their own farms to serve as postharvest assembly areas for dehusking, temporary piling, shelling and drying area, and; (c) where it is possible, the government to provide cemented interior roads among clustered farms which would not only facilitate mechanization and transport operations but at the same time serve as drying pavements during harvest season.

The development of appropriate mechanical dryers (suitable for ear corn and shelled corn) using biomass fuels to reduce drying cost should also be prioritized in the light of high fossil fuel cost especially in areas where harvesting season generally falls during the rainy season. The establishment of farmer-based community drying centers, with farmers as technology users and operators, should also be prioritized by concerned LGU following a public investment mode on the basis that operating drying facilities is not a financially viable enterprise for farmer-adopters and in recognition that under the present PPS the most practical way of controlling aflatoxin is at the farm level.

Facility loans should be provided to individual or group of farmers for the acquisition of shellers to augment the number of shelling machines available in the area especially during the peak of harvest season. Custom shelling is a profitable agribusiness enterprise with investment capital recoverable in almost two cropping seasons of corn. Government extension workers can work out a financing program with machine manufacturers or rural banks for the acquisition of machine by individual farmer or group of farmers on installment basis. With enough shellers in the community, the practice of long-duration piling could be avoided which in turn could reduce quality losses due to prolonged piling.

Strengthening viable cooperatives to serve as core credit and marketing conduits of farmers in a given production area will reduce the high cost of production due to high interest rates imposed by informal sources of credit and at the same time provide ready and higher markets for farmers' produce. The LGU can collaboratively work with viable cooperatives in their area to set up a marketing assistance program that will make use of the LGU funds as collateral or guarantee fund for lending institutions to allow cooperatives to acquire higher loans for production and trading operations.

Support studies on the factors that significantly influence the development of aflatoxin such as the effects of piling practices (piling of husked versus unhusked ear corn and bulk piling versus piling in bags), use of dehusker-sheller versus sheller, among others, should also be undertaken. Pilot testing study of a two-stage drying system appropriate to groups of small farmers should also be considered to further improve the quality of corn produced by the farmers especially in relatively wetter areas.

Acknowledgments: The authors would like to thank Ms. Amelita R. Salvador, senior author of the corn loss assessment project, for allowing the use of their quantity and quality data on corn. The data were generated from the same area covered by the project during the same year.

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Soil moisture conservation for improving maize yields through participatory micro-watershed approach in foothills of Shivaliks

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Abstract. The Shivalik ranges are a part of the Himalayan mountain chain that have been neglected for a long time and are now under a constant threat of erosion and floods. It covers over 20,000 km^2 area in the states of Himachal Pradesh, Punjab, Haryana and Jammu & Kashmir. Nearly 70 m ha of Indian land, mainly in the Himalayan slopes are critically degraded and need immediate attention. The prime cause for the backwardness of the Shivalik foothill region is reduction in soil fertility and productivity, due to massive soil erosion. Crop yields in the foothills are quite low and the situation warrants an efficient management of land and rainwater for conserving the *in-situ*. It was observed from studies that four ploughings are necessary for wheat to get optimum significant yield per unit of land. An increase in soil moisture content from 6.7 % in absence of ploughings to 19.4 % with four ploughings has been observed. On-farm experiments with maize in lower Shivaliks, showed that soil moisture storage increased to the tune of 9.3, 12.7 and 16.8 per cent while an increase of 18.1, 33.1 and 49.9 per cent in plant height was observed with shallow tillage, deep tillage and ridge and furrow treatments respectively over the control. Mulching in maize was also quite effective in conserving soil moisture and improving yields. There was 10.6 per cent increase in maize grain yield in ridge and furrow sowing of maize over control.

Keywords : moisture conservation, soil, land, maize, nutrient, foothill, rainfed

Introduction

The foothills of Shivaliks or submontaneous tract of N-W India is about 300 km long and 30 km wide, also known as *Kandi* area which more or less meets the boundary of the Shivalik range. The major part of the tract is rainfed because of more annual evapotranspiration compared to annual rainfall. Since in the area there is decline in land holdings, poor economic status of the farmers and the challenge lies in improving or raising the productivity of land in rainfed submontane region of not only in the state but country as well. One of the most important challenging tasks lies in in-situ moisture conservation especially in winter season due to occurrence of droughts in the area (Arora, 2006). However, most of the rainstorms received in summer (kharif) season are of short duration and high intensity whereas those received in winter (rabi) season are of low intensity and erratic in distribution in the area. All this results in serious problem of soil erosion through rainfall excess in summer monsoon months and soil moisture deficit in the winter months in the region. Therefore, the situation warrants an efficient management of both land and rainwater in the area for conserving the in-situ moisture and utilization as per the needs and requirements. The water table is deep to very deep and rainfall is the only scarce source of water in the area. The lack of irrigation facilities and large scale erosion put main limitations to the agricultural economy resulting in poor socio-economic status of the farmers of the area. The rainfed areas are mostly underfed from the point of view of application of inputs compared to outputs obtained (Hadda et al, 2000) However, the challenge of improving productivity in rainfed areas can be addressed by efficient utilization of nutrients and natural resources. One of the ways is to use the nutrients in an integrated manner from all possible resources and maximising the utilization of applied nutrients by crops (Acharya and Bandopadhyay, 2002). Thus, there is need to enhance crop yields through in-situ moisture conservation coupled with land, soil and nutrient management practices in the area.

Materials and Methods

In undulating agricultural sub-catchments in foothills of Punjab at village Kokowal-Majari, District Hoshiarpur (experiment I), group of 15 farmers was selected each representing a single unit of land in the village. Maize variety 'Prakash' was sown. Four treatments consisted of in-situ soil moisture conservation through land and soil management practices were adopted. These included shallow tillage, deep tillage and raised bed sowing which were compared with farmers practice. The farmers of the area generally follow the practice of sowing conventional (local) crop varieties without following any land management and moisture conservation practices. Mostly they cultivate the fields along the slope with application of only one-third of the recommended dose of nitrogenous straight fertilizers.

In another experiment at Ballowal saunkhari village of Nawanshahr district (experiment II), the treatments included five modes of straw mulch applications i.e. unmulched (control; M_o), fully covered plots (M_f), partial covered (lower 1/3rd covered; M_p) plots, Strip (M_s) and vertical (M_v) mulching. In the strip application, rice straw mulch was applied in 6" x 2" strips constructed in alternative rows and in vertical mulching rice straw applied in the holes (10 cm diameter and 15 cm deep) made with an auger. Thus there were 6 strips and 17 vertical holes per plot in the strip and vertical mulch treatments, respectively. Maize (cv. Parkash) was planted with 45 cm row-to-row and 22.5 cm plant-to-plant spacing. Rainfall was received in 31 rainstorms out of which 12 were erosive (produced runoff). A total of 547 mm rain was received during the monsoon season that was much below the normal rainfall of the area.

In foothills of Jammu (experiment III), the imposed treatments in maize included T_1= Farmers practice, T_2= recommended fertilizer+contour cultivation and sowing improved variety (GS-2) in lines across the slope, T_3= T_2+25% N replacement through farm yard manure, T_4= T_3 + addition of Zinc @ 25 kg $ZnSO_4$/ha and atrazine as pre emergence weedicide. The farmers practice included application of FYM (4 tonnes/ha) before sowing and application of only Urea (@ 40 kg/ha) as top dressing one month after sowing. Local variety of maize is sown through broadcasting.

The treatments were replicated thrice in Randomized Block Design (RBD). The periodic soil moisture samples were collected upto 120 cm depth before sowing and at different days after sowing (DAS). The crop was harvested at maturity from each treated plot and the grains were separated and yields were recorded.

Results and Discussion

Tillage & mulching practices in maize

Soil moisture storage, 40 days after sowing (DAS), increased by 11.7, 29.8 and 45.3 per cent with shallow tillage, deep tillage and raised bed sowing treatments respectively over the farmers practice. Although, it was observed that moisture storage was more in sub-surface layers at 40 DAS in comparison to surface (0-15 cm) layers in all the treatments (Table 1). Maximum amount of moisture upto lower depths (60-90 cm) was observed in raised bunds sowing treatment. This may be due to more absorption and less evaporation of moisture in the treatment. At 80 DAS, soil moisture storage increased to the tune of 9.3, 12.7 and 16.8 per cent with shallow tillage, deep tillage and raised bed treatments respectively over the farmers practice (Table 1). The more increase in soil moisture content may be because of better soil management practices and raised bed sowing of crop.

There was 10.6 per cent increase in maize grain yield in raised bed sowing treatment compared to farmers practice. However, in other treatments the grain yield was higher over control but the effect was non-significant. Increase in maize grain yield with moisture conservation practices have also been observed by Gaur (2002) in a watershed of Rajasthan state.

Mulch material was found to be quite effective in conserving soil moisture. However, it's role differed to a significant extent with different mode of mulch application. Observations of periodic soil moisture content of surface and sub-surface soil reveal from experiment II that all the modes of mulch application were more effective in conserving moisture at all the crop growth stages. As compared to control, at 40 DAS, soil moisture was higher to the magnitude of 3.80 and 1.80 % in surface and 4.20 and 2.30 % in sub-surface respectively in whole covered and partial covered plots by straw mulch (Table 2). The other two modes of mulch application also helped in conserving the soil moisture but the amount of water conserved was relatively small as compared to that under fully covered treatment. Similarly, at 60 DAS, gravimetric soil moisture was higher to the tune of 4.85, 2.30 and 1.05 % in 0-15 cm and 4.25, 2.75 and 1.80 % in 15-30 cm soil layers in M_f, M_p and M_s modes of mulch application over M_o (Table 2). At all the stages of crop growth, moisture storage was greater in sub-surface layers than surface soil layer. The greater

Table 1. Effect of different treatments on soil moisture storage in 90 cm depth and maize yield

Treatments	Moisture storage at 30 DAS (cm)	Moisture storage at 60 DAS (cm)	Grain yield (kg ha^{-1})
Farmers practice	10.0	21.2	2270.5
Shallow tillage	11.2	23.2	2344.4
Deep tillage	13.0	23.9	2435.6
Raised bed	14.5	24.7	2470.2
RDF (100%)	10.8	20.5	2525.2
RDF (75 %) + FYM	13.5	24.0	2568.5

Table 2. Effect of modes of mulch application on soil moisture storage

Mode of Mulch Application	Soil depth (cm)					
	0-15 cm			15-30 cm		
	40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Unmulched (M_o)	7.85	7.95	3.45	7.80	9.10	3.85
Vertical (M_v)	8.20	8.50	4.10	8.50	10.00	4.72
Strip (M_s)	8.75	9.00	5.00	9.35	10.90	5.40
Partial cover (M_p)	9.65	10.25	6.00	10.10	11.85	6.40
Whole cover (M_f)	11.65	12.80	8.20	12.00	13.35	10.15
LSD (*P*=0.05)	1.79	1.17	0.74	1.58	1.27	1.75

DAS : days after sowing

Table 3. Effect of modes of mulch application on grain and straw yield of maize

Mode of Mulch Application	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Unmulched (M_o)	2466	4074
Vertical (M_v)	2502	4352
Strip (M_s)	3163	5105
Partial cover (M_p)	3206	5353
Whole cover (M_f)	3912	5502
LSD (P=0.05)	104	256

the surface area covered by mulch material in a particular mode, the greater is its effectiveness in conserving soil moisture. Thus among different modes of mulch application the area covered varied as 98 % in fully covered followed by 33% in partially covered, 18% in strip mulching, 1.8 % in vertical mulched plots and 0 % in case of unmulched control.

It was also observed that fully covered plots had 156 % higher dry matter yield of maize as compared to the unmulched plots. Dry matter yield under different modes of straw mulch application viz. M_p, M_s and M_v was observed to be 99, 53 and 8 per cent higher than the unmulched control plots (M_o). The average dry matter yield of maize in the mulched plots were significantly higher than the unmulched plots which is because of more favourable influence of mulching on the soil (Weeraratna and Asghur, 1990; Gajera et al, 1998). The grain yield of maize was significantly higher in whole covered, partial covered and strip mulching plots as compared to the control plots. Mulch spread on the whole plot increased the grain yield by 58.6 % as compared to unmulched control (Table 3). In vertical mulched and control plots (unmulched) grain yield of maize was observed to be almost similar.

The mode of straw mulch application significantly affected the straw yield of maize. It was observed that straw yield in whole covered, partial covered and strip application plots was significantly higher by 35.05, 31.39 and 25.3 per cent respectively than the unmulched i.e. controlled plots (Table 3). The vertical mulching though had higher yield than the control plots but it could not reach the level of significance. Similar increase in maize fodder yields with mulch application in rainfed area of Punjab was also observed (Khera and Singh, 1998).

Contour cultivation in maize

Soil moisture storage at 45 days after sowing (DAS) increased by 15.0, 25.5 and 30.2 per cent with T_2, T_3 and T_4 treatments respectively over the farmers practice (control) during maize season. Although, it was observed that moisture storage was more in sub-surface layers at 45 DAS in comparison to surface (0-15 cm) layers in all the treatments (Table 4). This may be due to more absorption and less evaporation of moisture in the treatment. At 90 DAS, soil moisture storage increased to the tune of 20.3, 24.4 and 27.8 per cent with T_2, T_3 and T_4 treatments respectively over the farmers practice. The more increase in soil moisture content may be because of better land management in the form of contour cultivation across the slopes and FYM application.

There was 20.3, 35.6 and 49.4 per cent increase in maize grain yield in T_2, T_3 and T_4 treatment compared to farmers practice while the straw yield of maize was 18.0, 33.9 and 42.2 per cent higher in treatments T_2, T_3 and T_4, respectively over the farmers practice (Table 4). Increase in maize grain yield with improved soil moisture conditions and application of FYM have also been observed by Gaur (2002) in a watershed in Rajasthan and Arora and Hadda (2003) and Hadda et al (2005) in submontane region of Punjab. Also, application of weedicide has added effect on maize yields.

Table 4. Effect of contour cultivation on soil moisture storage and yield of Maize

Treatments	Soil moisture (cm/30 cm)		Grain yield ($kg\ ha^{-1}$)	Straw yield ($kg\ ha^{-1}$)
	45 DAS	90 DAS		
T_1 (Farmers practice)	17.4	21.2	1047	2125
T_2 (RDF+ contour cultivation and sowing improved variety in lines across the slope)	20.0	25.5	1260	2508
T_3 (T_2+25% N replacement through FYM)	21.1	26.4	1420	2845
T_4 (T_3+ addition of Zinc @ 25 kg $ZnSO_4$/ha and atrazine as pre emergence weedicide)	21.6	27.1	1564	3022
LSD (P=0.05)	1.20	2.40	114	282

DAS = days after sowing

The conducted experiment showed that adoption of improved moisture conservation practices through land and soil management helped in improving the crop yields of the rainfed areas although gradually but in a sustainable way.

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Effect of seed priming and its duration on seedling vigour and productivity of rainfed maize (*Zea mays* L.)

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Abstract. The experiment was conducted at Rajasthan college of Agriculture Udaipur, during rainy seasons of 2003 and 2004 in order to evaluate seedling vigour and growth of rain fed maize in response to select agrochemicals and to quantify the effect of seed priming duration. The experiment consisted of seed priming with six agrochemicals (100 ppm cycocel, 0.1 % succinic acid, 0.1 % thiourea, 1 ppm cytokinin, 2.5 % NaCl and 2.5 % KH_2PO_4) + water soaking and dry seed sowing and two soaking durations (6 and 12 hours). The experiment was laid out in randomized block design with four replication. Six hours seed priming with 0.1 per cent thiourea positively improved plumule and radical length along with fresh and dry weight of seedling over water soaks. It also improved plant height, dry matter /plant, LAI, grains/cob, grain weight/plant and shelling percentage consequently grain and stover yield over water soaking. The highest net returns and BC ratio were realized when seeds were primed with thiourea. Thus it is recommended that before sowing, six hrs seed priming with 0.1 per cent thiourea improved seedling vigour, mitigate early moisture stress and enhanced productivity of rain fed maize.

Key words: Seed priming, Agrochemicals, Priming duration, seedling vigour and yield

Introduction

Amongst Indian states, Rajasthan ranks first in respect to maize area. Here the crop is cultivated in about1.0m ha area of which 80 to 85 % is dependent on monsoon rains. Maize cultivation in the region represents a typical case of subsistence farming and crop failure leads to a felt in the very survival of the people by and large The long term rainfall data (Fig.1) indicate less than critical rainfall (above 30 mm per week) probability during seedling establishment (Solanki, 2006). Thus the farmers have a general fear of moisture stress at this stage. After the root appears and cells become vacuolated, the tissue is usually much more susceptible to injury from dehydration which results in poor crop stand and consequently low maize yield. Besides conventional approaches, use of agrochemicals through seed priming has opened new avenues for mitigating moisture stress at seedling stage. Keeping this in view, a study to evaluate seedling vigour and growth of rain fed maize in response to select agrochemicals and to quantify the effect of seed priming duration, was under taken.

Material and methods

A field experiment was conducted during rainy seasons of 2003 and 2004 under rain fed condition at Instructional farm Rajasthan College of Agriculture, Udaipur which represents a typical sub-tropical climate. It is situated at an altitude of 582.17 m above mean sea level. The soil was clay loam, alkaline (pH 8.5) in reaction, medium in available nitrogen (258.4 kg/ha), available phosphorus (10.5 kg/ha) and available potassium (285.3kg/ha).The experiment consisted of seed priming with six agrochemicals (100 ppm cycocel, 0.1 % succinic acid, 0.1 % thiourea, 1 ppm cytokinin, 2.5 % NaCl and 2.5 % KH_2PO_4) + water soaking and dry seed sowing and two soaking duration (6 and 12 hours). The experiment was laid out in randomized block design with four replications. As per treatment, each agrochemical was first dissolved in 100 liter water/ha. Seeds were primed in these solutions for desired period separately in plastic trays. There after seeds were allowed for surface

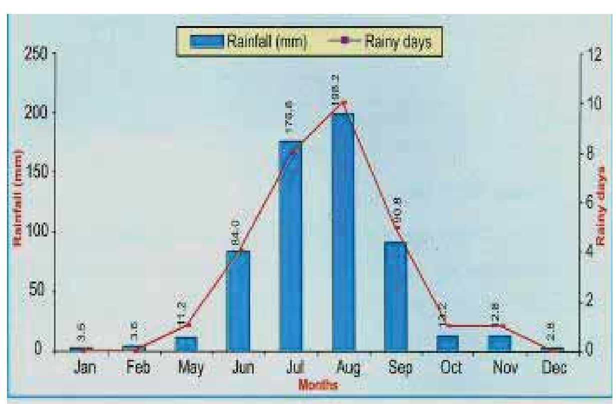

Fig1. Mean monthly rainfall and rainy days at Udaipur.

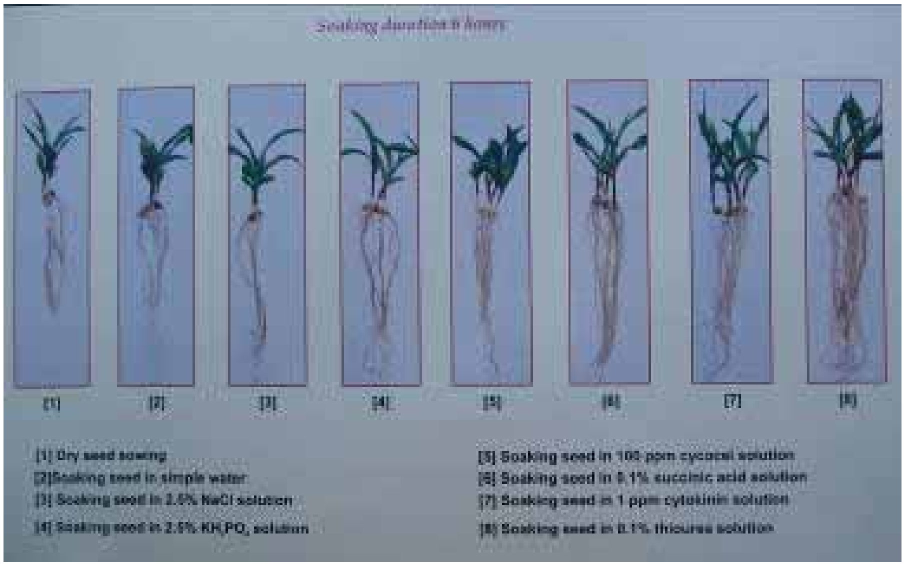

Fig.2. Effect of seed priming and its duration on seedling vigour

dry in shade and sown on the same day. As per recommendation a uniform dose 90 kg N and 40 kg P_2O_5/ha were applied to the crop. Full dose of P_2O_5 and 1/3rd dose of N were applied at the time of sowing through DAO and urea. The remaining N was applied in two equal splits at knee high and tassel initiation stage. The maize variety PHEM-2 was used as test crop. In order to control weeds, pre-emergence application of atrazine at 0.5 kg/ha followed by one hoeing and weeding at 25 days after sowing were carried out. The crop was sown in first fort night of July at 60 cm x 25 cm geometry and harvested in month October during both the years. The seedling stage encountered moisture stress during both the years due to less rain fall (12.5 and 24.5 mm per week) in month of June, however, received 445 and 556 mm total rain fall from June to October (Solanki, 2006). For study of seedling vigour, the experiment was conducted simultaneously in perforated poly bags and laid out in CRD. The poly bags were filled with sand, which was pass through 80 mm sieve. The sand was first wash with tap water and finally through distilled water. The bags were kept in field under natural condition in trench of 15 cm depth and at the time of sowing counted seeds were planted in these poly bags. Seedling from these poly bags were used for study of seedling vigour.

Result and Discussion

Control v/s rest

Seed priming with different agrochemicals positively enhanced germination percentage, plumule length, radical length, fresh weight of seedling at 15 days after sowing as well as dry matter, LAI, shelling per cent and P content of grain and stover of maize crop, however, failed to record perceptible variation in dry weight of seedling, yield attributes, yields, N content of grain and stover and economics.

Agrochemicals

Amongst agrochemicals, seed priming with thiourea bring about remarkable improvement in values of different parameter of seedling vigour, growth, yield attributes, grain yield and N and P content of grain and stover over water soaking and other agrochemicals. However, stover yield was highest under cytokinin followed by thiourea. Sahu and Singh (1995) have explained the role of thiourea in terms of –SH group which have diverse biological activities responsible for promotion of cell division in seedlings. It improves sucrose loading to phloem, there by translocation of photosynthates and thus improved dry weight and length of plumule and radical. At later stages its role in dry matter partitioning suggests better functional activities of each growth and reproductive structure and consequently improved grain yield. Despite higher grain yield under thiourea, the stover yield was low compared to cytokinin suggest that dry matter at harvest was efficiently allocated to cobs. This proves the profound role of sulphydryl compound in better partitioning of food material from source to sink. The results also corroborates the findings of Parihar *et al.* (1997). Further, the better development of root and its prolification suggested higher extraction of nutrient from soil profile and thus increased nutrient availability in grain and stover (Sahu and Singh, 1995).

The next best agrochemical in order of superiority was cytokinin (Table 1 and 2). It act as bioregulator and plays and important role in cell division and elongation processes in plant (Miller *et al.,* 1956). It appears that improvement in weight of seedling and length of radical and plumule under present investigation could partly be due to the beneficial effect of bioregulatory role in promoting cell division and elongation processes. This might have resulted in formation of higher leaves (LAI) their expansion and retention for longer period which lead to efficient photosynthetic activities and greater accumulation of photosynthates in plant and thus improved growth, yield attributes and yield. The result is in close agreement with findings of Jat, (2002).

The results also showed that seed priming with succinic acid tended to improve seedling vigour, growth, yield attributes, yields and N and P content of grain and stover compared to water soaking. Delvin and Watham (1986) opined that succinic acid may gets converted to succinic semi-aldehyde which in turn may be emanated by *y*-aminobutyric acid transminase to *y*- aminobutyric acid (GABA) which alternatively incorporates Mg to form chlorophyll. Thus both photosynthesis and respiration may be improved by succinic acid which plays an important role in stress mitigation and growth promotion.

Seed priming with cycocel failed to influence germination. Plumule and radical length were marginally reduced compared to water soaking. However, fresh and dry weight of seedling remained unaffected. Further, dry matter/plant, grains/cob, grain and stover yield improved significantly over water soaking treatment, however, most of yield attributes and growth parameters and nutrient content of seed and stover remained unaffected. It is well established fact that cycocel is an antigibberellin compound and when applied it produce endogenous gibberellic acid quantity. Therefore, the activities like apical growth, cell division and cell growth are inhibited resulting in reduced plumule and radical length. However, reduced apical growth increase thickness of cell wall which might have contributed to increase dry matter of seedling and crop consequently stover yield (Cathey, 1964).

Under present investigation NaCl and KH_2PO_4 failed to influence seedling vigour and other studied parameters compared to water soaking. During seed priming imbibitions of water is reduced mainly due to increase salinity under NaCl soaking. Ryan (1973) and Ramana and Ramdas (1978) reported that increase salinity delay in hydrolysis and mobilization of reserved proteins, and proteolysis is probably the primary but essential step towards synthesis of new proteins for seedling growth.

Table1. Effect of seed priming and its duration on seedling vigour, growth and yield attribute.

Treatment	Germination (%)	Plumule length	Radical length	Fresh weight (g/seedling)	Dry weight (g/seedling)	Dry matter (g/plant)	Plant height	LAI	Grains/ cob
Dry seed sowing	96.2	3.75	17.56	4.04	0.76	137.8	172	2.9	236
Rest	96.6	4.02	18.94	4.45	0.76	150.2	181.1	3.2	248
'F' test	*	*	*	*	NS	*	NS	*	NS
A. Seed Priming									
Soaking in water	97.4	3.84	18.47	4.24	0.76	142.3	175.9	3.04	238
NaCl	97.5	3.83	18.14	4.25	0.76	143.3	177.5	3.07	239
KH_2PO_4	97.8	3.84	18.28	4.32	0.76	145	176.4	3.13	241
Cycocel	97.2	3.8	18.18	4.33	0.77	157.3	173.6	3.14	241
Succinic acid	97.5	3.95	18.56	4.38	0.78	145.4	184.6	3.15	240
Thiourea	98.4	4.53	20.7	4.93	0.88	159.7	191.5	3.54	271
Cytokinin	97.7	4.32	20.32	4.71	0.8	158.5	188	3.38	266
CD (P=0.05)	NS	*	*	*	*	10.32	9.2	0.243	17.5
B. Soaking duration									
06 hours	97.7	4.02	18.91	4.44	0.79	150.2	179.6	3.2	248
12 hours	97.7	4.04	18.99	4.46	0.79	150.2	182.5	3.21	248
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at 5% level of significance

Table 2 Effect of seed priming and its duration on yield attribute, yield, nutrient contents and economics

Treatment	Grains/cob	Grain weight/plant	Shelling (%)	Stover yield (kg/ha)	Grain yield (Kg/ha)	N Contents		P Contents		Net returns	B:C ratio
						Grain	Stover	Grain	Stover	(Rs/ha)	
Dry seed sowing	49.5	54.5	64.2	5700	3653	1.690	0.650	0.400	0.130	16848	0.76
Rest	52.6	58.8	68.8	6078	3093	1.770	0.690	0.420	0.140	18317	0.76
'F' test	NS	NS	*	NS	NS	*	*	NS	NS	NS	NS
A. Seed Priming											
Soaking in water	47.5	53.3	65.1	5781	3707	1.701	0.660	0.400	0.136	16872	0.76
NaCl	51.6	56.7	67.8	5825	3733	1.704	0.670	0.400	0.139	16896	0.76
KH_2PO_4	50.8	56.6	67.8	5895	3753	1.706	0.660	0.410	0.137	16755	0.76
Cycocel	53.9	60.1	68.5	6393	4098	1.707	0.660	0.410	0.140	19521	0.77
Succinic acid	53	59.7	68.4	5910	3811	1.780	0.700	0.420	0.138	17352	0.78
Thiourea	59.9	64.2	73.5	6300	4346	1.951	0.770	0.760	0.149	20945	0.88
Cytokinin	54.1	60.8	70.4	6440	4129	1.852	0.720	0.440	0.146	19879	0.8
CD (P=0.05)	4.06	7.01	3.53	467	331	0.037	0.027	0.020	0.007	2077	*
B. Soaking duration											
06 hours	52.5	58.6	68.2	6019	3910	1.760	0.690	0.420	0.140	18091	0.79
12 hours	52.6	59	68.6	6136	3969	1.770	0.690	0.420	0.140	18543	0.79
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at 5% level of significance

Soaking duration:

Soaking duration failed to show any perceptible variation in all studied parameter of seedling vigour as well as maize crop.

Conclusion

It is recommended that before maize sowing, six hours seed priming with 0.1 per cent thiourea improves seedling vigour, mitigates early moisture stress and enhances productivity of rainfed maize.

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Performance of site specific nutrient management (SSNM) for maize on upland Lampung

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Abstract. Lampung is the third major maize producing province in Indonesia after East Java and Central Java. The average maize yield in Lampung is 3.4 t ha^{-1}, which is higher than the national average of 3.2 t ha^{-1}. Actual yields in farmers' fields range from 1.5 t ha^{-1} - 7.0 t ha^{-1}. To evaluate opportunities to increase the productivity of maize through site-specific nutrient management (SSNM), on-farm trials were conducted in five locations in Lampung i.e. four locations in Central Lampung District (Sidowaras, Binjai Ngagung, Watu Agung and Balai Rejo) and one location in South Lampung District (Trimulyo) during the 2004/2005, 2005/2006 and 2006/2007 rainy seasons. The experimental setup followed a standard protocol at all sites and included nutrient omission plots (PK, NK, NP) to estimate indigenous nutrient supplies, an NPK plot to measure yield response to fertilizer application and a farmers' fertilizer practice (FFP) plot in each farmer's field. An SSNM treatment plot was included in the second and third seasons. Each of the above treatments was paralleled by a plot with improved crop management practice (ICM) i.e. higher planting density, addition of lime and addition of magnesium. Results showed that yield response to fertilizer N, P and K application in these sites were: N = 2.3-4.1 t ha^{-1}; P = 0.6-2.0 t ha^{-1}; K = 0.3-2.4 t ha^{-1}. Attainable yield in the three seasons on average ranged from 7.6 t ha^{-1} to 10.6 t ha^{-1}. Yield in the SSNM treatment (with or without ICM) was significantly higher than the FFP indicating great opportunities for farmers to increase productivity and profitability with improved nutrient and crop management.

Key words: Nutrient management, fertilizer, agronomic efficiency, yield response, SSNM, Lampung

Introduction

Maize is an important economical crop in Indonesia and the acreage is currently about 3.5 million hectares that could produce 14 million tons of maize. The low yield attained (3.2 t ha^{-1}) is due to pest, diseases, weeds, low use of fertilizers, and low yielding varieties (Subandi *et al*, 1998).

Lampung is one of the major maize growing provinces in Indonesia that largely grow maize as commercial crop for feed. The potential area in this region is about 674,238 hectares, however, only 320,008–399,827 hectares are planted every year (Lampung Dalam Angka, 2006). Maize is planted in several dustricts (i.e. South Lampung, Central Lampung, East Lampung, North Lampung, Tulang Bawang, and Tanggamus), but the main maize production districts are East Lampung, South Lampung and Cetral Lampung which are situated from 60 m to 300 m above sea level. The soil type is mainly Ultisol with a slope of 8 – 15%. Maize is planted in irrigated lowland areas during the dry season after rice, in rainfed upland areas in the wet season, and in flood prone wetland areas. Majority of farmers grow hybrid corn varieties, open pollinated varieties (OPV), or seeds generated from hybrid varieties (F1 or F2). A few farmers use local varieties.

The average maize yield in Lampung is 3.4 t ha^{-1}, which is higher than the national average (3.2 t ha^{-1}). However, actual yields in farmers' fields range from 1.5 t/ha - 7.0 t/ha. Data collected from farmers in key maize production sites in Lampung showed that yield of maize is dependent on variety, season, soil and crop management implemented by farmers. Key production constraints are diseases (downy mildew, ear rot, sheath blight and rust), insect pests, weeds, low input fertilizer (potassium and phosphorus) and drought.

The general fertilizer recommendation for maize in Lampung is 300 kg urea, 100 -150 kg SP-36 and 100 kg KCl ha^{-1}, but most farmers apply urea and SP-36 without KCl while some farmers use urea and manure without SP-36 and KCl (Murni *et al* 2007). This general fertilizer recommendation and current farmers' fertilizer practice are not based on the nutrient requirements of the maize crop. The application of fertilizer does not meet the crop's need. Application of adequate quantities of nutrients is a key aspect in increasing maize productivity and production, but those nutrients should be balanced based on the plant requirement. According to Sawyer (*in* Ferguson *et al*, 2002), site-specific management of soil fertility inputs is an attractive and intuitive approach to increasing fertilizer use efficiency.

The concept for site-specific nutrient management (SSNM) was developed for irrigated rice in Asia (Dobermann *et al*., 2002; Witt *et al*., 2002) and is currently being disseminated in Indonesia (Samijan *et al*., 2003). SSNM has been suggested as a method to improve nutrient use efficiency by the plant. For example, Ferguson *et al* (2002) stated that site specific nitrogen management is a means of further increasing the efficiency in which N fertilizers are used and reducing environmental impact.

To evaluate opportunities to increase the productivity of maize through site-specific nutrient management (SSNM), on-farm experiments were conducted in the upland areas of Lampung during the rainy season in 2004/2005, 2005/2006, and 2006/2007.

Materials and Methods

On-farm experiments were conducted during the rainy seasons of 2004/2005, 2005/2006 and 2006/2007 in five locations i.e. four locations in Central Lampung namely Sidowaras, Binjai Ngagung, Watu Agung and Balai Rejo and one location in Trimulyo, South Lampung. Soils in Sidowaras, Binjai Ngagung and Trimulyo belong to the Kandiudult soil type while soils in Watu Agung and Balai Rejo belong to Kanhapludult soil. The experimental setup followed a standard protocol at all locations and included nutrient omission plots (PK, NK, NP) to estimate indigenous nutrient supplies, an NPK plot to measure yield response to fertilizer application, and a farmers' fertilizer practice (FFP) plot in each farmer's field. Preliminary estimates of yield responses and agronomic efficiencies from omission plots in the first season were used to calculate field-specific fertilizer recommendations for maize that were evaluated in an SSNM treatment plot in the following season. Each of the above treatments was paralleled by a plot with improved crop management practice (ICM), i.e. higher planting density, addition of lime and addition of magnesium. Farms are used as replicates. Individual plots sizes were 6 m x 6 m for treatments PK, PK+ICM, NK, NK+ICM, NP, NP+ICM, NPK and NPK+ICM and 6 m x 24 m for SSNM, SSNM+ICM, FFP and FFP+ICM. In crop 1 (2004/2005), the ICM treatment was the application of 1.5 t ha^{-1} lime and higher plant population (75 cm between rows and 17.5 between plants in a row) than the regular treatments (plant spacing of 75 cm x 20 cm). In the second and third crops (2006-2007), the ICM treatment was the application of 1.5 t lime ha^{-1} and 20.4 kg Mg ha^{-1}. Plant spacing for all treatments was 75 cm x 20 cm. Fertilizer rates in the omission plots, NPK, SSNM, and FFP treatments are shown in Table 1.

Urea was used as the N-source in plots with N application (NK, NP, NPK, and SSNM plots). Basal N (30%) was applied shortly after emergence (7 days after planting = dap), with side-dressings at V6 (40%) and V10 (30%) stages of maize, dibbled ± 5 cm deep as band along the maize rows. For plots with P application (PK, NP, NPK, and SSNM plots), the P-source was SP-36. All P fertilizer was applied together with the first N application shortly after emergence. The source of potassium in PK, NK, NPK, and SSNM plots was KCl. Half of K fertilizer was applied with the first N application shortly after emergence and the other half during the V10 stage, dibbled ± 5 cm deep as band along the maize rows. Farmers' fertilizer application were

Table 1. Fertilizer rates and application in the treatments.

Treatments/season	Fertilizar rates (kg ha^{-1})			Lime	Mg
	N	P_2O_5	K_2O	(t ha^{-1})	(kg ha^{-1})
Crop 1 (2004/2005):					
- Omision Plot	200	80	120	0,0	0,0
- Omision Plot+ICM	250	92	181	1,5	0,0
- FFP	135	67	48	0,0	0,0
- FFP+ICM	135	67	48	1,5	0,0
Crop 2 (2005/2006):					
- Omision Plot	200	80	120	0	0,0
- Omision Plot+ICM	200	80	120	1,5	24,5
- SSNM	129	71	148	0	0,0
- SSNM+ICM	129	71	148	1,5	24,5
- FFP	180	41	22	0	0,0
Crop3 (2006/2007):					
- Omision Plot	200	80	120	0	0,0
- Omision Plot+ICM	200	80	120	1,5	24,5
- SSNM	152	80	112	0	0,0
- SSNM+ICM	152	80	112	1,5	24,5
- FFP	235	34	43	0	0,0

splits twice for N (urea) that is the first application at 12 dap and the second application at 30 dap, while P (SP36) and K (KCl) applied all at 12 dap together with the first N application.

Before planting, composite soil samples were taken at 0 - 20 cm depth from each location. A composite soil sample was a mixture of 25 soil cores that were taken across the whole field following a zigzag pattern. Nutrients and fractions analyzed were particle size (clay, silt, sand; pipette method), soil organic C (Walkley-Black), total soil N (Kjeldahl), CEC and exchangeable K, Na, Ca and Mg (1 N NH_4-acetate), pH (KCl, 1:1), pH (H_2O, 1:1), Olsen-P (0.5 M $NaHCO_3$, pH 8.5), Bray-1 P (0.03 M NH_4F + 0.025 M HCl) and exchangeable Al.

Management practices implemented were based on the best recommendations and technologies available at each location, following the concept of integrated crop management. Maize varieties planted in the trial were Pioneer 21 at location one (Sidowaras) and BISI-2 at other locations (Binjai Ngagung, Watu Agung, Balai Rejo and Trimulyo). All treatments received full control from weeds, pests and diseases.

Grain yield was measured at final harvest around 7-10 days after physiological maturity (R6). Grain yield data were analyzed using Duncan Multiple Range Test (DMRT) to determine the differences among treatments. Yield responses to fertilizer NPK application were obtained from the difference between the yield in NPK (+/-ICM) and the relevant omission plot (0-N, 0-P, 0-K). Agronomic efficiency was measured as the amount of crop yield increase per unit nutrient applied with the formula:

$$AE_X = (Y_{NPK} - Y_{0X})/F_X$$

where:
AE_X = Agronomic efficiency of nutrient x (kg kg^{-1})
Y_{NPK} = yield (kg ha^{-1}) in a plot that received fertilizer nutrient X (kg ha^{-1})
Y_{0X} = yield in a plot without fertilizer nutrient X addition (kg ha^{-1})
Fx = Rate of nutrient X fertilizer applied.

Results and Discussion

General soil properties

Soil properties in the experimental sites are shown in Table 2. On an average soils are characterized as having clay loam texture, slightly acidic (pH H_2O: 5.0) with very low organic carbon (11.7±4.7 g kg^{-1}) and total N (0.94±0.32 g kg^{-1}), high status of P potential, P Olsen and P Bray-1 with soil test value of 428±256; 49±29 and 40±22 mg kg^{-1} P_2O_5 respectively and low exchangeable K (0.19±0.16 cmol kg^{-1}). The clay loam texture of the sites indicates a good environment for root development for maize (Isa *et al* 2005).

Yield response to fertilizer application

Yield response to fertilizer application was in the order N>K>P ranging on an average from 2.3 to 4.1 t ha^{-1} for N, from 0.5 to 2.0 t ha^{-1} for P and from 0.6 to 2.4 t ha^{-1} for K (Table 3). The magnitude of yield response to applied fertilizer is dependent on the indigenous nutrient supply and climatic conditions during the growing season. Yield responses to N, P, and K were higher in crop 2 and crop 3 where growing conditions were more favorable as indicated by the higher yields obtained in the NPK and SSNM treatment plots as compared to crop 1. Yield responses to fertilizer application are very variable among fields (data not shown) and/or seasons, thus, a nutrient management

Table 2. Soil Properties in the experimental site (average of 5 locations)

Soil Properties	Mean (0-20 cm)	Standard Deviation
Sand	32	29
Silt	21	12
Clay	47	25
Organic Matter		
C (g kg^{-1})	11.7	4.7
N (g kg^{-1})	0.94	0.32
C/N	13	1.79
Soil nitrate N (g kg^{-1})	128.2	26.3
P HCl 25% (mg kg^{-1})	428	256
P-Olsen (mg kg^{-1} P2O5)	49	29
P-Bray (mg kg^{-1})	40	22
pH (H2O)	5.0	0.34
Al3+ (cmol kg^{-1})	0.07	0.16
K Exchangeable (cmol kg^{-1})	0.19	0.16
CEC	10.95	3.93
Base Saturation (%)	54	9

Table 3. Yield response to Fertilizer N, P and K application based on NPK

Treatment	Yield Response (t ha^{-1})				
	Crop 1	Crop 2	Crop 3	Average	SD
PK	2.62	4.1	3.8	3.51	0,78
PK+ICM	2.25	3.5	2.9	2,88	0,63
NK	0.67	2.0	1.8	1,49	0,72
NK+ICM	0.54	1.3	1.2	1.01	0,41
NP	1.57	1.7	2.4	1.89	0,45
NP+ICM	0.57	0.8	1.1	0,82	0,27

Notes : *) n =2 x 5 sites (+/- ICM x 5 sites) = 10 ,
SD = Standard Deviation.

Tabel 4. Agronomic efficiencies based on NPK and SSNM treatments.

	Treatment	Agronomic Efficiencies (kg /kg)				
		Crop 1	Crop 2	Crop 3	Average	SD.
AEN	NPK	13	21	19	18	4
	NPK+ICM	9	18	15	14	3
AEP	NPK	19	57	51	43	0
	NPK+ICM	14	37	34	28	11
AEK	NPK	16	17	24	19	4
	NPK+ICM	4	8	11	8	4

Notes : *) n =2 x 5 sites (+/- ICM x 5 sites) = 10 ,
SD = Standard Deviation.

strategy that is robust to handle such variability is clearly required.

Agronomic efficiencies

Agronomic efficiency (AE) is one of several indicators of nutrient use efficiency. It is expressed as the yield increase per unit fertilizer applied (kg grain per kg fertilizer). Agronomic efficiency is a measure of the ability of a plant to transform the amount of fertilizer applied into economic yield (grain). It is a good indicator of the congruence between plant nutrient demand and the quantity of nutrient released from applied fertilizer. Table 4 shows the agronomic efficiency for N, P, and K measured from the NPK treatment plots. On an average, agronomic efficiencies ranged from 9 to 21 kg grain per kg fertilizer N applied, from 14 to 57 kg grain per kg fertilizer P applied, and from 4 to 24 kg grain per kg fertilizer K applied. The low AE measured in the NPK treatment plots was due to the very high rates of fertilizer applied in these plots to make sure that nutrients are not limiting the yield. Nitrogen use efficiency was improved with more appropriate fertilizer N rates and better timing of N application practiced in the SSNM treatments.

Yield comparison

Data on grain yield from the various treatments (Table 5) shows that on an average, grain yields in omission plots increased in the order PK (5.9 t ha^{-1}) < NP (7.8 t ha^{-1}) < NK (7.9 t ha^{-1}). This shows that N is the most limiting nutrient affecting maize yield, whereas P and K supply are equally limiting factors.

Across sites, attainable yields measured in NPK and SSNM plots on average ranged from 7.4 t ha^{-1} to 10.9 t ha^{-1} while yields in the FFP plot on average ranged from 6.4 t ha^{-1} to 8.7 t ha^{-1}. This clearly shows that a substantial yield gap of about 1 to 2 t ha^{-1} exists between farmers' actual yield and what is attainable with optimal crop and nutrient management. Differences in grain yield among the seasons are mainly attributed to seasonal fluctuations in climate and other environmental factors. In the case of crop 3, the high yields attained even in the omission plots are attributed to the long dry period before the maize cropping season which enabled crop residues to decompose and become available to the maize crop.

5. Yield Comparison among the treatments for three crops

Treatments	Grain Yield (t ha^{-1})		
	Crop 1	Crop 2	Crop 3
PK	4.78 e	5.0 f	7.1 f
PK+ICM	5.47 de	5.9 ef	7.4 ef
NK	6.73 abc	7.1 de	9.1 bcd
NK+ICM	7.18 ab	8.1 bcd	9.1 bcd
NP	5.83 cde	7.4 cd	8.5 def
NP+ICM	7.15 ab	8.6 abc	9.2 bcd
NPK	7.4 ab	9.1 ab	10.9 a
NPK+ICM	7.72 a	9.4 a	10.3 ab
SSNM	-	8.4 abc	10.1 ab
SSNM+ICM	-	8.9 ab	10.1 ab
FFP	6.41 bcd	7.0 de	8.7 cde
FFP+ICM	7.02 ab	-	-
CV (%)	12.02	12.2	11.6

Note: SSNM = Site Specific Nutrient Management, ICM = Improve crop management, and FFP = Farmers' Fertilizer Practice. Number followed by the same letters in each column is not significant different at 5% DMRT

In general, ICM treatments resulted in higher yields compared to plots without ICM. This indicates that ICM, mainly liming, had a positive effect in the soil reaction particularly in increasing the availability of nutrients to the plant.

Grain yield in the NPK plots was on an average 0.5 t ha^{-1} higher than in SSNM plots although this difference is not statistically significant. This shows that the nutrient management strategy employed in the SSNM treatment can give equally high yields as the NPK plot. Grain yield in SSNM was significantly higher than the FFP in both season 2 and season 3. On an average, SSNM generated a yield gain of 1.5 t ha^{-1} (19%) over the FFP. This clearly indicates that SSNM provides substantial opportunities for farmers to increase productivity and profitability of maize through improved crop and nutrient management strategies.

Conclusion

There are substantial opportunities for maize farmers in Lampung to increase their productivity and profitability with SSNM. Yield increases of about 1.5 t ha^{-1} over current farmer fertilizer practice can be achieved with improved timing of N application and more balanced fertilizer use in SSNM. Yield responses of about 2.3-4.1 t ha^{-1}, 0.6-2.0 t ha^{-1}, 0.3-2.4 t ha^{-1} can be expected with the application of fertilizer N, P and K respectively. Yield responses, particularly to fertilizer N, are highly variable among fields and/or seasons. Thus, the SSNM strategy for nitrogen with total N rate, split N applications and dynamic N management provides assurance that additional yield can be attained in years more favorable than the average.

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Fertilizer Recommendation Based on the SSNM Approach in Upland Karo District, North Sumatra

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Abstract. North Sumatra is the most important maize production area in Indonesia after East Java, Central Java, Lampung and NTT. Productivity here is low but there are opportunities to increase it. The purpose of this study was to look for a suitable dosage of N, P_2O_5 and K_2O to increase maize production and profitability in this province. Our study was conducted during Aug-Dec 2007 at Tigabinanga in Karo district. The experiment was arranged in a split-plot design consisting of two factors (variety and nutrient management) with three replications. The varieties were P 12, BISI 9, NK 22 and DK 3, and the nutrient management treatments were absence of fertilizer, SSNM + PD1, SSNM + PD2 and FFP. The SSNM + PD1 and SSNM + PD2 treatments used the same fertilizer recommendation (N = 160 kg ha^{-1}, P_2O_5 = 70 kg ha^{-1} and K_2O = 80 kg ha^{-1}). The fertilizer levels used in farmers' practice were N = 300 kg ha^{-1}, P_2O_5 = 90 kg ha^{-1} and K_2O = 60 kg ha^{-1}, applied without covering. The results indicated that the recommendation N = 160 kg ha^{-1}, P_2O_5 = 72 kg ha^{-1} and K_2O = 90 kg ha^{-1} increased productivity by 1.3 t ha^{-1}, and farmers' income by 15.93%.

Key words: Maize, recommendation NPK, variety, income increase

Introduction

Maize is the most important cereal crop after paddy in Indonesia, with the total sown area reaching 3.4 million ha in 2006. But average productivity is low at 2.8 t ha^{-1} (Deptan 2007).

North Sumatra has potential for maize development and can join East Java, Central Java, Lampung and NTT as a major maize-producing region in Indonesia. The areas with potential for maize development in North Sumatra are (BPS 2007) the districts of Simalungun (59 604 ha), Karo (50 182 ha), Dairi (25 645 ha), Deli Serdang (19 027 ha) and Langkat (17 236 ha).

Maize is a crop that requires 27.4 kg N, 4.8 kg P and 18.4 kg K per ton of grain yield. To increase crop productivity to 7.5-12 t ha^{-1} and to replenish nutrients lost during cropping, farmers require site-specific nutrient management which depends on climate and soil conditions, genetic factors (variety) and the application technique (Cooke 1985).

Maize cropping in the Inceptisols of Karo district has not yet resulted in optimal production, the reason for which is that site-specific nutrient management (SSNM) here has not been based on local soil characteristics. Inceptisols are low in N. As a response to this problem, farmers here generally apply fertilizer in excess of common recommendations. Hence research is required to assess the exact SSNM requirement for these Inceptisols.

The objective of our research was to identify the location-specific recommendations for maize in the Inceptisols of Tigabinanga subdistrict of Karo, thereby helping farmers increase their production and income.

Materials and Method

Our study was carried out in Aug-Dec 2007 in Tigabinanga subdistrict of Karo district at a dryland location 600-700 m above mean sea level. Nutrient and soil analysis of the location was done by the Assessment Institutes for Agricultural Technology (AIAT) laboratory in North Sumatra.

We used a factorial completely randomized block design with 2 factors (variety and fertilization) and 3 replications. The first factor was variety (V) which consisted of 4 levels (varieties): V1 = P 12; V2 = BISI 9; V3 = NK 22; and V4 = DK 3. The second factor was nutrient management (P), consisting of 4 levels: P0 = Control; P1 = SSNM + PD1; P2 = SSNM + PD2; and P3 = FFP. So there were 4 × 4 × 3 = 48 units. The fertilizer doses used in the four treatments are presented in Table 1.

Table 1. Fertilizer dosage[1] used in the nutrient management treatments.

Block	Fertilizer composition	Control (kg ha^{-1})	SSNM[2]+PD1 (kg ha^{-1})	SSNM+PD2 (kg ha^{-1})	FFP (kg ha^{-1})
I	Urea	0.00	355.56	302.22	450.00
	SP 36	0.00	355.56	355.56	300.00
	KCl	0.00	268.33	268.33	150.00
II	Urea	0.00	355.56	302.22	400.00
	SP 36	0.00	283.33	283.33	300.00
	KCl	0.00	133.33	133.33	200.00
III	Urea	0.00	355.56	302.22	450.00
	SP 36	0.00	213.89	213.88	250.00
	KCl	0.00	200.00	200.00	250.00

1. Dosage based on the earlier results from three planting seasons (Aug 2004-Dec 2005).
2. SSNM = Site-specific nutrient management.

Table 2. Weight of 100 seeds (g) of maize varieties under different nutrient management recommendations.

Treatment	V1 (P 12)	V2 (BISI 9)	V3 (NK 22)	V4 (DK 3)	Average
P0 (control)	37.80	37.77	37.87	37.20	37.66c
P1 (SSNM+PD1)	43.57	42.90	44.13	42.97	43.39a
P2 (SSNM+PD2)	41.87	41.23	42.13	41.50	41.68b
P3 (FFP)	41.37	40.93	41.63	41.20	41.05b
Average	41.15a[1]	40.70b	41.44a	40.72a	

1. Numbers followed by the same letter were nonsignificantly different at 5% level based on the Duncan Test.

Fertilization was done 7 DAP with an application of 30% of urea, 100% SP 36 and 50% of KCl. Another application of 35% of urea was given 25 DAP, and another of 35% of urea and 50% of KCl after 50 DAP to the SSNM + PD1 and SSNM + PD2 treatments. The farmers' practice treatment was given two applications only: 50% at 25 DAP and 50% at 50 DAP. Thinning was done for the control and SSNM + PD1 treatments at 5-7 DAP, leaving one plant/ seed. No thinning was done for the SSNM + PD2 and FFP treatments.

Weed control was done manually and pesticides were used as recommended. Crop husbandry was done from the planting stage to the ready-for-harvest stage. The crop was harvested from a sample of 6 plots of 4 x 3 m. The following data on production, agronomic efficiency and ratio of profitability were computed.

- Weight of 100 seeds (g)
- Production (t ha^{-1})
- Agronomic efficiency (kg seed per kg fertilizer)
  1. Agronomic efficiency of N = (Production with NPK – Production with 0 N)/amount of fertilizer N
  2. Agronomic efficiency of P = (Production with NPK – Production with 0 P)/amount of fertilizer P
  3. Agronomic efficiency of K = (Production with NPK – Production with 0 K)/ amount of fertilizer K
- Ratio of profitability

$$\frac{\text{The yielded net profit}}{\text{Expense spent on treatment}} \times 100\%$$

To assess the effect of the treatments analysis of variance (tested F) at 5% and 1% levels and continuation test DMRT were applied.

Results

Weight of 100 seeds

Table 2 presents the 100-seed weight of the four varieties of which NK 22 (V3) had the highest average of 41.44 g and and BISI 9 (V2) the lowest average of 40.70 g. Statistical analysis indicated that NK 22 was significantly different from BISI 9 and DK 3 (V4).

From Table 2 we know that among fertilization treatments the highest average weight of 100 seeds was for the treatment SSNM + PD1 (P1) at 43.39 g, and the lowest was for the control treatment at 37.66 g. Statistical analysis indicated that the SSNM + PD1 treatment was significantly different from the control, SSNM + PD2 and FFP treatments.

Productivity

Table 3 shows that the highest productivity was attained by the SSNM + PD1 treatment with an average of 11.3 t ha^{-1}. The lowest average was for the control with 3.2 t ha^{-1}. The SSNM + PD1 treatment was significantly different from the control, SSNM + PD2 and FFP treatments.

Agronomic efficiency

The highest agronomic efficiency of N was given by the SSNM + PD1 (Pl) treatment with an average of 25.9 kg seed per kg N and the lowest efficiency average of 0 kg seed per kg N was given by the control (Table 4). The SSNM + PD1 treatment was significantly different from control and the FFP treatments.

The highest agronomic efficiency of P (33 kg seed per kg P) was given by variety NK 22 (V3) and the lowest (30.6 kg seed per kg P) by BISI 9 (Table 5). NK 22 was significantly different from P 12 (V1), BISI 9 (V2) and DK 3 (V4).

As regards the fertilizer treatments, the highest agronomic efficiency of P was (40.4 kg seed per kg P) was achieved by SSNM + PD1 (P1) and the lowest (0 kg seed per kg P) by the control treatment. Statistical analysis

Table 3. Productivity (t ha^{-1}) of maize varieties under different nutrient management treatments.

Treatment	V1 (P 12)	V2 (BISI 9)	V3 (NK 22)	V4 (DK 3)	Average
P0 (control)	3.16	3.19	3.17	3.1	3.2c
P1 (SSNM+PD1)	11.28	11.24	11.44	11.24	11.3a
P2 (SSNM+PD2)	10.43	10.42	10.73	10.52	10.5ab
P3 (FFP)	10.02	9.97	10.03	9.98	10.0b
Average	8.72a[1]	8.71a	8.84a	8.71a	

[1.] Numbers followed by the same letter in the same column were nonsignificantly different at 5% level based on the Duncan Test.

Table 4. Agronomic efficiency of N (kg seed per kg N) of maize varieties in different nutrient management treatments.

Treatment	V1 (P 12)	V2 (BISI 9)	V3 (NK 22)	V4 (DK 3)	Average
P0 (control)	0.0	0.0	0.0	0.0	0.0d
P1 (SSNM+PD1)	25.8	25.6	26.8	25.6	25.9a
P2 (SSNM+PD2)	20.5	20.4	22.4	21.1	21.1b
P3 (FFP)	17.9	17.6	18.0	17.7	17.8c
Average	21.4a[1]	21.2a	22.4a	21.4a	

[1.] Numbers followed by the same letter in the same column were nonsignificantly different at 5% level based on the Duncan Test.

Table 5. Agronomic efficiency of P (kg seed per kg P) of maize varieties under different nutrient management treatments.

Treatment	V1 (P 12)	V2 (BISI 9)	V3 (NK 22)	V4 (DK 3)	Average
P0 (control)	0.0	0.0	0.0	0.0	0.0d
P1 (SSNM+PD1)	40.1	39.6	42.2	39.6	40.4a
P2 (SSNM+PD2)	29.1	29.0	33.0	30.3	30.3b
P3 (FFP)	23.8	23.1	23.9	23.2	23.5c
Average	31.0b[1]	30.6b	33.0a	31.0b	

[1.] Numbers followed by the same letter in the same column were nonsignificantly different at 5% level based on the Duncan Test.

indicates that treatment P1 was significantly different from P0, P2 and P3.

The highest average agronomic efficiency of K (7.9 kg seed per kg K) was given by NK 22 and the lowest (6.4 kg seed per kg K) by BISI 9 (Table 6). The variety NK 22 was significantly different from P 12 (V1), BISI 9 (V2) and DK 3 (V4).

As for the four fertilization levels, the highest average agronomy efficiency of K (12.7 kg seed per kg K) was attained by SSNM + PD1 (P1) and the lowest (0 kg seed per kg K) by the control treatment. The P1 treatment was significantly different from the P0, P2 and P3 treatments.

Profitability ratio

The highest profitability ratio (B:C ratio) of 1.72 was given by the SSNM + PD1 treatment and the lowest (0.35) by the control treatment (Table 7). The SSNM + PD1 treatment was significantly different from the control, SSNM + PD2 and FFP treatments.

Analysis

The results show that NK 22 is better adapted to the location than the other varieties, thereby giving it better productivity and agronomic efficiency. Fisher (1992) reported that the adaptation process is influenced by many factors such as sunshine intensity, light, temperature and humidity. The maize variety that is capable of better adapting to an environment will have better growth and production.

The fertilizer treatment SSNM + PD1 had a significant effect on crop production and proved to be better than the farmers' practice treatment. The average productivity achieved under the SSNM + PD1 fertilization treatment was 11.3 t ha^{-1} in comparison with 10.0 t ha^{-1} achieved under the farmers' treatment. As per Sanchez (1993), N plays an important part in the production of chlorophyll which has a central role in photosynthesis, leading to a better crop. Nutrient P supports the growth of roots, and plays an important role during the ripening and flowering stages. Lack of P lowers production. Lingga (2001) reported that K has an important function in metabolic processes like photosynthesis and respiration and in sugar translocation during the formation of some proteins and extracts, rendering assistance in the opening and closing of stomata and regulating water-use efficiency.

The results show that the agronomy efficiency of N, P and K for the SSNM + PD1 treatment was higher than the farmer treatment. The agronomic efficiency of N (AEN) was 25.9 kg seed per kg N (low); the agronomic efficiency of P (AEP) 40.4 kg seed per kg P (medium); and the agronomic efficiency of K (AEK) 12.7 kg seed per kg K (low). This yielded a fertilization recommendation of N = 160 kg ha^{-1}, P_2O_5 = 72 kg ha^{-1} and K_2O = 90 kg ha^{-1}. It means

Table 6. Agronomic efficiency of K (kg seed per kg K) of maize varieties under different nutrient management treatments.

Treatment	V1 (P 12)	V2 (BISI 9)	V3 (NK 22)	V4 (DK 3)	Average
P0 (control)	0.0	0.0	0.0	0.0	0.0d
P1 (SSNM+PD1)	12.5	12.2	13.8	12.2	12.7a
P2 (SSNM+PD2)	5.4	5.3	7.9	6.2	6.2b
P3 (FFP)	2.0	1.6	2.1	1.7	1.8c
Average	6.6b[1]	6.4b	7.9a	6.7b	

[1.] Numbers followed by the same letter in the same column were nonsignificantly different at 5% level based on the Duncan Test.

Table 7. Profitability ratio (B:C ratio) of maize varieties under different nutrient management treatments.

Treatment	V1(P 12)	V2(BISI 9)	V3(NK 99)	V4(DK 3)	Average
P0 (control)	0.40	0.35	0.35	0.31	0.35c
P1 (SSNM+PD1)	1.71	1.70	1.74	1.71	1.72a
P2 (SSNM+PD2)	1.65	1.63	1.70	1.66	1.66ab
P3 (FFP)	1.51	1.50	1.51	1.50	1.51b
Average	1.32a	1.30a	1.33a	1.30a	

[1.] Numbers followed by the same letter in the same column insignificantly different at 5% level based on the Duncan Test.

that to increase production by 25.9 kg, 1 kg N is needed. This is as per the finding of Witt (2007) that higher agronomic efficiency at a location allows use of appropriate levels of fertilization to yield optimal production.

In the farmers' fertilization practice treatment, the AEN was 17.8 kg seed per kg N (low); AEP 23.5 kg seed per kg P (low); and AEK 1.8 kg seed per kg K (low). According to Witt (2007), maize plant growth needs N during three phases: early growth, formation of flowers and filling of seed. If in these phases the value of LCC is <4, vegetative and generative growth of the crop will be affected, causing low yield.

The profitability ratio (B:C ratio) for the SSNM + PD1 treatment was 1.72 while the farmers' practice treatment gave only 1.51, the advantage to farmers from the SSNM + PD1 technology being 15.93%..

The effect of interaction between variety and nutrient management on maize growth and production indicates that treatment of variety and management of nutrients show insignificant influence on all parameters. This is as per Sutedjo (1992). Each treatment factor has a different character of influence and hence will yield different relationships in influencing crop growth and production.

Conclusions

The appropriate fertilization recommendation for Tigabinanga subdistrict is 160 kg ha^{-1} N, 72 kg ha^{-1} P_2O_5 and = 90 kg ha^{-1} K_2O. This recommendation resulted in a 1.3 t ha^{-1} higher yield than the farmers' fertilization practice and a 15.39% increase in income. The interaction between the variety and nutrient management factors showed insignificant influence on all parameters. It indicates that all hybrid varieties have the same potency to increase production and farmers' income.

We suggest that during every planting season one plot be made an omission plot to get an accurate recommendation. Production of maize in Tigabinanga can be increased with 3 applications of N, P and K by a dibber and then covered.

Acknowledgement

We thank Dr. Christian Witt and Dr Sania Saenong of the Assessment Institutes of Agricultural Technology North Sumatra for their valuable inputs to this research.

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Fertilizer Recommendation for Open-Pollinated Maize in the High Uplands of Tegal District in Central Java

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Abstract. Maize yields in the highlands of Central Java are low because of the use local varieties and inadequate agricultural practices. Improvement of varieties, appropriate plant spacing and precise fertilizer recommendations can lead to higher yields. Our study assessed Integrated Crop Management (ICM) recommendations on fertilizer use and plant density for maize open-pollinated and local varieties in Kedawung village in Bojong subddistrict of Tegal district in Central Java during May-Aug 2007. The site is located in the high uplands 900 m above sea level with an Andisol soil type and annual rainfall of 3500 mm. Our on-farm research had 7 treatment combinations (including variety, plant density, fertilizer recommendation) with 3 replications. The varieties studied were Bisma, Sukmaraga, Srikandi Kuning, Anoman, Yellow Local and White Local. The plant spacing treatments were 75 × 20 cm and farmers' practice. The fertilizer recommendations included urea 350-425 kg ha^{-1} and Phonska 300 kg ha^{-1}, combined with LCC for the third and fourth fertilizer application. The fertilizer and plant spacing recommendations were also implemented with the two local (Yellow and White) varieties to compare the results with the cultivation of these same varieties under farmers' practices. The variety Sukmaraga under recommended plant spacing and fertilizer use gave a significantly better yield than White Local, Anoman and farmers' practices (DMRT 15%), whereas Bisma and Srikandi Kuning were only better than White Local and farmers' practice. The local varieties under existing farmers' practices yielded only about 2.25 t ha^{-1} of dry grain. By using open-pollinated superior varieties, increasing plant density, and applying precise fertilizer recommendations, maize yield can be increased by about 108-172%. The yield increases we recorded for each variety were Anoman (108%), Srikandi Kuning (128%), Bisma (155%) and Sukmaraga (172%). Moreover, introduction of plant spacing and fertilizer recommendations to cultivation of White Local and Yellow Local increased yield by about 134% for Yellow Local and 113% for White Local. Intensification of maize cultivation of open-pollinated superior varieties and local varieties increased yield by an average of 5.3 t ha^{-1} dry grain, or about 135% over cultivation under existing farmers' practices.

Key words: Maize, open-pollinated, high uplands, fertilizer, recommendation

Introduction

In Indonesia, local varieties account for about 20% of maize production and new superior varieties about 80%. Among the latter, 56% of them are using open-pollinated varieties (Pingali 2001 cited in Mejaya *et al.* 2007). Nugraha *et al.* (2002, cited in Mejaya *et al.* 2007) reported that the production area of new superior varieties is still dominated by open-pollinated variety (OPVs), and that they account for about 48% of the total area. Many farmers still use OPVs because their seeds are cheaper than that of hybrids.

Most people in living in the highlands of Central Java consume maize as a staple food. Generally, maize yields in the high uplands are low at about 2-3 t ha^{-1} (Prastuti *et al.* 2007) because farmers there continue to use local varieties and practise minimal crop management.

Intensification of maize cultivation through improved crop management can increase yields. Toward that objective, we conducted an assessment of improved crop management (ICM) practices (use of superior varieties, appropriate plant spacing and precise fertilizer recommendations) in Kedawung village in Bojong subdistrict of Tegal district in Central Java.

Methodology

The assessment was conducted during the dry season (May-Aug) of 2007. The experimental site was located 900 m above sea level with an Inceptisol soil type and annual rainfall of about 3700 mm. Our on-farm research had 7 treatment combinations (including variety, plant density, fertilizer recommendation) with 3 replications. The varieties studied included Bisma, Sukmaraga, Srikandi Kuning, Anoman, Yellow Local and White Local. The two plant spacing treatments were 75 × 20 cm and farmers' practice. The fertilizer recommendation included urea 350-425 kg ha^{-1} and Phonska 300 kg ha^{-1} combined with LCC for the third and fourth fertilizer application (Samijan *et al.* 2005). These recommendations on fertilizer use and plant spacing were also implemented for both the local varieties in our

Table 1. Treatments used in the assessment of variety, fertilizer use and plant density recommendations for OPV and local maize varieties in highland areas of Tegal district in Central Java, 2007.

Treatment	Existing farmers' practices	Experimental practices
Fertilizer		
Urea (kg ha^{-1})	225	350 (100-125*-125*)
NPK (kg ha^{-1})	-	300** (150-150-0)
Organic (kg ha^{-1})	0-10.000	2.000
Varieties	Yellow Local, White Local (yellow and white grain color)	Bisma, Sukmaraga, Srikandi Kuning, Anoman, Yellow Local White Local
Plant spacing (cm)	(70-80) × 40	70 × 20
Number of seeds per hill	3-4	1
Other techniques	2 times	3 times, combined with LCC
Seed treatment	No	Yes
Source of seed	Farmers' own	Certified seed

*Recommendation 100-150 kg ha^{-1} depending on LCC.
**NPK Phonska (15:15:15).

study, Yellow Local and White Local, in order to compare their results with that achieved by these varieties under farmers' practices. The treatments used in our study are presented in Table 1.

The data we collected for the assessment included flowering date at 50%, 1000-grain weight, grain weight per plot and grain yield per hectare (moisture content 15.5%).

Results and Discussion

Site characteristics

Our study area lies at an altitude of about 800-1000 m above sea level. The soils are Inceptisols (*Typic Dystrudepts*). The annual rainfall (Fig. 1) in this location is about 3700 mm with 7 wet months and 3 dry months (BPP Bojong 2002). Farmers practise mixed cropping of food crops (maize, cassava) and vegetables (carrot, cabbage, chilli, leaf onion and mustard). Maize is normally planted during the end of the wet season and up to the dry season (Jan-Sep). Farmers plant maize at different times depending on the kind of crop they had planted in the previous season.

Growth and yield

Our observations relating to plant growth and yield components were as follows.

Table 2.

Practice	Fertilizer application I	II	III	Extra
Vegetative flowering	Leaf 3	Leaf 6-8	Leaf >10	50%
Time	7 DAT	21-25 DAT	>50 DAT	
Other guidance		BWD	BWD	BWD <4
Urea	100	125-150	125-150	75

Source: Witt 2005.

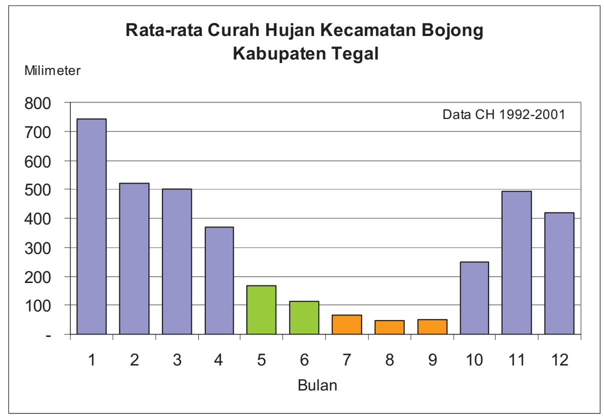

Figure 1. Rainfall and solar radiation in Bojong subdDistrict in Tegal district of Central Java province, Indonesia.

Days to 50% flowering. The number of days of 50% flowering was not significantly different between the newly introduced varieties and the local varieties, being around 62-69 days after seedling. The difference of less than a week may be due to the adaptability of the new varieties in the new agroecology. This indicates that the new varieties have potential to substitute the local varieties.

1000-grain weight. As shown in Table 3, 1000-grain weight of the new varieties was not significantly different from that of the local varieties (at DMRT 5%). Nevertheless, at DMRT 10% new variety Sukmaraga and local variety Yellow Local (with improved crop management) had more grain weight than the other varieties. On the basis of this parameter, Sukmaraga may be suitable for farmers to adopt.

Grain yield. The results show that the yields recorded by the newly introduced varieties and the two local varieties cultivated under recommended plant spacing and fertilizer use were not significantly different at DMRT 10%. There, however, was a difference between the yields recorded by all the varieties (local as well as new varieties) under improved crop management (plant spacing and fertilizer

Table 3. Plant growth and yield components of seven varieties in the high upland areas of Central Java, Indonesia.

Variety	Days to 50% flowering (FD_{50}) DMRT 5%	Weight of 1000 grain (WG_{1000}) (g)	Weight of grain per 12m² plot (WG_{12}) (kg) DMRT 10%	Grain yield per ha ($GY_{15.5}$) (kg)	Grain yield ($GY_{15.5}$) (kg ha^{-1}) DMRT 15%
Bisma	66a	415ab	8.99b	5741b	5741cd
Sukmaraga	66a	504b	9.60b	6138b	6138d
Srikandi	69a	380a	8.05b	5132b	5132cd
Anoman	63a	436ab	7.36b	4684b	4684bc
Yellow Local*	62a	507b	8.33b	5281b	5281cd
White Local*	62a	388ab	7.44b	4803b	4803b
Farmers' existing cv.	62a	-	3.53a	2253a	2253a

recommendation) in contrast with yields recorded under existing farmers' practices (DMRT 5%).

In the higher multiple-range test of Duncan (DMRT 15%), Sukmaraga under improved crop management performed significantly better in terms of yield than White Local, Anoman and farmers' practices, whereas Bisma and Srikandi were better than White Local and farmers' practice. Local varieties (White Local and Yellow Local) with improved crop management also performed significantly better than the same local varieties grown under farmers' practices (DMRT 5-15%).

Local varieties cultivated under existing farmers' practices only yielded about 2.25 t ha^{-1} dry grain. In contrast, improved crop management of open-pollinated superior varieties cultivated under increased plant density and precise use of fertilizer recommendations yielded about 108-172% more. The yield increases recorded by each new variety were: Anoman (108%), Srikandi (128%), Bisma (155%) and Sukmaraga (172%). Moreover, introduction of recommendations on plant spacing and fertilizer use to local maize varieties (Yellow Local and White Local) increased their yield by about 134% for Yellow Local and 113% for White Local. Improved crop management with open-pollinated superior varieties and local varieties yielded an average of 5.3 t ha^{-1} (135% increase) compared with existing farmers' practices.

Conclusions

- Improved crop management with optimum plant density and recommended fertilizer use can improve grain yield over farmers' existing local varieties.
- The average grain yield achieved by improved crop management (superior varieties, optimum plant density and recommended fertilizer use) was about 5.3 t ha^{-1}, or 135% higher than that achieved with farmers' practices.
- Introduction of new open-pollinated varieties with improved crop management increased yields by about 108-172% over yields achieved with farmers' existing practice, with variety Sukmaraga yielding the highest.
- Improved crop management of local varieties can improve yield by about 113-134% over farmers' existing practice.

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Effect of N, P and K Fertilization on Maize in Grobogan District of Central Java

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Abstract. Maize is a strategic commodity which needs more attention to establish and improve yields. One of the key factors in maize production is nutrient sufficiency for optimal yield. To improve fertilization effectiveness and efficiency, we need to assess the response to fertilization. Our research was aimed at assessing the effectiveness of N, P and K fertilization in lowland Vertisols in Toroh and Purwodadi subdistricts in Grobogan district of Central Java during three cropping seasons (2004-2006). We used the 'omission plot' approach with 5 replications in 5 villages. The N, P and K fertilizer ratios applied in the 3 cropping seasons were: 200:30:75 kg ha^{-1} in 2004; (200:35:100 kg ha^{-1} in 2005; and 229:92:40 kg ha^{-1} in 2006. Maize planting in the irrigated lowlands is done during the dry season (Jun-Oct) after the cropping of rice-rice. The experimental site in Grobogan was a Vertisol with heavy clayey texture; pH neutral to basic (7-8); low in organic C and soil N; high Ca, Mg and base saturation; and serious soil cracking in the dry season. Treatment Yield 2004 (t ha^{-1}) Yield 2005 (t ha^{-1}) Yield 2006 (t ha^{-1})Minus N 2.35 1.70 1.47Minus P 7.45 9.05 8.96Minus K 8.20 9.20 9.39Complete NPK 8.65 10.45 10.81The results show that the minus-N (omission) plots yielded an average of only 1.8 t ha^{-1} grain (MC 15.5%). From the minus-P plots, the average yield was 8.5 t ha^{-1}, and from the minus-K plots 8.9 t ha^{-1}. The average yield from the NPK plots was 10 t ha^{-1} (MC 15.5%). that It was clear that the highest response of maize was to N fertilization, much more than to P or K. The maize response to N, P and K fertilization was N 8.1 t ha^{-1}, P_2O_5 1.5 t ha^{-1} and K_2O 1.0 t ha^{-1}.

Key words: Maize, response, fertilizer, Vertisols, lowlands.

Introduction

Grobogan Regency is one of the largest areas of lowland maize production in Central Java. Maize here is a primary source of income for farmers after rice. It is a strategic commodity that needs more attention from researchers to establish and improve yields. One of the key factors in maize production is nutrient sufficiency with site-specific, balanced fertilization.

The concept of site-specific nutrient management (SSNM) considers the indigenous supply of nutrients at a particular location and assesses the amount of nutrients needed by way of balanced fertilization (Dobermann and Fairhurst 2000; Witt and Dobermann 2002). Since 2002, the concept of SSNM has been used for maize in Nebraska, USA to realize the yield potential (Dobermann 2003 cited in Subandi *et al.* 2004). Site-specific nutrient management is an approach toward precise delivery of nutrients (dosage, kind and time of application) by considering plant needs and soil capacity (Makarim 2003).

Maize plants need N, P and K more than other nutrients. Deficiencies often are of these nutrients. In order to improve fertilization effectiveness and efficiency in maize, it is necessary to know the crop's response to fertilization. This study was conducted to assess the effectiveness of N, P and K fertilization in Toroh and Purwodadi subdistricts of Grobogan Regency in Central Java.

Methodology

Our research was conducted during the dry season (Jun-Oct) of three years (2004-2006) after rice. The source of irrigation for the experimental plots was local rainfall with additional irrigation from canals or rivers.

The site was a lowland Vertisol with heavy clayey soil texture, pH 7-8, low soil organic carbon (C) and nitrogen (N), high content of Ca, Mg and a high level of base saturation. One of the unique characteristics of Vertisols is soil cracking during the dry season.

The fertilization dosages and treatments used in the study are presented in Table 1.

Table 1. Fertilizer dosage and treatments used in the study of fertilization response in Grobogan district, Central Java, 2004-2006.

Variable	2004		2005		2006	
	ICM[1]	Regular	ICM	Regular	ICM	Regular
N (kg ha^{-1})	200	200	200	200	200	200
P_2O_5 (kg ha^{-1})	30	30	35	35	35	35
K_2O (kg ha^{-1})	75	75	100	100	100	100
Organic manure (kg ha^{-1})			3000		5000	
Plant spacing (cm)	75×17.5	80×20	75×20	75×20	75×20	75×20

Fertilizer treatment Minus N (+PK); Minus K (+NP); Minus P (+NK); Complete (+NPK)

[1.] ICM = improved crop management.

To test fertilizer response, we used the 'omission plot' approach in 5 replications spread over 5 locations. We recorded data on local climatic conditions, soil physical and chemical status, maize yield, yield response to fertilizer application and agronomic efficency. Data analysis used the simple model of descriptive statistics by referring to theSSNM model (Witt 2005)

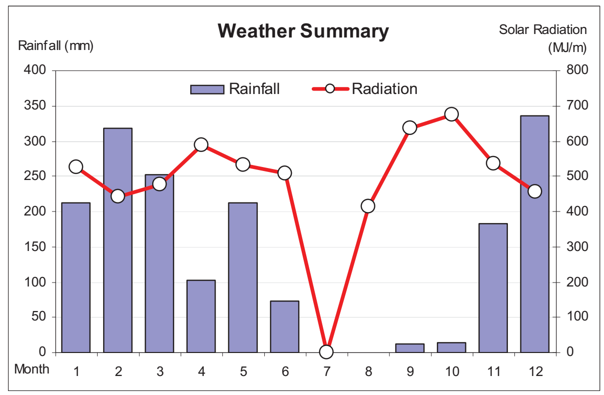

Figure 1. Annual rainfall and solar radiation in Grobogan district, Central Java.

Results and Discussion

Site characteristics

The experimental site was an irrigated lowland Vertisol (*Typic Hapluderts*). The average monthly rainfall is about 150 mm with 5 wet months and 5 dry months (Fig. 1). The cropping pattern practised is rice-rice-maize. Usually, maize is planted in the dry season (Jun-Oct), and is dependent on the minimum rainfall and additional irrigation from canals or rivers.

The soils at the experimental site (Table 2) have a heavy clayey texture with pH $\geq$8. The soil organic carbon (C) content is low (1.0-2.0%) with very low soil N content (<0.1%). The soil status of P_2O_5 and K_2O (HCl 25%) is very high: 1.580 mg 100 g^{-1} (P_2O_5) and 310 mg 100 g^{-1} (K_2O). The extractable nutrient content of P is also high: >20 ppm for P Olsen and >15 ppm for P Bray. Generally, the capacity of exchangeable cation (CEC) in Grobogan is moderate (>16 me) and is dominated by exchangeable cation Ca (>20 cmol kg^{-1}) and Mg (>2.0 cmol kg^{-1}). The domination of exchangeable Ca is evident from the high $CaCO_3$ content (17.6%) in the soil.

Some of the soil problems in the context of maize production here are: (1) low soil organic carbon and nitrogen; (2) high soil pH which may cause deficiency of nutrients such as Zn, Cu, Fe and Mn; (3) Vertisols with clay mineral type 2:1 (*montmorilonit*) may cause K deficiency because of strong fixation in the clay mineral interlayer; and (4) excessive soil cracking during the long dry season.

Table 2. Soil properties of experimental location.

Soil properties (0-20 cm of soil surface)	Value
Soil pH (1:2.5 H_2O)	8.3
Soil organic C (g kg^{-1})	1.18
Total soil N (g kg^{-1})	0.09
Soil nitrate N (mg kg^{-1})	80.6
Exchangeable K (cmol$_c$ kg^{-1})	0.3
Exchangeable Ca (cmol$_c$ kg^{-1})	59.5
Exchangeable Mg (cmol$_c$ kg^{-1})	3.4
Capacity of exchangeable cation (pH7)	24.3
Potential P_2O_5 (HCl 25%) (mg 100 g^{-1})	1580
Potential K_2O (HCl 25%) (mg 100 g^{-1})	310
Extractable P (Olsen-P, mg kg^{-1})	42
Extractable P (Bray-1 mg kg^{-1})	15
$CaCO_3$ (%)	17.6
Texture	Heavy clay

Soil type: Typic Hapludert, very fine texture, mixed, isohyperthermic

Table 3. Yieldof maize (t ha^{-1}) from experimental plots in Grobogan district in Central Java, 2004-2006.

Fertilizer treatment	Grain yield (MC 15.5%)			
	2004	2005	2006	Average
Minus N	2.35	1.70	1.47	1.8
Minus P	7.45	9.05	8.96	8.5
Minus K	8.20	9.20	9.39	8.9
NPK	8.65	10.45	10.81	10.00

Grain yield

The results (Table 3) show that the average grain yield from the NPK plot during 2004-2006 was 10.00 t ha^{-1}. Yield data from the omission plots (-N, -P, -K) indicated that omission of K fertilizer up to 100% (-K plot) decreased yield by about 10%. Omission of P fertilizer up to 100% (-P plot) decreased yield about 15%. Omission of N up to 100% (-N plot) decreased yield by about 82%.

Yield response to N, P and K

From the difference between the yields recorded by the NPK plot and each of the omission plots (-N, -P, -K), we can assess the yield response to the respective fertilizer (N, P or K). The average yield response of maize to N, P and K fertilizer application during the three crop seasons is shown in Table 4.

The results show that the maize crop in the lowland Vertisols of Grobogan district in Central Java has a very high response (8.1 t ha^{-1}) to N fertilizer, much higher than the yield response to P and K, which was 1.5 t ha^{-1} and 1.0 t ha^{-1} respectively. As per these findings, N fertilizer has the most important effect on the growth and yield of maize, whereas P and K have a lesser effect.

Agronomic efficiency

Agronomic efficiency (AE) is the amount of yield increase per unit of fertilizer nutrient applied (kg grain per kg fertilizer nutrient). By assessing the AE of a fertilizer nutrient, the amount of fertilizer needed for a crop can be predicted. The AEs of N, P and K for maize in Grobogan district are shown in Table 5.

The results show that N and P fertilizer have medium-high agronomic efficiencies of 41 kg kg^{-1} and 44 kg kg^{-1} respectively. Fertilizer K has a low agronomic efficiency of 11 kg kg^{-1}. These data indicate that N and P fertilizer are effective in increasing maize grain yield in the lowland Vertisols of Grobogan district. On the other hand, K fertilizer application has very low effectiveness, possibly because of the interlayer fixation of K by montmorilonit mineral (type 2:1).

Table 4. Yield response of maize to NPK fertilizer application, 2004-2006.

Fertilizer application	Yield response (t ha^{-1})*			
	2004	2005	2006	Average
N	6.3	8.8	9.3	8.1
P	1.2	1.4	1.9	1.5
K	0.5	1.3	1.4	1.0

*Calculated data from average grain yield.

Table 5. Agronomic efficiencies of N, P, K fertilizer for maize in Grobogan district, Central Java, 2004-2006.

Fertilizer	Agronomic efficiency (kg kg^{-1})*			
	2004	2005	2006	Average
N	32	44	47	41
P	40	40	53	44
K	6	13	14	11

*Calculated data from yield response and fertilizer rate.

Conclusions

1. The lowland Vertisols of Grobogan district have high soil pH, low soil organic carbon and N and mineral interlayer fixation of K nutrient. There is soil cracking during the dry season.
2. Maize grain yield from complete fertilizer (NPK) plots was 10 t ha^{-1}. Omission of N fertilizer decreased grain yield 82%. Omission of P fertilizer decreased yield by 15% and omission of K fertilizer decreased yield by 10%.
3. Maize yield response to N fertilizer was highest at 8.1 t ha^{-1}. The yield response to P fertilizer was 1.5 t ha^{-1} and that of K fertilizer 1.0 t ha^{-1}.
4. N and P fertilizers have a medium-high agronomic efficiency of 41 kg kg^{-1} (N) and 44 kg kg^{-1} (P), but K fertilizer has a low AE of only 11 kg kg^{-1}.

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Effect of N, P, K Dosages on Grain Yield and Protein Quality of QPM Variety QP4 and Normal Maize Variety LVN 10 in Thai Nguyen, Vietnam

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Abstract. This study assessed the effect of 5 levels of nitrogen (0 N, 60 N, 120 N, 180 N, 240 N), 5 levels of phosphorus (0 P_2O_5, 40 P_2O_5, 80 P_2O_5, 120 P_2O_5, 160 P_2O_5), and 5 levels of potassium (0 K_2O, 40 K_2O, 80 K_2O, 120 K_2O, 160 K_2O) on two maize cultivars, the quality protein maize (QPM) variety QP 4 and normal variety LVN 10. The experiment was conducted in Thai Nguyen, Vietnam over three cropping seasons: the spring and autumn-winter crops of 2005 and the spring crop of 2006. For both QP 4 and LVN 10, mean grain yield at the level of 240 N was higher than the control level (0 N) by 138.5% and 156.5% respectively. Similarly, protein content at this level of N was higher than control by 51.5% and 16.3%; lysine levels were higher by 123.6% and 68.4%; and methionine levels by 100% and 51.7%, respectively. For QP 4 the highest economic return resulted from the level of 180 N, while for LVN 10 it was at the 240 N level. At the 160 P_2O_5 level, mean grain yield of QP 4 and LVN 10 were higher than that of the control (0 P_2O_5) by 89.2% and 102.4% respectively. Similarly, protein content was higher by 20.8% and 5.5%; lysine levels were higher by 48.4% and 30.0%; and methionine levels by 28.3% and 23.7%, respectively. The highest yield and economic efficiency of QP 4 and LVN 10 were achieved at the level of 120 P_2O_5. At the level of 160 K_2O the mean grain yield of QP 4 and LVN 10 increased by 102.9% and 117.9% in comparison to control (0 K_2O) respectively; protein content was similarly higher by 9.7% and 2.7%; lysine levels higher by 38.1% and 29.2%; and methionine levels by 22.3% and 17.3%, respectively. The highest yield and economic efficiency of QP 4 and LVN 10 were at the level of 120 K_2O. The effect of N, P and K dosages on the grain yield of QPM variety QP 4 was lower than normal maize variety LVN 10. The effect of N, P and K dosages on the protein quality of QPM variety QP 4 was higher than that of normal maize variety LVN 10. The effect of nitrogen on grain yield and protein quality was the most clearly evident among the three nutrients studied.

Key words: Nitrogen, normal maize, phosphorus, potassium, protein, QPM

Introduction

Nitrogen is the most important nutrient affecting maize yield (Bennet 1993). Sinclair and Muchow (1995) stated that maize yield increases in the past decades have been closely related to the supply of nitrogen. Maize yields are also influenced by phosphorus (Evangelista 1999). Potassium is necessary for cytoplasmic activity, control of stomata and resistance to pests, drought and low temperatures. Potassium plays a role in the promotion of photosynthesis, carrying the products of photosynthesis to the grain (Afendulop 1972) and participating in the making of the energy-rich compound ATP which is related to the synthesis of starch and protein (Tysdale *et al*. 1985). However, studies of the effects of N, P and K on grain yield and protein and essential amino acid (lysine, tryptophan, methionine) levels in QPM and normal maize varieties have been few.

In view of this, we conducted a study on the effect of different dosages of N, P and K on the productivity and protein quality of a quality protein maize (QPM) variety QP4 and a normal variety LVN 10 in Thai Nguyen, Vietnam.

Materials and Methods

Using urea (46% N), superphosphate (16% P_2O_5) and Kaliclorua (60% K_2O), we conducted an experiment on three maize crops: the spring and autumn-winter crops of 2005 and the spring crop in exhausted soil with a sand mix in 2006 at the Thai Nguyen University of Agriculture and Forestry. The experiment with two elements was arranged in a split-plot design with three replications. Fertilizer was the major element, and variety the accessory element (G_1: QP 4 and G_2: LVN 10). The following fertilizer levels were used. For the nitrogen experiment (base: 10 t of muck + 80 kg P_2O_5 ha^{-1} + 80 kg K_2O ha^{-1}): N_1: 0 kg N ha^{-1} (control); N_2:

60 kg N ha^{-1}; N_3: 120 kg N ha^{-1}; N_4: 180 kg N ha^{-1}; N_5: 240 kg N ha^{-1}. For phosphorus (base: 10 t of muck + 120 kg N ha^{-1} + 80 kg K_2O ha^{-1}): P_1: 0 kg P_2O_5 ha^{-1} (control); P_2: 40 kg P_2O_5 ha^{-1}; P_3: 80 kg P_2O_5 ha^{-1}; P_4: 120 kg P_2O_5 ha^{-1}; P_5: 160 kg P_2O_5 ha^{-1}. For potassium (base: 10 t of muck + 120 kg ha^{-1} N + 80 kg ha^{-1} P_2O_5): K_1: 0 kg K_2O ha^{-1} (control); K_2: 40 kg K_2O ha^{-1}; K_3: 80 kg K_2O ha^{-1}; K_4: 120 kg K_2O ha^{-1}; K_5: 160 kg K_2O ha^{-1}. The main plot was 44.1 m^2 (10.5 × 4.2 m), and the subplot 21 m^2 (5 × 4.2 m). Sowing was done in 6 lines per plot with plant spacing of 70 × 25 cm (density of 5.7 ten thousand plants ha^{-1}). Cultivation was done according to the recommended practices of the National Maize Research Institute and CIMMYT, test norm of fertilizer of 10TCN216-95. Quality analysis was done at the Central Laboratory of the Thai Nguyen University of Agriculture and Forestry.

Results and Discussion

Effect of N

Effect of different levels of N on yield. Under the effect of different nitrogen rates, the real revenue yields ranged from 2.44 t ha^{-1} (0 N) to 5.82 t ha^{-1} (240 N) for QP 4 and from 2.53 t ha^{-1} (0 N) to 6.49 t ha^{-1} (240 N) for LVN 10. Yield at the 240 N level was higher by 138.5% for QP 4 and by 156.5% for LVN 10 when compared to control (0 N). All nitrogen fertilization levels (60-240 N) resulted in significantly higher real revenue yield than the treatment without nitrogen. The 0 N treatment gave the lowest real revenue yield, followed by the nitrogen levels of 60–120 N. At nitrogen levels of 180-240 N, the real revenue yield was similar at 95% confidence intervals. At the 120-240 N levels, the real revenue yield for QP 4 was lower than for LVN 10 but at the 0-60 N levels the real revenue yields for both QP 4 and LVN 10 were similar at 95% confidence intervals.

Effect of different levels of N on protein quality. At different levels of N, total protein content ranged from 7.48% (0 N) to 11.33% (240 N) for QP 4 and from 7.66% (0 N) to 8.92% (120 N) for LVN 10. Protein levels increased positively with increasing levels of N, being 51.5% higher than control at 240 N for QP 4 and 16.3% higher for LVN 10. All fertilization levels of N resulted in higher protein content than control. The 240 N level gave the highest protein level while the 120 N and 180 N levels yielded the same protein content. The quality protein maize (QPM) variety QP 4 recorded higher levels of protein at equivalent N levels than LVN 10 at 95% confidence intervals. The effect of different levels of N on the protein level in QP 4 was significantly higher than in LVN 10.

Lysine levels at different rates of N fertilization ranged from 1.91% (0 N) to 4.27% (240 N) for QP 4 and from 1.58% (0 N) to 2.66% (240 N) for LVN 10. Lysine content increased gradually with increasing levels of N. At 240 N, it was 123.6% higher than control in QP 4 and 68.4% in LVN 10. All the N rates experimented with resulted in higher lysine content than control, the highest being attained at 240 N. The 120 N and 180 N levels resulted in the same lysine content. The variety QP 4 had higher levels of lysine at equivalent rates of N than the variety LVN 10 at 95% confidence intervals. Thus, the effect of nitrogen levels on lysine content was greater in QP 4 than LVN 10.

At different N rates, methionine content in the two maize varieties ranged from 1.70% (0 N) to 3.40% (240 N) for QP 4 and from 1.49% (0 N) to 2.26% (240 N) for LVN 10. Methionine content gradually increased with increasing levels of N. At 240 N, it was higher than control by 100.0% for QP 4 and by 51.7% for LVN 10. All rates of N recorded higher methionine levels than control, the highest being recorded at 240 N. Nitrogen rates 120 N and 180 N resulted in the same methionine level. The variety QP 4 had higher levels of methionine at equivalent N rates than LVN 10 at 95% confidence intervals. Thus, the effect of different levels of N was greater in QP 4 than LVN 10 in terms of methionine content. Our research results correspond with the results reported by Sinclair and Muchow (1995), (Schrader 1984) and Bennet (1993).

Effect of P

Effect of different levels of P on yield. Increasing levels of P clearly resulted in an increase in real revenue yields. These ranged from 2.88 t ha^{-1} (0 P_2O_5) to 5.45 t ha^{-1} (160 P_2O_5) for QP 4 and from 2.98 t ha^{-1} (0 P_2O_5) to 6.03 t ha^{-1} (160 P_2O_5) for LVN 10. At the 160 P_2O_5 level, real revenue yield increased by 89.2% over control (0 kg ha^{-1} P_2O-$_5$) for QP 4 and by 102.4% for LVN 10. All fertilization rates of P (40-160 P_2O_5) recorded higher real revenue yield than the control. The P levels 120 P_2O_5 and 160 P_2O_5 recorded the same real revenue yield at 95% confidence intervals. Real revenue yield was lower in QP 4 than LVN 10 at P levels 80-160 P_2O_5, but P levels 0 P_2O_5 and 40 P_2O_5, real revenue yields were similar in QP 4 and LVN 10 at 95% confidence intervals. In short, the effect of P on the two maize varieties was very clear, the yields increasing positively with increasing levels of P. These results confirm the results reported by Evangelista (1999).

Effect of different levels of P on protein quality. At different levels of P, protein content in the two maize varieties ranged from 9.05% (0 P_2O_5) to 11.03% (120 P_2O_5) for QP 4 and from 8.07% (0 P_2O_5) to 8.88% (120 P_2O_5) for

LVN 10. Protein content increased with increasing levels of P and stopped at 120 P_2O_5. All the experimental P levels resulted in higher protein content than control. At levels 120 P_2O_5 and 160 P_2O_5, the protein content was equivalent. The maize variety QP 4 recorded higher protein levels at equivalent P fertilization rates than LVN 10 at 95% confidence intervals.

At different rates of P, lysine content in the two maize varieties ranged from 2.87% (0 P_2O_5) to 4.26% (160 P_2O_5) for QP 4 and from 2.10% (0 P_2O_5) to 2.73% (160 P_2O_5) for LVN 10. The lysine level gradually increased with increasing P. At the 160 P_2O_5 level lysine content was higher than control (0 P_2O_5) by 48.4% in QP 4 and by 30.0% in LVN 10. All the fertilization rates of P resulted in higher lysine level than control, the highest level being recorded at 120-160 P_2O_5; The variety QP 4 showed higher lysine levels than LVN 10 at 95% confidence intervals.

The effect of different levels of P on methionine levels in the two maize varieties was very clear. Methionine content ranged from 2.51% (0 P_2O_5) to 3.22% (160 P_2O_5) for QP 4 and from 1.73% (0 P_2O_5) to 2.14% (160 P_2O_5) for LVN 10. The methionine levels gradually increased with increasing P. At 160 P_2O_5 the methionine level increased by 28.3% over control (0 P_2O_5) in QP 4 and by 23.7% in LVN 10. All fertilization rates of P recorded a higher methionine level than control, the 120-160 P_2O_5 levels obtained the highest methionine level. The variety QP 4 had higher methionine levels than LVN 10 at equivalent rates of P at 95% confidence intervals. Thus, the effect of different levels of phosphorus on the levels of protein, lysine and methionine was higher in QP 4 than LVN 10.

Effect of K

Effect on yield. Potassium also had a clear effect on maize real revenue yield, which gradually increased with tincreasing levels of K. The yield increases ranged from 2.78 t ha^{-1} (0 K_2O) to 5.64 t ha^{-1} (160 K_2O) for QP 4 and from 2.79 t ha^{-1} (0 K_2O) to 6.08 t ha^{-1} (160 K_2O) for LVN 10. At 160 K_2O, real revenue yield increased by 102.9% in QP 4 and 117.9% in LVN 10 over control (0 K_2O). The effect of different levels of K on real revenue yield was smaller in QP 4 than LVN 10. All experimental rates of fertilization of K (40 K_2O-160 K_2O) led to higher real revenue yield than control. At 120 K_2O and 160 K_2O, the real revenue yield was similar at 95% confidence intervals. The variety QP 4 showed the same real revenue yields as LVN 10 at 95% confidence intervals. At the same levels of K (120-160 K_2O), the real revenue yield in QP 4 was lower than in LVN 10 but at levels 0-80 K_2O their yield was the same at 95% confidence intervals. The highest yield was recorded at 160 K_2O. These results correspond with the results reported by Nguyen Trong Thi và Nguyen Van Bo (1999).

Effect on protein quality. The etotal protein content in the two maize varieties ranged from 10.15% (at 0 K_2O) to 11.13% (at 160 K_2O) for QP 4 and from 8.29% (0 K_2O) to 8.55% (80 K_2O) for LVN 10. At 160 K_2O, the protein level increased by 9.7% in QP 4 and by 2.7% in LVN 10 over control (0 K_2O). The two maize varieties fertilized at 80-160 K_2O showed higher levels of protein than the control treatment. At 0-40 K_2O levels, protein content was lowest. The variety QP 4 had higher levels of protein at the same rate of K fertilization than LVN 10 at 95% confidence intervals. The effect of different levels of K on protein content in QP 4 was very clear, whereas in LVN 10 it was unclear.

Lysine content at different levels of K ranged from 2.99% (at 0 K_2O) to 4.13% (160 K_2O) in QP 4 and from 2.19% (0 K_2O) to 2.83% (160 K_2O) in LVN 10. Lysine content gradually increased with increasing levels of K and at 160 K_2O, it increased by 38.1% in QP 4 and by 29.2% in LVN 10 over their respective controls. All rates of fertilization of K recorded higher levels of lysine than the control; The K levels 80-160 K_2O had the same lysine content. The QPM variety QP 4 had higher levels of lysine than LVN 10 at 95% confidence intervals. Also, as with protein content, the effect of different rates of K fertilization on lysine levels was very clear in QP 4 but not so in LVN 10.

Methionine content ranged from 2.65% (at 0 K_2O) to 3.24% (160 K_2O) in QP 4 and from 1.79% (0 K_2O) to 2.16% (120 K_2O) in LVN 10. At 160 K_2O, it was 22.3% higher than control in QP 4 and 17.3% higher than control in LVN 10. All rates of K fertilization showed higher levels of methionine than the control treatment. The K levels 80 K_2O and 160 K_2O gave the same level of methionine. The variety QP 4 had higher levels of methionine than LVN 10 at 95% confidence intervals. potassium levels clearly affected the levels of protein, lysine and methionine in the QPM variety QP 4 but not so clearly in the normal maize variety LVN 10.

Table 1 shows the effect of different levels of N, P, and K on some major factors of QP 4 and LVN 10. At 240 N, 160 P_2O_5 and 160 K_2O, the increments in these norms are much higher than the respective control treatments (0 N, 0 P_2O_5 and 0 K_2O). The effect of different levels of N, P, K on grain yield of the QPM variety QP 4 is lower than on the grain yield of LVN 10 (Fig. 1). In contrast, for the quality norms, the effect of N, P and K on QP 4 is higher than LVN 10. The effect of N on the yield and quality of protein was the most obvious among the three elements, N, P and K.

Table 1. Increments (%) achieved by nitrogen fertilization level 240 N, phosphorus level 160 P_2O_5 and potassium level 160 K_2O over their respective controls in two varieties QP 4 and LVN 10 (mean of 3 crops).

Variable	N		$P\text{-}_2O_5$		K_2O	
	QP 4	LVN 10	QP 4	LVN 10	QP 4	LVN 10
Yield	+138.5	+156.5	+90.2	+102.8	+102.9	+117.9
Total protein	+51.5	+16.3	+20.8	+5.5	+9.7	+2.7
Lysine/protein	+123.6	+68.4	+48.4	+30.0	+38.1	+29.2
Methionine/protein	+100.0	+51.7	+28.3	+23.7	+22.3	+17.3

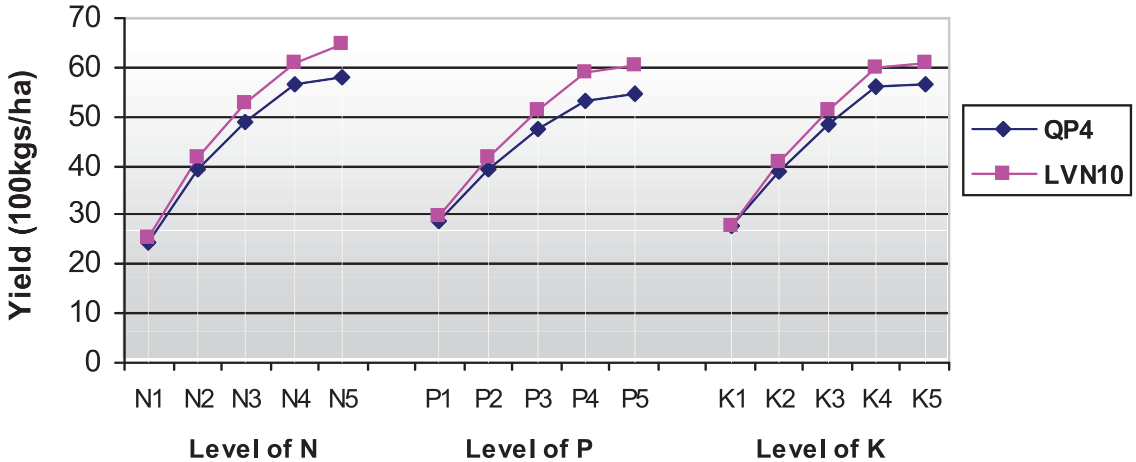

Figure 1. Effect of different levels of nitrogen, phosphorus, potassium on maize yields in QP 4 and LVN 10.

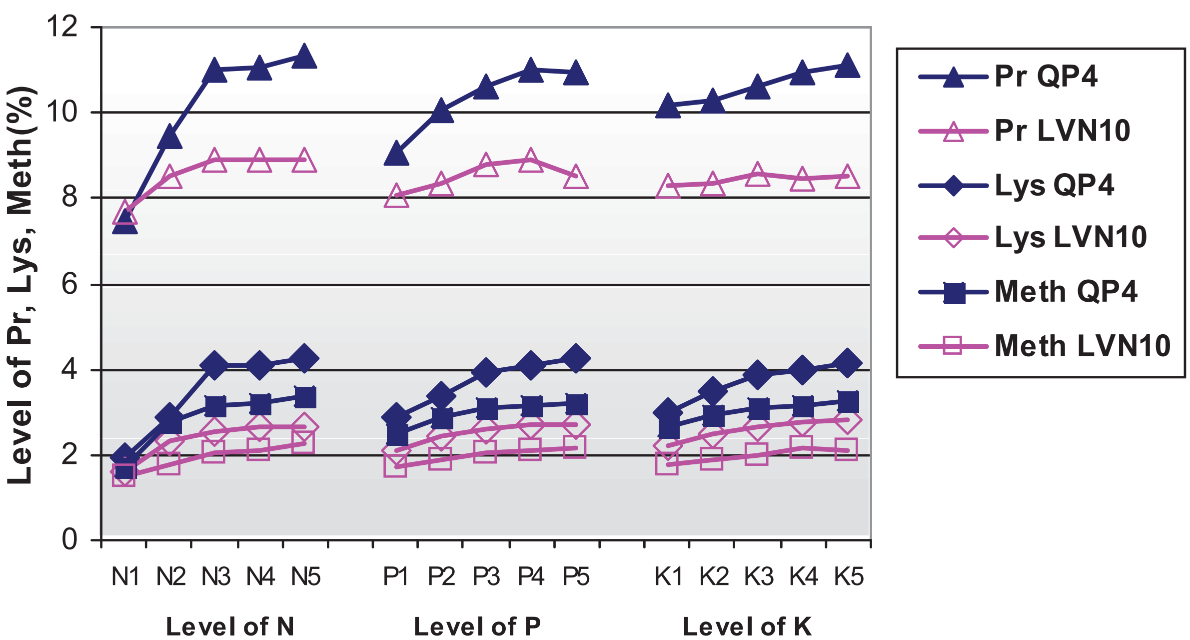

Figure 2. Effect of different levels of nitrogen, phosphorus and potassium on protein (Pr), lysine (Lys) and methionine (Meth) content in varieties QP 4 and LVN 10.

Figure 2 shows that the effect of different levels of N, P and K on protein, lysine and methionine content was greater for QP 4 than LVN 10. Especially, the K levels had less effect on the level and quality of protein in maize variety LVN 10.

Economic efficiency

Nitrogen. Fertilized at the rate of 180 kg ha^{-1}, N had the highest economic efficiency in QP4 but for LVN10 the highest efficiency was attained at the level of 240N.

Phosphorus. The highest economic efficiency in both QP 4 and LVN 10 was achieved at the 120 P_2O_5 level.

Potassium. The 120 K_2O level produced the highest economic efficiency in both QP 4 and LVN 10.

Conclusions

The different levels of N, P and K had a greater effect on grain yield in QP 4 than in LVN 10, but the level and quality of protein in QP 4 was higher LVN 10. Of the 3 elements studied, N had the greatest effect on grain yield and quality of protein in both QPM and LVN 10. The effect of different rates of potassium on the level and quality of protein in the QPM variety QP 4 was very clear but unclear in LVN 10.

At 240 N, grain yield increased by 138.5% in QP 4 and by 156.5% in LVN 10; protein content increased by 51.5% in QP 4 and by 16.3% in LVN 10; lysine levels increased by 123.6% in QP 4 and by 68.4% in LVN 10; methionine increased by 100% in QP 4 and by 51.7% in LVN 10. For QP 4, the highest grain yield would be attained if fertilized at the rate of 180 kg ha^{-1} N and for LVN 10 it would be at the 240 N level. Protein, lysine and methionine levels would be highest at the 240 N level.

Grain yield increased by 89.2% over control in QP 4 and 102.4% in LVN 10 at the level of 160 $P_2O_{5.}$ At this P level, protein content increased by 20.8% over control in QP 4 and by 5.5% in LVN 10; lysine increased by 48.4% in QP 4 and by 30.0% in LVN 10; and methionine increased by 28.3% in QP 4 and by 23.7% in LVN 10. For both varieties, yield was highest at the level of 120 P_2O_5. At the 120-160 P_2O_5 level, protein, lysine and methionine levels were highest.

When compared to control (0 K_2O), grain yield increased by 102.9% in QP 4 and 117.9% in LVN 10 at the 160 K_2O level; protein content increased by 9.7% in QP 4 and by 2.7% in LVN 10 at the same level; lysine increased by 38.1% in QP 4 and by 29.2% in LVN 10; and methionine increased by 22.3% in QP 4 and by 17.3% in LVN 10. With QP 4 and LVN 10, at the level of 120 K_2O, the yield would be the highest. At the levels of 80-160 K_2O, the levels of protein, lysine and methionine were higher than the levels attained at 0-40 K_2O.

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Nutrient Management in Rainfed Lowland Rice-Maize Cropping Systems of Indonesia

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Abstract. Rice-maize is an important rotation in the rainfed lowland areas of Indonesia. Low crop yields associated with poor nutrient-use efficiency are the major production constraint in these areas. Therefore, improving nutrient-use efficiency by balancing and phasing nutrients for both crops in a systemic perspective is a key concern. Toward that objective, an experiment was conducted at the ICERI experimental farm in Maros during the wet and dry seasons of 2007 with four tranches (phasings) of NPKS fertilizer in a rice-maize rotation in a randomized block design with four replications. Dividing the combination of four fertilizers (600 kg ha^{-1} urea + 200 kg ha^{-1} SP 36 + 200 ha^{-1} KCl + 100 kg ha^{-1} ZA) into two tranches, 50% for rice and 50% for maize, gave the highest yield of both rice (5.01 t ha^{-1}) and maize (8.30 t ha^{-1}). This finding suggests that for higher productivity in a rainfed lowland ecosystem fertilizer nutrients should be applied in phases with a systemic approach.

Key words: Cropping system, rice-maize, nutrient management, rainfed lowland.

Introduction

Maize development is a priority task for the Indonesian Government because it is an important feed and industrial material whose demand has been increasing (Deptan 2002). In Indonesia maize is generally cultivated in dryland areas but its production is not sufficient to meet national demand. Increasing domestic demand has spurred the cultivation of maize in irrigated lowlands and rainfed lowlands. The growth rate of maize planting area in the lowlands and rainfed lowlands has been estimated at 10-15% and 20-30% respectively (Kasryno 2002).

Maize development in the lowlands holds more promise compared to drylands. To boost productivity of rice and/or maize in the rice-maize cropping system, it needs efficient fertilizer management to improve the nutrition status of the soil. Cambirato and Frederick (1994) reported that N fertilization of maize increased the NO_3-N of soil for the succeeding wheat crop, leading an increase in dry material, N content and grain yield of wheat. However, fertilizer rates for rice-maize cropping systems are conventionally calculated separately for each commodity regardless of the fertilizer residue of the preceding crop.

Fertilizer applied to soil is not entirely taken up by the plant. Only 55-60% of the nitrogen (Patrick and Ready 1976), 20% of the phosphorus (Hangin and Tucker 1982; Goswani *et al*. 1990), 50-70% of the potassium (Tisdale and Nelson 1985) and 33% of the sulfur (Morris 1987) is absorbed. Therefore, when using inorganic fertilizer for rice-maize cropping in the lowlands, the fertilizer residue of each commodity should be considered. If not, it may result in an imbalance in the soil's nutrient levels. Accumulation of P may lead to deficiency of Zn; K accumulation may restrict the absorption of Ca; and sulfur (S) accumulation may change the soil pH.

Materials and Methods

This experiment was conducted in a rainfed lowland plot at the Maros experimental farm of ICERI in the wet and dry seasons of 2007. We used a randomized block design with four replications, and the plot size was 6 m × 4 m. The rates of NPKS fertilizer used in the different tranches are given in Table 1, the total amounting to 600 kg urea + 200 kg SP 36 + 100 kg KCl + 100 kg ZA ha^{-1}. Rice was planted in the wet season (Jan-May). The first plowing was done in a dry condition and the second harrowing in saturated conditions. The land was prepared using a tractor/hoe for levelling. The rice variety Cisantana was planted with 20 × 20 cm spacing, one seedling per hill. Fertilizers ZA and SP 36 were applied in two phases: 50% fertilizer applied 7 days after planting (DAP) and 50% 25 DAP; KCl was applied in 50% doses at 7 DAP and 40 DAP. Urea was applied three times: 7 DAP, 25 DAP and 40 DAP. Pest and disease management was done by monitoring and control if disease occurred.

The maize variety Bima-3 Bantimurung was planted immediately after rice was harvested (Jun-Sep 2007) with no tillage. Planting was done at the rate of two seeds per hole with 75 cm × 20 cm spacing. Pruning to one plant per hill was done at 6 DAP. Weeds were controlled with the herbicide Glifosfat (active ingredient) at the rate of 2 L ha^{-1}. Fertilizing was done beside the plant at 7 DAP with ZA and SP 36 applied whole, urea applied at the rate of 110 kg ha^{-1} and 50% of KCl. The remaining dose of urea and KCl was applied at the V9 phase (40 DAP.)

Data collection

Soil samples were collected before the planting of rice and maize. The sampling method was random at 0-40 cm depth over the whole plot and then composited. Plant height was measured from the base of the stem to the tip of the highest leaf at harvest time. Grain yield was measured on 3 m × 3 m size per plot and converted to 14% water content.

Results and Discussion

The soil at the research site was classified as an Entisol with silty clay texture, moderately acidic pH, low N content, moderate K level, high CEC and basin saturation (Table 2). Such soils are good enough to support maize growth although they still need additional fertilizer especially in soils with low nutrient status.

Recordings of rice plant height at harvest showed that if 75% (450 kg urea + 75 kg ZA + 150 kg SP 36 + 150 kg KCl ha^{-1}) of the total fertilizer dosage was given to rice, it gave the highest plant height, even though the result was not significantly different from the other fertilization treatments. However, allocation of 75% of the total fertilizer dosage to rice would have a predominance of urea, which accelerates vegetative growth in rice but on the other hand decreases generative growth and affects grain yield. Application of 50% (300 kg urea + 50 kg ZA +100 kg SP 36 + 100 kg KCl ha^{-1}) of the total fertilizer to rice gave the highest yield of rice grain (5.01 t ha^{-1}), while the same rate of fertilizer allocated to maize gave a yield of 8.30 t ha^{-1}. This means that 300 kg of urea ha^{-1} was enough for optimum maize grain yield. Zhao *et al.* (2006) reported that optimum N fertilizer dramatically decreased NO_3-N flow to the deeper layers of the soil. Even so, N would be available to the succeeding crop.

Allocation of 75% (450 kg urea + 75 kg ZA + 150 kg SP 36 + 150 kg KCl ha^{-1}) of the total fertilizer to rice gave a high enough yield of maize grain (6.62 t ha^{-1}). This phenomenon indicated that residual fertilizer from rice was available to the succeeding maize. Steven *et al.* (2005) reported that the N residue in the soil only accrued to the succeeding crop if the N rate had exceeded optimum. Our study indicated that apportioning 50% of the total fertilizer dosage to rice and maize in a rice-maize cropping system attained the highest total grain yield (13.30 t ha^{-1} in our study) compared to the other fertilizer allocations we studied. This finding suggests that for higher productivity in a rainfed lowland ecosystem, fertilizers should be applied in appropriate allocations taking the cropping system as a whole.

Table 1. Fertilizer dosage used for rice-maze cropping in a rainfed lowland area, Maros, Indonesia, 2007.

Treatment	Dosage[1] of NSPK (kg ha^{-1})	
	Rice	Maize
A (control)	250	350
Rice	50	50
Maize	100	100
	100	100
B	450	150
Rice 75%	75	25
Maize 25%	150	50
	150	50
C	300	300
Rice 50%	50	50
Maize 50%	100	100
	100	100
D	150	450
Rice 25%	25	75
Maize 75%	50	150
	50	150

[1.] Total fertilizer dosage for rice-maize cropping system: 600 kg urea + 100 kg ZA + 200 kg SP 36 + 100 kg KCl ha^{-1}.

Table 2. Soil analysis before rice planting in rice-maize cropping system in Maros, Indonesia, 2007.

Variable	Value	Status
Texture		
Clay (%)	22	Silty clay
Silt (%)	43	
Sand (%)	35	
pH H_2O (1:2.5)	5.90	Moderately acid
pH KCl (1:2.5)	4.88	
Organic C (%)	0.99	Low
N total (%)	0.10	Low
C/N	9.9	Low
P (Baray I (ppm)	13.70	Moderate
Kdd (me/100 g)	0.45	Moderate
Cadd (me/100 g)	18.45	High
Mgdd (me/100 g)	1.11	Moderate
Nadd (me/100 g)	0.58	Moderate
H^+	0.29	-
CEC (me/100 g)	27.82	High
Basin saturate (%)	74	Very high

Table 3. Plant height and grain yield of rice and maize under different allocations of NPKS fertilizer[1], Maros, Indonesia, 2007.

Treatment	Rice		Maize	
	Plant height (cm)	Grain yield (t ha^{-1})	Plant height (cm)	Grain yield (t ha^{-1})
A. Control	122.4ab	4.90ab	204.3a	8.11a
B. Rice (75%) Maize (25%)	120.1ab	3.91b	208.1ab	6.62b
C. Rice (50%) Maize (50%)	122.4a	5.01a	210.4ab	8.30a
D. Rice (25%) Maize (75%)	120.4ab	4.41ab	215.7b	8.41a
CC (%)	5.0	12.3	3.0	8.2

[1.]Total fertilizer used in rice-maize cropping system: 600 kg urea + 100 kg ZA + 200 kg SP 36 + 100 kg KCl ha^{-1}.

Conclusions

Allocation of the total NPKS fertilizer dosage (600 kg urea + 200 kg SP 36 + 200 KCl + 100 kg ZA ha^{-1}) in equal proportions, 50% to rice and 50% to maize in a rice-maize cropping system gave the highest yield of both rice (5.01 t ha^{-1}) and maize (8.30 t ha^{-1}). This finding suggests that fertilizer use efficiency and productivity of a rice-maize cropping system in the rainfed lowlands of Indonesia would be optimized if the fertilizer dosage was appropriately apportioned to the two crops.

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Response of Promising Maize Cultivars to Different Nitrogen Levels during Winter

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Abstract. Maize plays a vital role in the economic status of marginal and poor farmers in rural Nepal. In the lowland plains of Nepal, wheat is being gradually replaced by winter maize because of the latter's higher yield potential and demand for food and feed purposes. This experiment was aimed at identifying the optimum dose of nitrogen for two pipeline maize genotypes. The field experiment was conducted at the National Maize Research Program (NMRP), Rampur, Chitwan during the winter season (Sep-March) of two consecutive years. The experiment was laid out in a two-factor factorial randomized complete block design with four replications. Two maize varieties, Deuti (ZM-621) and Arun-4, were tested at eight nitrogen levels (0, 30, 60, 90, 120, 150, 180 and 210 kg ha^{-1}). The results revealed that increasing nitrogen levels from 0 to 180 kg ha^{-1} increased the grain yield of both cultivars. However, there was no significant effect on grain yield between 90 and 180 kg ha^{-1} N. Application of 30, 60, 90, 120, 150, 180 and 210 N kg ha^{-1} increased grain yield by 19, 27, 30, 41, 54, 59 and 49% over control, respectively. Yield-attributing characters like the number of harvested ears per unit area, number of grain per ear, and 1000-grain weight contributed to increased yield with increasing nitrogen levels up to 90 kg ha^{-1}. Deuti (ZM-621) yielded more (5.7 t ha^{-1}) than Arun-4 (5.0 t ha^{-1}). Net return increased with increasing N levels, the maximum (2.58) being achieved at 180 N kg ha^{-1}. The cost:benefit ratio too was highest at 180 N kg ha^{-1}. There was no significant interaction effect between maize cultivars and nitrogen levels for all the tested parameters, suggesting that both cultivars responded similarly to different rates of N.

Keywords: Cultivars, experiment, marginal, promising, nitrogen, potential

Introduction

Maize (*Zea mays* L.) is the second most important staple food crop after rice in terms of area and production in Nepal. Maize is grown over 0.87 million ha with an average yield of 2.2 t ha^{-1} (Government of Nepal 2007), and contributes about 6.8% to the agricultural gross domestic product. Maize contributes largely to food security in the hills whilst in the accessible areas it is gradually becoming a commercial commodity due to the growth of the poultry and other industries (Pathik 2001). The average annual growth rate for maize yield in Nepal is 1.05% (Poudyal and Ransom 2001). On the other hand, demand for maize has been estimated to grow by 6-8% per annum over the next two decades to meet the increased food demand from the increasing population and the growing livestock feed industry (Pathik 2001). In recent years, increased use of high-yielding crop varieties in intensive cropping systems has led to a substantially higher demand for nutrients. Locally available sources of nutrients, mainly farmyard manure (FYM), compost and biologically fixed nitrogen (N), are not sufficient to meet the crop demand. Depletion of organic matter due to high soil runoff and poor soil management are at the center of the overall problem of soil fertility decline. Nitrogen, the major plant nutrient, is very low in Nepalese soils (Tripathi and Shrestha 2001). Most of maize the research done in Nepal recommends a nitrogen level of 120 kg ha^{-1}. This blanket recommendation, however, does not address all maize growing conditions. Fertilizer recommendations must consider the risk of overapplication as well as underapplication to the profitability of farmers. The need for research that addresses the interests of both small-scale as well as commercial maize farmers has been realized. This need is further justified by observations that the bulk of maize in the country is produced by small-scale farmers one the one hand and maize farming in the plains and irrigated areas where high input supply is suitable for higher yield on the other. Therefore, it is necessary to identify the response of the promising genotypes to different levels of N. The objectives of our study were:

- To identify the optimum N dose for the maize varieties Deuti (ZM 621) and Arun-4 in the winter season; and
- To determine the effect of different levels of N on maize growth, yield and yield attributes.

Materials and Method

Our field experiment was conducted at the research farm of the National Maize Research Program, Rampur, Chitwan situated in central Nepal at 27°372 N latitude and 84°252 E longitude at an elevation of 228 m. The location has subtropical hot and humid climate with a minimum temperature range of 5-10°C in January and a maximum range of 34-38°C in April-May. Total N and organic matter are at a moderate level whereas available P_2O_5 and K_20 are higher. The soil is moderately acidic. Our experiment was laid out in a two-factor factorial randomized complete block design (RCBD) with four replications in which the two maize varieties constituted one factor and eight doses of nitrogen the other. Interrow spacing was 75 cm and plant-to-plant 25 cm. All plots received 60 kg ha^{-1} phosphorus as single super phosphate and 40 kg ha^{-1} potash as muriate of potash at sowing. Every plot except the 0 kg ha^{-1} N treatment received half the dose of N as urea at sowing. The remaining half dose of N was side-dressed twice with urea at 30 days after sowing (DAS) and at the silking stage. Two seeds per hill were planted with a planter at 5 cm depth and thinned to one plant at 21 DAS. Weeding was done manually at 21 DAS and earthing up at 45 DAS. Other cultural practices were followed as recommended for improved maize cultivation in Nepal. Parameters like the number of green leaves per plant, leaf area index (LAI), plant and ear height, male and female flowering, physiological maturity, lodging, barren plants per plot, and yield-attributing characters like the number of harvested ears, ear length and circumference, kernels per ear, and 1000-grain weight were recorded. Similarly, stover yield, above-ground dry matter, harvest index and grain:stover ratio, grain nitrogen uptake and nitrogen-use efficiency (agronomic efficiency, apparent N-recovery percentage, and physiological efficiency) were also calculated. Economic analysis of the benefit:cost ratio, net income and percentage increment in net income over control were also estimated.

Results and Discussion

We observed a significant effect on the number of kernels per ear between the two cultivars. Deuti (393) produced significantly more kernels per ear than Arun-4 (364). Grain number per ear depends upon the potential number of reproductive ovules which is established early in plant development, and is fixed at the 5th leaf stage of crop growth (Below 1996). Similarly, the growth duration of a cultivar might cause variation in the grain number per ear. Adhikari *et al.* (2004) and Gurung *et al.* (2001) also observed less grain per ear in early-maturing cultivars than in full-season ones. Kernels per ear differed significantly due to the different N levels. Increasing the N level increased the number of kernels per ear. Irrespective of the variety, the number of kernels per ear was highest (410) at 180 kg ha^{-1} N. It was significantly higher than the number of kernels per ear obtained from 0 to 90 kg ha^{-1} N but statistically similar with the other N levels (Table 1).

We detected a variety effect on grain yield. Deuti produced significantly higher grain yield (5.7 t ha^{-1}) than

Table 1. Response of two maize cultivars to different N levels, 2005-06, National Maize Research Program, Rampur, Chitwan, Nepal.

Treatment	Grain yield (t ha^{-1})	Dry matter (t ha^{-1})	Grain N uptake (kg ha^{-1})	Number of kernels per ear	1000-grain weight (g)
Cultivar					
Deuti	5.7a[1]	11.3a	108a	393a	326a
Arun-4	5.0b	10.2b	94b	364b	313b
Nitrogen level (kg ha^{-1})					
0	4.0d	7.7c	76e	331d	268d
30	4.7cd	9.4bc	88de	359cd	304c
60	5.0bcd	9.9b	93cde	372bc	311c
90	5.2abc	10.1b	97bcd	378bc	316bc
120	5.6abc	11.2ab	106abcd	379abc	341a
150	6.1ab	12.3a	118ab	401ab	347a
180	6.3a	12.7a	119a	410a	329abc
210	5.9ab	12.3a	112abc	395ab	340ab
LSD	1.02	1.8	19.2	28.5	22.5
SE (±)	0.358	0.632	6.7	10.0	7.9
CV%	19	17	19	8	7

[1.] Treatment means followed by the same letter/letters within a column are not significantly different between cultivars and among N levels respectively as per DMRT at 5% level of significance.

Arun-4 (5.0 t ha^{-1}). Deuti is a full-season variety with a maturity period of 143 days whereas Arun-4 is an early-maturing variety with a maturity period of 120 days. It has been reported that full-season varieties yield more than early-season ones because they get more time for photosynthesis and use more resources than early varieties. Nitrogen levels significantly influenced grain yield. The highest grain yield (6301 kg ha^{-1}) was obtained at 180 kg ha^{-1} N, which was statistically higher than yields obtained at 0, 30 and 60 kg ha^{-1} N but not significantly different from yields at 90, 120, 150 and 210 kg ha^{-1} N.

In general, grain yield increased with increasing N levels up to 90 kg ha^{-1}. This was in agreement with results reported by Tripathi and Pathak (1999) and Kamprath *et al.* (1982). Greenwood (1976) reported that N can influence photosynthesis by affecting the leaf area itself or through changes in photosynthetic rate per unit leaf area. Grain yield increases with increase in maximum LAI only up to a point. When there is more LAI, there is more photosynthesis, more assimilates, and more grain yield. Kamprath *et al.* (1982) reported that the increase in maize yield after N application is largely due to an increase in the number of ears, total dry matter distributed to the grain and increase in the average ear weight.

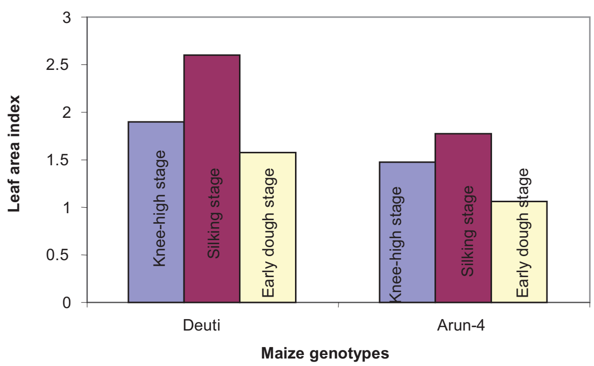

Figure 1. Response of two maize cultivars to leaf area index.

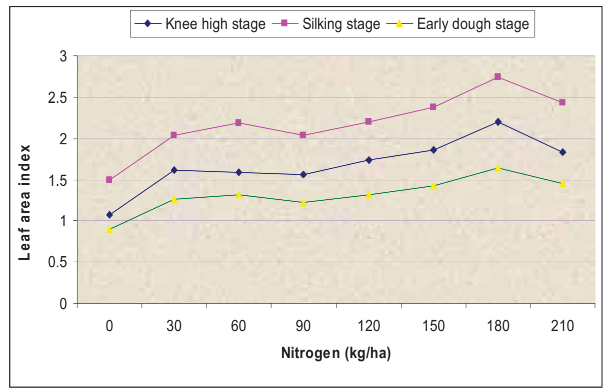

Figure 2. Influence of different nitrogen levels on leaf area index measured at different stages of the maize crop.

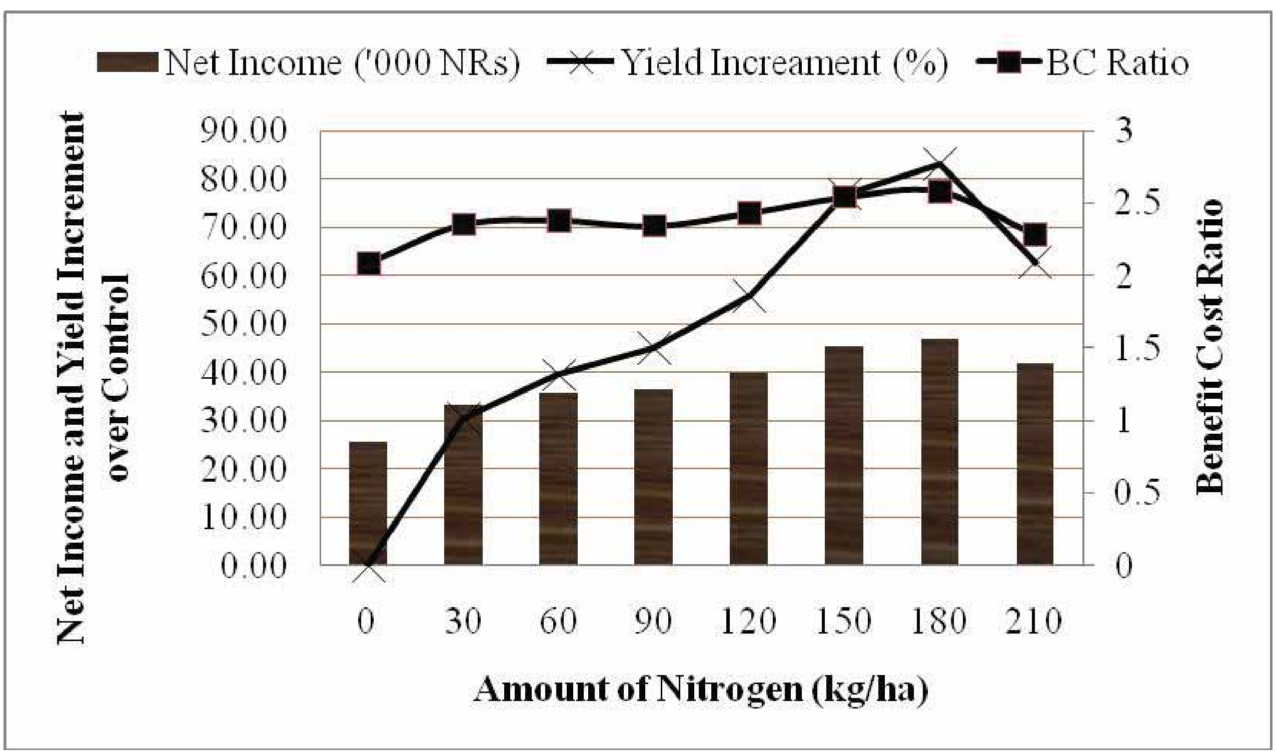

Figure 3. Influence of nitrogen levels on net economic return, benefit:cost ratio and yield increment over control.

There was a significant difference between the two varieties in terms of the above-ground dry matter. Deuti (11.3 t ha^{-1}) produced significantly more dry matter than Arun-4 (10.2 t ha^{-1}). The LAI for Deuti was higher than Arun-4.

Nitrogen levels significantly influenced the above-ground dry matter accumulation. Dry matter increased with increasing nitrogen up to 180 kg ha^{-1} and then declined at 210 kg ha^{-1}. Grain yield and stover yield also followed the same trend. Dry matter is directly related to grain and stover yields. As with grain yield and stover yield, there was good correlation between yield-attributing characters and higher dry matter, which accumulates up to 180 kg N ha^{-1} and then declines at 210 kg ha^{-1} because of the decrease in photosynthesis. Greenwood (1976) reported that photosynthesis increases dry weight as well as the metabolic energy for crop development. There was no significant difference between the two varieties for their interaction with nitrogen levels for above-ground dry matter production.

There was, however, a significant difference between the varieties in terms of the N uptake by grain. Deuti absorbed a significantly higher amount of N (108 kg ha^{-1}) than Arun-4 (94 kg ha^{-1}). Nitrogen uptake also differed significantly due to different N levels. It was highest (119 kg ha^{-1}) at 180 kg ha^{-1} N. It was statistically similar at 120, 150 and 210 kg ha^{-1} N but significantly higher than at all other N levels. Similarly, N uptake at 150 kg ha^{-1} was significantly higher than N uptake at 0 to 60 kg ha^{-1} but statistically at par with 90, 120 and 210 kg ha^{-1} N.

Net income was higher for Deuti (NR 42 060, equivalent to US$ 546 ha^{-1}) than Arun-4 (NR 34 149, equivalent to US$ 444 ha^{-1}). The grain yield and stover yield of Deuti were higher than Arun-4. Therefore, net return was higher for Deuti. The highest net benefit (NR 46 880, equivalent to US$ 609 ha^{-1}) was observed at 180 kg ha^{-1} N followed by 150 kg ha^{-1} N (NR 45 290, equivalent to US$ 488 ha^{-1}) and the lowest (NR 25 590, equivalent to US$ 332 ha^{-1}) at 0 kg ha^{-1} N levels. Based on average data, the highest (2.6) benefit:cost ratio (B/C) was observed at 180 kg ha^{-1} N followed by 150 kg ha^{-1} N (2.5); the lowest ration (2.1) was at 0 kg ha^{-1} N. In general, the B/C ratio increased with increasing N levels up to 180 kg ha^{-1} N. The maximum increment in net income (83%) over control was observed at 180 kg ha^{-1} N followed by 150 (77%) and 210 (63%) kg ha^{-1} N. An average of 31, 39, 42 and 56% higher increment in net income was obtained over control with the application of 30, 60, 90 and 120 kg ha^{-1} N, respectively.

Conclusion

Grain yield of maize increased with increasing nitrogen levels. The highest yield was obtained at 180 kg ha^{-1} N. Application of 30, 60, 90, 120, 150, 180 and 210 kg ha^{-1} nitrogen increased grain yield by 19, 27, 30, 41, 54, 59 and 49% over control (4.0 t ha^{-1}), respectively. Yield-attributing characters like number of ears per unit area, number of grain per ear, 1000-grain weight, above-ground dry matter production and net economic return increased with increasing nitrogen levels. There was no significant interaction between maize cultivars and nitrogen levels for all the tested parameters.

Acknowledgement

We thank the Hill Maize Research Project (HMRP) for funding our research and participation in the 10th ARMW. We also thank Mr. K. Adhikari, the then Coordinator; Mr. Diwakar Sharma, Senior Scientist; Mr. B. Pandey, Technical Officer; Mr. N. Dhakal and Mr. H. Bohra, Technicians of the NMRP, Rampur for their respective contributions to our effort.

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Opportunity and Challenges in Maize Farming with Limited Irrigation

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Abstract. This study on the opportunities and challanges presented by maize farming in irrigated lowlands was carried out in South Sulawesi, Indonesia in 2005. The study was carried out through a semiintensive survey and a Participatory Rural Appraisal. Information on maize farming in irrigated lowlands was collected by interviewing farmers, farmer group leaders and field officials. The results of the study suggest that there is potential for maize in the irrigated lowlands especially in the dry season. This opportunity is due to several factors: (i) depletion of river water in the dry season because of which rice cropping becomes difficult and maize would be a potential alternative; (ii) rainfall may be sufficient for maize cultivation; and (iii) technically, maize cultural practices in irrigated lowlands are easier and more efficient because no land preparation is needed and no-till cultivation using herbicide could be viable. However, alongside this opportunity, there are several challanges as well. These include: (i) farmers may still prefer rice as it is a priority crop; (ii) landowners want to plant rice because by convention they get a higher crop share (50:50) from their tenants, but for maize there is no such regulation; (iii) farmers are less knowledgeable about maize cultural practices in the lowlands; (iv) facilities supporting postharvest processing of maize such as grain shellers, drying floors and storage facilities are not available; and (v) uncertain grain prices and marketing.

Key words: **Irrigated lowlands, opportunity, challenge, cultural practices, maize**

Introduction

Maize is important in Indonesia not only to fulfill the people's food needs but also to meet the demand of the feed industry (Suryana 2006). Domestic demand for feed purposes is about 4.9 million t and is likely to reach 6.6 million t by 2010 (Pangan 2006). There are opportunities of exporting animal and poultry feed to neighboring countries like Japan and South Korea which import about 18 million t of feed every year (Suk 2006). Demand for feed has been increasing every year and domestic maize production is not enough to meet it. About 400 000 t had to be imported in 2006 (Heryanto *et al.* 2006).

Maize production can be increased through crop intensification (Damarjati *et al.* 2005). It is a crop with potential under limited irrigation in the dry season. Due to increasing demand, there has been changed in the cropping pattern in irrigated lowlands from mainly rice-rice-maize to rice-maize-maize.

Barru and Pinrang are two districts in South Sulawesi which have large tracts of irrigated lowlands, but maize cultivation has not been growing here as expected (Bahtiar *et al.* 2005). In Pinrang, maize is grown in 37 565 ha under full irrigation and 852 ha under partial irrigation, whereas in Barru only 1,458 ha is under maize with partial irrigation system (BPS 2003).

Materials and Method

This study was carried out in 2005 to investigate the opportunities and challenges before maize production under limited irrigation in Barru and Pinrang districts on the west coast of Sulawesi Selatan province in Indonesia. These districts are characterized by large tracts of low-lying irrigated areas where a maize-based cropping system is emerging, and the government has shown some interest in investing resources in maize development here. The study sites were identified in consultation with agriculture officers, considering two factors: (a) the chosen district must have a large extent of low-lying irrigated areas; (b) and an operational government program. Nine villages were thus selected (six in Barru and three in Pinrang).

Data were collected through interviews with key informants/respondents, and by using maps and secondary data. The information collected from interviews was basically technical, economic and social in nature. The data were analyzed using a sociocultural approach considering the respondents' income. Quantitative data such as profit and loss were compared between maize and rice cultivation and subjected to cost and revenue analysis and the R/C ratio was computed.

The study was focused on two main problems: availability of land resources and sufficiency of water for maize planting under limited irrigation.

Results and Discussion

Potential of maize in lowlands with limited irrigation

About 37 565 ha are under full irrigation in Pinrang district, and 852 ha under partial irrigation. There is limited possibility of growing rice during the dry season but maize can be grown easily. However, from our discussions with agricultural officers in the study villages, we learnt that not all lowlands with limited irrigation can be used for maize cultivation. About 1060 ha in Barru district and 650 ha in Pinrang can be brought under maize cultivation.

Potential use of water resources

The main sources of irrigation in these lowlands are canals and rainfall. Availability of water from the canals for rice cropping in the dry season has always been problematic. Irrigation only reaches plots near the tertiary canals. In Barru district, there is no assured irrigation for rice, and the water available from the irrigation canals is not enough for rice. Similar conditions exist in Suppa subdistrict in Pinrang district. Irrigation water from the canals is not adequately available for rice during the dry season. While pumping water from the primary canals may serve that need, we understood from our field visits that the irrigation system as a whole is in ruins. Against that context, maize has become an alternative crop for these farmers.

Another source of water is from rainwater/rainfall. If there is timely rainfall early in the rainy season, farmers prefer to grow rice, and there is less likelihood of them going in for maize rather than rice in the dry season, regardless of the fact that there may be a shortage of water during the generative stage of rice in July and August. But if rainfall is limited in the very beginning of the season, farmers consideer maize as a safer option.

Based on cost:benefit analysis, revenue obtained from green maize was higher than dry-season rice in Barru district. Our analysis revealed that revenue obtained from rice in the dry season was Rp. 4 340 000 ha^{-1} in contrast with Rp. 10 977 000 ha^{-1} for maize (Table 1).

In Pinrang, using hybrid maize Pioneer 11 on a demonstration plot cultivated by the Tunas Harapan farmer group under the supervision of the agricultural service, the benefit worked out to Rp 2 302 250 ha^{-1} (Table 2). Hybrid maize cultivation fetched a revenue/cost ratio of 1.78, which indicates that it is suitable for development (Simatupang 2003).

The cultural practices used by the farmers were suitable enough for high-yielding cultivars such as Pioneer, BISI and A4, and the farmers seem to realize the benefits of fertilizer application and water pumps. The depth of underground water in this area was 6-7 m, and 3-5 wells are needed to irrigate 1 ha of land. However, not all places had water sources. To drill one well, it cost Rp. 55 000 plus Rp. 150 000 for pipes and other material.

Table 1. Costs (ha^{-1}) and returns (ha^{-1}) of dry-season rice and maize grown on semitechnical irrigation, Barru subdistrict, Barru district, Indonesia, 2005.

Description	Dry-season rice	Maize
Land preparation	2 tractor plowings: Rp. 400 000	1 tractor plowing: Rp.200 000
Seed	25 kg (Rp.1500 kg^{-1}) =Rp. 37 500	Glutinous 20 kg (Rp. 3500 kg^{-1}) =Rp. 70 000
Planting	Wages: Rp. 400 000	Food cost for mutual cooperationRp. 150 000
Weeding	DMA: Rp. 56 000	Gramoxone: Rp. 48 000
Fertilizer	4 bags NPK Pelangi (Rp.120 000 per bag) = Rp. 480 000. 2 bags urea (Rp. 55 000 per bag) = Rp. 110 000. Total: 590 000	5 bags urea (Rp. 55.000 per bag) = Rp. 275 000
Harvesting, yield and yield value	Wages 1:6 or 1:7. Production 5 t ha^{-1}. Nonhulled rice grain @ Rp.1200 kg^{-1} = Rp. 6 000 000 ha^{-1}. Production sharing 50:50. Tenant obtained Rp. 3 000 000	Green maize production 62 500 ears (156 bags) @ Rp. 200 = Rp.12 500 000 ha^{-1}. Production sharing: 1 for landowner, 3 for tenant. Tenant obtained Rp. 9 375 000
Marketing cost	Bag 55 pieces @ Rp.1500 = Rp. 82 500. Pick up at house.	Transportation: Rp. 3500 per bag × 156 bags = Rp. 546 000 + bag cost 156 × Rp.1500 = Rp. 234 000. Total = Rp. 780 000
Benefit	Rp. 6 000 000 – Rp. 1 566 000 = Rp. 4 434 000. Tenant obtained Rp. 2 217 000 ha^{-1}	Rp.12 500 000 – Rp. 1 523 000 =Rp. 10 977 000. Tenant obtained Rp. 6 174 500 ha^{-1}
R/C ratio	3.83	8.21

Table 2. Costs and returns of maize farming in Suppa subdistrict in Pinrang district, Indonesia, 2005.

Description	Quantity	Price per unit (Rp. unit^{-1})	Value (Rp ha^{-1})
Pioneer 11 seed	18 kg	32 000	576 000
Urea	200 kg	1100	220 000
ZA	100 kg	1100	110 000
SP 36	100 kg	1600	160 000
KCl	100 kg	1800	180 000
Soil cultivation	once	500 000	500 000
Irrigation (cost of diesel fuel)	150 L	4500	675 000
Gramoxone herbicide	1 bottle	48 000	48 000
Dursban insecticide	1 bottle	15 000	15 000
Decis insecticide	1 bottle	20 000	20 000
Food for planting	once	100 000	100 000
Transportation cost to market	5000 kg	50	250 000
Cost of bags	63 pieces	1500	93 750
Total cost			2,947,750
Production	5000 kg	1050	5 250 000
Benefit			2 302 250
R/C ratio			1.78

Conclusion

There are opportunities of growing maize on irrigated lowlands in these districts of Indonesia because (a) land suitable for maize is available; (b) the cost of cultivation and cultural practices required for maize are easy for farmers; and (c) farmers see the possibilities of growing maize not only for household food purposes but also as a commercial proposition. However, there are several constraints as well such as erratic and high rainfall during maize planting (April-May) which may cause heavy lodging and wilting due to the poor drainage system. Moreover, the choice of crop to be planted is determined by landowner/landlord, and they tend to prefer rice because it gives them a higher share of the returns. Further, the maize marketing system is less familiar to farmers here. Despite these constraints, however, there are great opportunities of expanding maize cultivation in low-laying areas during the dry season.

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Agronomic Characteristics and Yield Potential of Promising Maize Hybrids in Dryland Agroecosystems of Western Nusa Tenggara

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Abstract. Maize is a major crop in Nusa Tenggara Barat (NTB) province of Indonesia. It is mainly cultivated in dryland areas during the rainy season. With demand increasing nationally, the prospects for maize cultivation are promising. To raise productivity in order to meet this growing demand, use of hybrids with high yield potential is needed. Therefore, evaluation of the suitability of new hybrids/inbred lines for dryland agriculture is necessary before they are released to farmers. The objective of this study was to assess the potential yield of hybrids of harapan grown in dryland agroecosystems. The study was carried out in Perigi village in Suela subdistrict, Lombok Timur, during the 2005/2006 rainy season. The location is a center of dryland maize production. Fourteen hybrids were tested using a randomized complete block design with four replications. For control purposes, Bima-1 and BISI-2 hybrids were used. Each plot consisted of 2 rows 5 m long with 0.75 × 0.20 m plant spacing. Of the 14 tested hybrids, 4 more grain yield than the controls. These include Nei92002/Mr4 (9.22 t ha^{-1}), Mr4/B11-209 (8.95 t ha^{-1}), G193/Mr4 (8.53 t ha^{-1}) and B11-136/Mr14 (8.30 t ha^{-1}). To know more about these hybrids, further tests are needed at several locations, both drylands as well as lowlands, before they are released commercially.

Key words: Maize, yield potential, hybrid, dryland

Introduction

Maize is an important commodity in Nusa Tenggara Barat (NBT) province because it has a strategic role in meeting the food needs of the people and the demand of feed industries in the region. Maize harvested area in the province in 2005 was 39 380 ha, with an average productivity of 2.45 t ha^{-1} (AANTB 2005), which is less than the national productivity (3.1 t ha^{-1}) (Directorate General of Food Crops 2005). The introduction of superior hybrids has contributed to improvement of maize productivity at both regional and national levels. However, dissemination and adoption of the introduced materials has been slow.

Besides higher yield, hybrids have several advantages like tolerance to pests and diseases, greater response to fertilizers, more homogenous growth and uniform ears, and higher number and heavier grain (Jugenheimer 1985). However, the environmental conditions for maize farming are highly varied across locations to which hybrids tend to be sensitive. Soemartono (1995) reported that to improve maize production in this region, hybrids tolerant to heterogeneous environments are needed.

A good number of hybrids have been developed in Indonesia in addition to those brought in from other countries. However, before hybrids are commercially released, they must go through a series of evaluations for yield and adaptability in comparison to existing well-adapted hybrids.

Materials and Methods

This study was carried out in Perigi village in Suela subdistrict of Lombok Timur district during the rainy season of 2005/2006. The experimental location was characterized by dryland agriculture under arid climatic conditons. The study was arranged in a randomized complete block design in which 14 hybrids were replicated four times. These hybrids were compared with two hybrid controls (Bisi-2 and Bima-1). Each hybrid was grown on a two-row plot 5 m long with 75 × 20 cm spacing. Two seeds per hill were sown which were thinned to 1 at two weeks of sowing.

About 38 kg N, 36 kg P_2O_5 and 30 kg K_2O were applied per plot. The main fertilization was done 7 days after planting using 1/3 of the dose of N and the full dose of P_2O_5 and K_2O as basal dressing. The remaining N was applied at one month after sowing. The sources were urea, SP-36 and KCl. Weeding was done twice, first at two months after planting and the second at 4 months after planting, during which heaping (earthing-up) was done. Plots were kept free of pests and diseases. Irrigation was given once every 2-3 weeks considering the soil moisture. Harvesting was done when seeds showed the symptoms of physiological maturity or when the maize husk turned yellow-brown.

The agronomic traits recorded were plant height, ear height, male and female flowering, number of harvested plants and number of harvested ears per unit area, plant aspects, husk cover, ear aspects and production. The quantitative data were analyzed by computing analysis of variance (ANOVA) using Genstat (Discovery version 3) for each set of data. The LSD was computed to compare treatments.

Results and Discussion

Table 1 shows data on summarized agronomic traits like flowering, plant height, height of ear position and productivity of the hybrids. Analysis of plant height observations showed significant differences among the tested hybrids. Mr4/B11-209 hybrid (237 cm) had the tallest plant height and B11-209/Mr14 (128 cm) the tallest ear position. Plant height was closely related to biomass production. The ear position could also ease the harvesting and enabled youngsters to participate in the harvest.

The male and female flowering ranged from 54 to 58 days and 55 to 59 days, respectively.

We detected a variety effect on grain yield. Nei92002/Mr4 produced the highest grain yield (9.22 t ha^{-1}). The other higher-yielding hybrids were Nei92002/Mr4 (9.22 t ha^{-1}), Mr4/B11-209 (8.95 t ha^{-1}), G193/Mr4 (8.53 t ha^{-1}) and B11-136/Mr14 (8.30 t ha^{-1}). These hybrids could be promoted in NTB. Subandi (1985) reported that genotype-environment interaction leads to better selection. Some genotypes are good in certain environments but not in others.

The effective plants and number of ears per plot ranged from 46 to 48 and 45 to 48, respectively (Table 2). It means that some plants had 2 ears per plant (prolific).

Almost all the tested hybrids were good with respect to ear aspects, ear height, husk aspects and tolerance to pests or diseases.

In terms of yield, Nei9008/Mr 14 (14.3%) and B11-209/Mr14 (11%) were better than the control Bima-1, and Nei9008/Mr14 (12.3%) and B11-209/Mr14 (9%) were better than the control BISI-2 (Table 3). Some of the hybrids were, however, inferior to the controls: E54-2/Mr14, Mr4/B11-132, B11-132/MR14, etc.

Table 1. Summary of agronomic traits of hybrids tested on dryland at Perigi, Lombok Timur in Nusa Tenggara Barat province of Indonesia, rainy season 2005/2006.

Line/variety	Flowering age (day)		Plant height (cm)	Ear height (cm)	Grain yield (t ha^{-1})
	Male	Female			
Nei92002/Mr4	55	56	217	113	9.22
E54-2/Mr14	56	57	224	116	7.28
CML 165/Mr4	54	56	218	108	7.81
E45/Mr14	57	58	205	103	7.64
B11-209/Mr14	57	58	239	128	7.83
B11-126/Mr14	58	59	221	118	7.59
G180/Mr14	55	56	233	117	8.13
Mr4/B11-132	54	55	231	123	7.55
CML 431/Mr14	55	56	234	121	8.23
B11-157/MR14	57	58	233	120	7.70
B11-136/Mr14	55	56	223	125	8.30
Mr4/B11-209	57	58	238	125	8.95
G193/Mr4	57	58	226	122	8.53
B11-132/MR14	55	56	223	112	7.56
Bisi-2 (control)	56	57	242	137	8.07
Bima-1 (control)	55	57	233	119	8.21
CV (%)	1.5	1.6	3.4	4.9	6.6
LSD_{05}	1.22	1.31	11	8.4	0.75

Table 2. Summary results of number of plants, ears, husk and ear aspects of several hybrids grown on dryland at Perigi, Lombok Timur in Nusa Tenggara Barat province of Indonesia, rainy season 2005/2006.

Hybrid	No. of harvested plants per plot	No. of harvested ears per plot	1000-grain weight (g)	Husk aspect (1-5 scale[1])	Ear aspect (1-5 scale)
Nei92002/Mr4	48	48	40	1.00	1.00
E54-2/Mr14	47	47	38	1.50	1.50
CML 165/Mr4	46	46	35	2.00	1.25
E45/Mr14	48	48	35	1.25	2.00
B11-209/Mr14	46	46	35	1.25	1.00
B11-126/Mr14	46	45	38	1.00	1.50
G180/Mr14	48	47	37	1.25	1.25
Mr4/B11-132	49	47	38	1.50	2.00
CML 431/Mr14	48	47	38	1.00	1.00
B11-157/MR14	47	48	35	1.00	1.50
B11-136/Mr14	47	47	35	1.25	1.25
Mr4/B11-209	48	49	39	1.50	1.00
G193/Mr4	48	47	36	1.50	1.25
B11-132/MR14	47	48	38	1.25	1.75
Bisi-2 (control)	48	48	38	1.25	1.25
Bima-1(control)	47	47	39	1.00	1.25
CV (%)	2.4	4.3	3.8	-	-
LSD 5 %	1.65	2.86	7.20	-	-

[1.] 1= best and 5 = worst.

Conclusions

- The tested hybrids were relatively similar in plant height and ear length.
- Of the 14 tested hybrids, six were higher yielding than the controls. They include Nei92002/Mr4 (9.22 t ha^{-1}), Mr4/B11-209 (8.95 t ha^{-1}), G193/Mr4 (8.53 t ha^{-1}), and B11-136/Mr14 (8.30 t ha^{-1}).
- These hybrids need to be further tested for wider adaptation and promoted accordingly.

Table 3. Comparative yield benefit (%) of experimental hybrids over two controls Bima-1 and BISI-2.

Hybrid	Production (t ha^{-1})	% relative to Bima -1	% relative to BISI-2
Nei92002/Mr4	9.22	114.3*	112.3*
E54-2/Mr14	7.28	88.7	88.7
CML 165/Mr4	7.81	96.8	95.1
E45/Mr14	7.64	94.7	93.0
B11-209/Mr14	7.83	97.0	95.4
B11-126/Mr14	7.59	94.0	92.5
G180/Mr14	8.13	100.7*	99.0
Mr4/B11-132	7.55	93.6	91.9
CML 431/Mr14	8.23	100.2*	100.2*
B11-157/MR14	7.70	95.4	93.8
B11-136/Mr14	8.30	101.1*	101.1*
Mr4/B11-209	8.95	110.9*	109.0*
G193/Mr4	8.53	105.7*	103.9*
B11-132/MR14	7.56	93.7	92.1
Bima 1	8.07	100.0	-
Bisi 2	8.21	-	100.0

* Better than controls Bima-1 and Bisi-2.

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Sustainable Techniques for Maize Production on Sloping Lands

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Abstract. The northern mountainous region of Vietnam accounts for more than 30% of the maize production in Vietnam. Maize yields have nearly doubled from 1.6 t ha^{-1} in 1995 to 3.1 t ha^{-1} in 2007. Maize area in the region in 2007 was 400 000 ha with an average yield of 3.1 t ha^{-1}. Total production stood at 1.2 million t, up by 150 000 t over 2006. Maize contributes greatly to hunger eradication and poverty reduction in the mountainous region. However, this has led to farmers destroying forests on the very steep slopes to grow maize, causing severe soil erosion. This has raised concerns over the future of maize cultivation. There is an urgent need to introduce more sustainable techniques for maize production on sloping lands.

This paper deals with the development of sustainable techniques to reduce soil erosion by mulching with suitable materials or direct seeding with mini-tillage. Using living or dried plant materials as mulch and direct seeding with mini-terraces was found very promising in helping to reduce soil erosion by up to 96% and increase maize yield by at least 20%. These techniques are being further developed and are being transferred to other similar production areas.

Key words: Sustainable, soil mulch, soil erosion, direct seeding, mini-terrace, maize

Introduction

Mountainous and hilly regions occupy three-fourths of the natural inland territory of Vietnam. In general, these lands are not favorable for agriculture, especially hillsides that have been denuded of vegetation. Sloping lands are distributed over all 9 ecological regions of Vietnam, but mostly in the Northern mountainous, Central and Tay Nguyen regions. Records show that in the 1940s forest cover in Vietnam used to be 43%, and then was reduced to 25% during the 1980s. Today, it has improved to 36.7% thanks to reforestation efforts supported by the people and the international community.

The more gentle slopes with an incline of less than 15º (accounting for 21.9%) are used for agriculture and forestry. Lands with an incline of 15-25º occupy 16.4% of the total area. The rest of such lands (61.7%) are very steep slopes (more than 25% inclination). Due to the shortage of productive land, farmers in the mountainous regions have no choice but to grow food crops on these very steep slopes. Soil erosion occurs at a very high rate and intensity on these slopes. Crops like rice (upland) and maize can be grown for two or three seasons followed by cassava as the last crop of a cultivation cycle. The fallow period has decreased from more than 25 years to 3-5 years which is not long enough for soil fertility to be restored. Yields are decreasing year after year because of continuous cropping.

The sloping land area for annual crops, mainly maize and upland rice, is about 545 000 ha but faulty cultivation practices lead to very rapid soil erosion. Maize yields for instance dwindle, and availability of arable land for the crop is decreasing. To meet their food demands, farmers resort to slash-and-burn practices, leading to frequent natural hazards and deepening of poverty. There is an urgent need to help these farmers with more effective and sustainable sloping agriculture land technology (SALT).

Maize plays a very important role in the life of farmers in the mountainous region. It is the staple food of the H'Mong people. Maize has in recent years become a high income crop and its area has been increasing very fast. Husson *et al.* (2001) reported that 62% of the households (HHs) receive income from maize. The crop contributes up to 15% of these households' total income. As a result of hybrid maize research and development, maize yields increased from 2.1 t ha^{-1} in 1995 to 3.96 t ha^{-1} in 2007. However, in 'old' fields, maize yields tend to dwindle due to soil erosion and land degradation. Available data show that soil loss in sloping lands is 200 t ha^{-1} $year^{-1}$. So, farmers are forced to increase maize planting area by clearing forests. In 2007, maize planting area in the Northern mountainous regions increased to 385 000 ha (an increase of 13 000 ha annually). Maize cultivation has now spread to the top of hills and mountains. This is ecologically dangerous.

Results and Discussion

Since 1999, the French Centre for International Cooperation in Agricultural Research for Development (CIRAD) and the Vietnam Agricultural Science Institute (VASI), then the Northern Mountainous Agriculture and Forestry Science Institute (NOMAFSI), jointly implemented a project on agricultural systems in the northern mountainous regions in Cho Don district of Bac Kan province. Called the SAM project, it promoted ecologically based methods using organic materials (mainly vegetative matter from different sources) to preserve and improve soil fertility and physical properties to get higher and stable yields at lower cost. It achieved promising results within a short time and attracted the attention of researchers, project planners, local farmers and decision-makers.

The technologies that the project developed are mulch-based direct-sowing systems (DMC or SCV). The DMC techniques help recover lands damaged by slash-and-burn shifting cultivation. These techniques have been further studied and successfully applied to maize cultivation on sloping lands. The following are the results of these activities.

Reduction in soil erosion

Experiments were conducted with different vegetative materials to mulch the soil before sowing. Eroded soil from the experimental and control plots was collected in soil traps at the foot of the hills, then air dried and weighed to comupte as tons per ha per year. The results are shown in Table 1.

At the Northern Mountainous Agriculture and Forestry Science Institute (NOMAFSI), the experiments used mulch consisting of herbicide-sprayed weed residues. The control treatment consisted of normal farmers' practices including normal land preparation, burning of all vegetative residues and no soil mulch (Fig. 1). Even with a small amount of weed leaves and stems left on the soil surface, the reduction of soil erosion was clear (Table 2).

Similar results were obtained from tea fields at the establishment stage (1 year old) (Table 3). With farmers' practices, the amount of eroded soil was 47.8 t ha^{-1}, whereas soil loss was only 13.9 t ha^{-1} and 18.2 t ha^{-1} with Gramoxone- and Glyphosate-treated plots, respectively.

These data clearly show that the soil mulch helped control soil erosion, contributing to more effective and durable use of sloping land for crop cultivation in general and maize in particular.

Growth and development of maize plants

Data obtained from various experiments on maize with and without soil mulching showed that maize plants grew much better in mulched plots than nonmulched (Table 4; Fig. 2).

Figure 1. Maize plot with soil mulch showing less soil erosion (foreground) than the control plot without mulch (back).

Table 1. Effects of soil mulch on soil erosion in Na Ri district of Bac Kan province, Vietnam, 2004.

Treatment	Eroded soil (t ha^{-1} year^{-1})	Decrease (t ha^{-1})	Decrease %
Mini-terraces			
Maize on sloping lands without soil mulch (control)	16,407	0	0
Maize on mini-terraces without soil mulch	1,584	14 823	90.3
Maize on mini-terraces with soil mulch	1,005	15 402	93.9
Sloping lands			
Maize on sloping lands without soil mulch (control)	9.3	0	0
Maize on sloping lands with soil mulch	2.3	7.0	75.3

[1.] 1= best and 5 = worst.

Table 2. Soil erosion in different mulching treatments with maize.

Treatment	Soil erosion ($t\ ha^{-1}$)	Decrease over control ($t\ ha^{-1}$)	Decrease over control (%)
C (control)	71.17	0	0
T1 (Gramoxone)	11.67	59.50	84
T2 (Glyphosate)	29.17	42.00	59
$LSD_{0.05}$	5.1		
CV (%)	11.2		

Table 3. Soil erosion in different treatments from tea field.

Treatment	Soil erosion ($t\ ha^{-1}$)	Decrease over control ($t\ ha^{-1}$)	Decrease over control (%)
C (control)	47.8	0	0
T1 (Gramoxone)	13.9	33.9	70.9
T2 (Glyphosate)	18.2	29.6	61.9

Table 4. Effect of soil mulching on maize plant height, Van Chan district, Vietnam.

Treatment[1]	Maize plant height at stage V8 (cm)	Ear height (cm)	Plant height at harvest (cm)
C	57	70	165
T1	653	79	173
T2	69	81	176
T3	69	83	178
$LSD_{0.05}$	4.84	3.05	2.91
CV(%)	1.14	0.72	0.69

[1.] C = Control; T1 = mini-terrace without mulch;
T2 = mini-terrace with soil mulch; and
T3 = mini-terrace with cowpea as live mulch.

As a result of mulching, maize biomass was higher compared to control (Table 5). Dry biomass was higher in all experimental plots, but with soil mulch, ie, T3 and T2, it was highest (17% and 15% over control respectively). This is very important if biomass is going to be used as mulch for the next crop.

Maize yield

Obviously, maize plants benefit from the nutrition provided by mulch decomposition. Maize yield was much higher in the mulched plots. Table 6 shows the results of experiments from 15 sites in 8 districts in the northern mountainous regions of Vietnam.

Figure 2. Maize without soil mulch (foreground) and showing much better growth and development with soil mulch (background).

Table 5. Dry biomass of maize under different treatments ($t\ ha^1$).

Treatment[1]	C	T1	T2	T3
Dry biomass	3.6	3.8	4.1	4.2
Increase over control ($kg\ ha^{-1}$)		0.255	0.527	0.594
Increase over control (%)	0	7	15	17

[1.] C = control; T1 = mini-terrace without mulch;
T2 = mini-terrace with soil mulch; and
T3 = mini-terrace with cowpea as live mulch.

Table 6. Effects of soil mulching on maize yield at different sites.

Maize variety	Yield ($t\ ha^{-1}$) Control (without mulch)	With mulch	Increase (%)	District
LVN 10	1.97	3.29	67.2	Cho Don (3 sites)
DK888	2.20	3.46	57.3	Na Ri
CP 999	6.49	8.48	30.7	Mai Son
LVN10	3.97	6.82	58.2	Song Ma (3 sites)
LVN10	1.95	4.44	78.8	Tuan Giao (2 sites)
LVN10	3.26	4.84	48.5	Dien Bien Dong (2 sites)
LVN10	1.48	2.92	97.3	Phong Tho (2 sites)
LVN10	42.0	60.7	44.5	Van Chan
Average			62.6	

It can be seen from Table 6 that maize yields increased in all the sites (at least by 30.7% in Mai Son district, and at most by 97.3% in Phong Tho district). The average increase was 62.6%.

On the very steep slopes (with an incline of more than 25º) soil erosion occurs at a very rapid rate. The results of the SAM project experiments showed that mini-terrace construction with soil mulching is very promising (Table 7) in arresting soil erosion.

Weed growth

Weed infestation tends to be very high in dryland cultivation systems. So weed control plays an important role in increasing crop yields on sloping lands. Our results show that mulching can greatly reduce weed growth, hence reducing investment in weed control (Table 8).

The results also showed that soil mulching greatly reduced the labor needed for maize cultivation (Table 9). Only 5 days were spent on weeding in T3, and 10 days in T2, while farmers using common practices (control) spend 60 days for weeding. This clearly shows mulching helps reduce drudgery for women as weeding is carried out mainly by them.

Improvement of soil properties

Beside control of soil erosion, soil mulch can improve soil properties by providing nutrients when it is decomposed. Soil samples were taken from different treatments and analyzed. The results are shown in Table 10.

It can be seen from Table 10 that mulching can improve soil fertility properties. The amount of moveable Al^{3+} was reduced from 7.39 me $100g^{-1}$ to 4.68 me $100g^{-1}$, while pH and other good parameters increased dramatically, especially available P_2O_5 and K_2O (Fig. 3).

Lessons Learned

Difficulty of seedling emergence from vegetative cover: The mulch layer should not be thicker than 10 cm. Fields should be mulched at least 15 days before sowing. It is also advisable not to allow the mulch to completely cover the seed hill.

Table 7. Effects of mini-terracing and soil mulching on maize yield.

Field designation	Treatment	Yield (t ha^{-1}) 2000	2001	2002
1002-1	Mini-terrace + *Cassia rotundifolia*		1.44	2.39
1002-2	Mini-terrace + *Arachis pintoi*		1.77	4.01
1003-3	Mini-terrace + *Brachiaria ruziziensis*		1.59	2.11
1004-2	Sloping field (no mini-terrace)	1.68	0.44	0.93
1010-1	Mini-terrace + *Arachis pintoi*		1.18	4.17
1012-3	No mini-terrace + hand weeding		1.07	
	Mini-terrace with soil mulch			3.77

Table 8. Weed biomass under different mulching treatments, Van Chan district of Yen Bai province, Vietnam.

Treatment[1]	C	T1	T2	T3
Number of weed species	14-16	12-14	12-14	10-12
Weed biomass (kg ha^{-1})	406.7	273.3	113.3	60.0
Decrease over control (kg ha^{-1})		133.3	293.3	346.7
Decrease over control (%)	100	67.2	27.9	14.8

[1.] C = control; T1 = mini-terrace without mulch; T2 = mini-terrace with soil mulch; and T3 = mini-terrace with cowpea as live mulch.

Table 9. Labor spending on maize cultivation under different mulching treatments[1] (days ha^{-1}).

Treatment[1]	C	T1	T2	T3
Sowing, fertilization	20	25	30	30
Insecticide spraying	5	5	5	5
Weeding	60	30	10	5
Gathering mulch materials			10	10
Sowing legumes as live mulch				15
Harvest	30	30	30	30
Total	115	95	90	95

[1.] C = control; T1 = mini-terrace without mulch; T2 = mini-terrace with soil mulch; and T3 = mini-terrace with cowpea as live mulch.

Table 10. Results of analysis of soil samples under different mulching treatments[1] in Van Chan district, Vietnam.

Parameter	Value C	T1	T2	T3
pH_{KCl}	3.64	4.06	4.05	4.17
Al^{3+} (me $100g^{-1}$)	7.39	7.12	6.76	4.68
CEC (me $100g^{-1}$)	8.15	8.65	9.44	10.43
OM (%)	1.79	1.84	2.04	2.24
P_2O_5 available (mg $100g^{-1}$)	6.83	8.47	8.76	10.00
K_2O available (mg $100g^{-1}$)	9.37	9.45	10.30	13.07

[1.] C = control; T1 = mini-terrace without mulch; T2 = mini-terrace with soil mulch; and T3 = mini-terrace with cowpea as live mulch.

Figure 3. Improvement of soil properties as a result of soil mulching.

Transmission of diseases from mulch materials: This can be overcome by using disease-free materials of other crops. In addition, one can use crop rotation to cut the circle of disease development.

Need for more labor for gathering mulch materials: This is true only for the first season. Labor for mulching is paid off by the 80% reduction in labor required for weeding. For the next season, one can use the *in situ* crop and weed residues so that there is no need to gather mulch materials. In addition, farmers can produce mulch materials on-the-spot or use locally available sources.

Conclusions and suggestions

- Maize cultivation on sloping lands can be made more effective and sustainable by using mulch-based crop cultivation techniques which have multiple benefits.
- The two main impacts of soil mulch are soil erosion control and soil improvement by supplying organic matter and creating good conditions for crop growth and development.
- Mulch helps to increase maize yield steadily with reduced input use, less use of labor, mineral fertility and agrochemicals, facilitating the formation of an ecological production system.
- On very steep slopes, mini-terracing in combination with soil mulching can be a most effective measure to make maize production more effective and sustainable.
- It is necessary to conduct more research, training and transfer of these technologies on a wider scale.
- There is a need to advocate the use of crop residues and agro-byproducts as a gift of nature to mulch and improve soil properties.

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Site-Specific Nutrient Management for Maize in the Philippines

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Abstract. Increasing the productivity of maize requires effective use of nutrients from naturally occurring and fertilizer sources. On-farm trials were conducted at key production sites in Isabela, Bukidnon and Nueva Ecija/Tarlac in the Philippines with one or two maize hybrids each year during 2005-2007 to develop and evaluate a new site-specific nutrient management (SSNM) approach for irrigated and favorable rainfed maize environments. Attainable yield was estimated from treatments with an ample supply of fertilizer nutrients. Soil indigenous nutrient supplies were estimated as grain yield in omission plots where nutrients other than the omitted ones were not limiting yield (0-N, 0-P, 0-K kg ha^{-1}). Fertilizer rates for attainable yields were calculated based on two-season estimates of the yield responses to fertilizer N, P and K and expected nutrient-use efficiencies. The results indicate that attainable yield is in the range of 6-10 t ha^{-1} in Isabela, 6-8 t ha^{-1} in Bukidnon and 8-10 t ha^{-1} in Nueva Ecija/Tarlac. Fertilizer recommendations based on the SSNM approach increased yield on average by 1.0 t ha^{-1} and net benefit by 5170 PhP ha^{-1} (US$125 ha^{-1}) compared to the farmers' practice across all sites and seasons. The SSNM concept for maize is now being evaluated on a wider scale using farmer participatory approaches.

Key words: Maize, site-specific nutrient management, omission plot, attainable yield

Introduction

Maize is the second most important cereal crop after rice in the Philippines. Opportunities of increasing yield and profitability with traditional varieties including white corn, a staple food for about 20% of the population, are modest as these varieties have limited yield potential. Yellow corn is a major component of feed for the livestock and poultry industry, as well as a source of raw material for the food and manufacturing sector. In 2006, yellow corn comprised 61% of the total corn production in the country with a share of about 43% of the total harvested maize area (BAS 2008).

While the total area planted to white corn decreased in the 1990s, total maize production and average yield in the Philippines increased due to the expansion of yellow corn and the adoption of modern varieties and production technologies (DA 2008). The last four years in particular have seen renewed interest among farmers in planting maize, largely driven by advantageous net revenues compared to other crops due to high corn prices. Farm gate prices of corn increased from an average of Php 6.8 kg^{-1} in 2003 to Php 10.3 kg^{-1} in 2007. During the same time, total harvested maize area increased by 0.25 million ha, or 10%, from 2.40 million ha to 2.65 million ha. Total maize production reached 6.74 million t in 2007, a 47% increase from the 2003 production level (FAO 2008; BAS 2008). However, national maize production is insufficient to meet the increasing domestic demand particularly from the growing livestock and poultry industry. This prompted the Philippine government to import maize supplies worth US$ 42.7 million in 2005 (FAO 2008). Although considerable achievements have been made in varietal improvement and production technologies for yellow corn leading to yield increases of 31%, average national yield is low (2.4 t ha^{-1}) because the share of total maize area cropped to modern varieties grown on favorable irrigated and rainfed land is relatively low. Knowledge on attainable yield, exploitable yield gaps and constraints to improving productivity at the field level is still limited.

In 2005, the IPNI Southeast Asia Program in partnership with the University of the Philippines in Los Baños and the Department of Agriculture – Bureau of Agricultural Research (DA-BAR) launched a national initiative to increase the productivity and profitability of maize farming through site-specific, integrated crop and nutrient management. The project is part of a multinational research initiative and aims to (i) quantify and understand the yield potential of maize in favorable environments of Southeast Asia and (ii) develop and evaluate a new site-specific nutrient management (SSNM) approach and best crop management practices for maize through on-farm research in major agroecological zones of the Philippines, Indonesia, and Vietnam (Witt *et al.* 2008b). This paper will report on results from on-farm experiments with hybrid maize conducted at three major production sites in the Philippines during 2005-2007.

Materials and Method

On-farm trials were conducted in the three key maize-producing provinces of Isabela, Bukidnon and Nueva Ecija/ Tarlac following the cropping calendar of maize at the three project sites. Maize is planted in both the dry (DS) and wet (WS) seasons in Bukidnon and Isabela while in Nueva Ecija, it is planted starting mid-October after harvest of wet-season rice. All farms are dependent on rainfall for irrigation except those in Nueva Ecija where shallow water pumps are used.

General crop and field management was performed by collaborating farmers in these on-farm trials, but with guidance from researchers, particularly on implementing planting density and nutrient management to ensure good control of major production factors impacting the crop response to nutrient application. The varieties used were farmer-selected modern hybrid maize varieties. Farmers at all project sites generally follow a 60 × 20 cm plant spacing (83 333 plants ha^{-1}). To evaluate the optimum plant population and plant spacing required for high yield, planting distance and population density treatments were included in the experiments. Plant spacing in the 2005 WS were 60 × 20 cm and 75 × 20 cm (66 667 plants ha^{-1}). During the 2006 DS and WS, plant spacing of 60 × 20 cm, 75 × 20 cm and 60 × 25 cm (66 667 plants ha^{-1}) were evaluated. A plant spacing of 60 × 20 cm was used during the 2007 DS and WS at all project sites. All planting density treatments were included in the analysis of attainable yield but only treatments with plant spacing of 60 × 20 cm were used to compare results of SSNM and NPK treatments with the farmers' fertilizer practice.

Yield responses to the application of fertilizer N, P and K and associated agronomic efficiencies (kg grain increase per kg fertilizer nutrient applied) were estimated during four cropping seasons (2005 WS to 2007 DS). The treatments included nutrient omission plots with ample supply of all nutrients except the omitted nutrient (0 N, 0 P and 0 K) to estimate the nutrient-limited yield, a treatment with ample application of fertilizer N, P and K to estimate attainable yield and a farmer's fertilizer practice (FFP) to obtain the actual yield in farmers' fields for comparison. The following fertilizer rates were used in each treatment: NPK (200 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1}, 120 kg K_2O ha^{-1}; N omission (no fertilizer N but fertilizer P and K applied); P omission (no fertilizer P but fertilizer N and K applied); K omission (no fertilizer K but fertilizer N and P applied) and FFP (farmers' fertilizer practice).

Fertilizer N was commonly applied in three splits with 30% N given at crop establishment or at planting and 35% each top-dressed at growth stages V6-8 and V10-12. All P was applied at planting while K was applied in two equal splits at planting and V10-12. Nitrogen treatments in SSNM and NPK with two N split applications at planting and V8-10 were added in the 2006 DS to 2007 DS crops. Farmers commonly applied fertilizer N in two split applications with 50% at planting and 50% top-dressed latest at 45 days after planting while all fertilizer P and K were applied at planting. Fertilizer rates in the NPK and omission plot treatments were deliberately high to avoid any nutrient limitations. Before the 2007 DS, fertilizer rates for SSNM treatments were calculated following the approach described by Witt et al. (2008a) based on yield responses to fertilizer application obtained from the 2005-2006 experimental data, the expected agronomic efficiencies and adjustments for nutrient removal with grain and straw (only for P and K). The calculated SSNM fertilizer rates were included as a treatment in the 2007 DS and 2007 WS on-farm trials.

Treatments in each farmer's field were not replicated. Instead, data from at least five farmers' fields at each project site were used as replicates for statistical analysis. Plot size for each treatment was 6 × 6 m except for the single large SSNM plot (6 × 24 m) during the 2007 DS and WS. The FFP plot was the remaining farmers' field. All soil and agronomic data were collected at the single field/single treatment level. Soil samples were taken from two depths (0-15 and 15-20 cm) before the first cropping season to determine general soil properties. Initial soil chemical and physical properties at each site were analyzed following the standard methods used in the Philippines. Grain yield and final plant population were measured in a central 6 × 6-m plot using 12-m harvest rows (4 rows of 3 m length) at maturity. A 6-plant sample was collected at physiological maturity (R6 or black layer stage) to determine the yield components and the harvest index. In the FFP and SSNM treatments, two duplicate samples were taken because treatment plots were larger than in the NPK and omission plot treatments. Grain yield was reported in t ha^{-1} and adjusted to a standard moisture content of 15.5%.

Results and Discussion

Initial soil characteristics

Soil organic matter and soil organic carbon were high and moderate, respectively, in Bukidnon, but low at the other two sites. Total soil N was low to very low at all field sites. Soils pH was generally moderately to acidic. In Bukidnon, soil texture was clayey to clay loam. All farms were P deficient assuming a critical value of 15-20 mg kg^{-1} for Bray-P_2 at which yield responses to fertilizer P are expected. With soil exchangeable K of 0.61 $cmol_c$ kg^{-1}, soil K availability was moderate assuming a critical value of 0.4 $cmol_c$ kg^{-1}. Exchangeable Ca and Mg were also low. In Isabela, soil properties indicated moderate to high supply of P, Ca, and Mg, but exchangeable K was moderately low. Soils had loam to light clay soil texture. In Nueva Ecija/ Tarlac, the soil test values for P and K were below the critical level in all fields indicating high nutrient removal with harvested crop products in the intensive rice-maize systems. Soil textural classes ranged from sandy loam to loam.

Attainable yield, nutrient-limited yield and yield response to fertilizer application

Attainable yield is an important measure in the development of site-specific fertilizer recommendations. In this study, we defined attainable yield as yield achieved in farmers' fields with best management practices including water, pest and general crop management in the absence of nutrient limitations. The yield achieved in the NPK treatments was thus considered a representative indicator of attainable yield at project sites.

Attainable yield was in the range of 6-10 t ha^{-1} in Isabela, 6-8 t ha^{-1} in Bukidnon, and 8-10 t ha^{-1} in Nueva Ecija/Tarlac. The higher yield attained in Nueva Ecija/Tarlac is likely related to existing supplemental irrigation facilities at this site (irrigated rice-maize system). The attainable yield varies from season to season and year to year due to small-scale variations in soil moisture and seasonal differences in climatic conditions. Optimal economic yield is often linked to attainable yield (Buresh and Witt 2008). The maximum attainable yield in any given season could be close to the yield potential if management is excellent and weather conditions are very favorable. Seasonal differences in attainable yield were often accompanied by differences in indigenous nutrient supplies as indicated by grain yield measured from the omission plots. Thus, yield responses to the application of fertilizer N, P and K were less variable than attainable yield among fields and/or seasons.

In general, yield responses to fertilizer application followed the order N>P=K. Yield response to fertilizer N ranged from 1 to 4 t ha^{-1} in Isabela, 1 to 2 t ha^{-1} in Bukidnon and 3 to 4 t ha^{-1} in Nueva Ecija/Tarlac. Yield response to fertilizer P and K fertilizer ranged from 0.5 to 1.0 t ha^{-1} at all sites. In Isabela, the yield response to fertilizer N was higher during the dry season (3-4 t ha^{-1}) compared to the wet season (1-2 t ha^{-1}). Water as a yield-limiting factor was probably the main cause of the lower yield response to fertilizer N observed in Bukidnon and Isabela compared to Nueva Ecija. However, despite soil test values for P and K being below critical levels in Nueva Ecija, yield responses to fertilizer applications were moderate which may be related to residual effects from high P and K fertilizer use in the previous rice crop. Attainable yield and measured yield responses to fertilizer application were used to calculate site-specific fertilizer N, P and K requirements for high, economic yield. Fertilizer P and K rates were further adjusted to avoid soil nutrient depletion.

The agronomic and economic performance of SSNM

The actual yield in farmers' fields (FFP) was in most cases lower than the attainable yield in NPK treatments indicating significant opportunities for farmers to increase productivity and profitability (Fig. 1).

The agronomic and economic performance of SSNM was evaluated in more detail for all project sites as shown in Table 1. The derived net benefit covers only the price of harvested grain minus the total cost of fertilizer and seed while other variable costs were not included in the computation. The results showed that significantly greater yield (up to +28%) and net benefit (up to +20%) were achieved with SSNM compared to the farmers' practice despite larger investments in fertilizer (up to +100%). For example, in Isabela during the 2007 DS, grain yield in SSNM plots was 2 t ha^{-1} higher than in FFP. This translated to a higher net benefit of PhP 14 200 in the SSNM plot despite incurring 100% more on fertilizer costs compared to FFP. In Nueva Ecija/Tarlac, similar yields of about 8.8 t ha^{-1} were obtained in the FFP and SSNM plots during the 2007 DS. Note that fertilizer P and particularly K was increased with SSNM at the expense of fertilizer N.

Conclusions and Recommendations

The SSNM concept has demonstrated significant agronomic and economic potential to enhance the productivity and profitability of maize farming in favorable

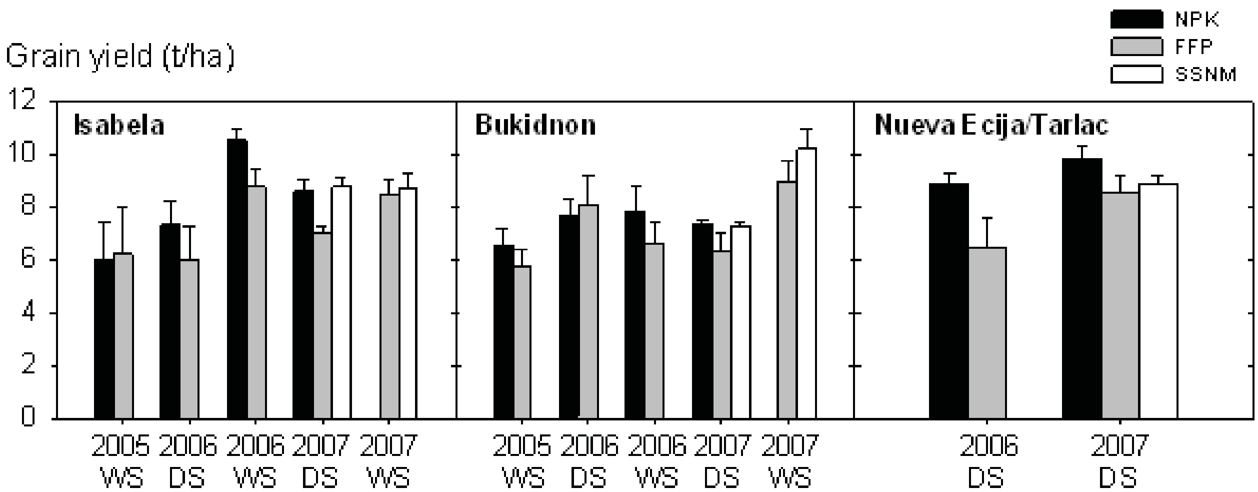

Figure 1. Grain yield in treatments with ample application of fertilizer N, P and K (NPK), farmers' fertilizer practice (FFP) and site-specific nutrient management (SSNM) in Isabela, Bukidnon and Nueva Ecija, the Philippines, 2005-2007. Planting densities were 60 × 20 cm in all treatments. Fertilizer was applied in three N split applications in NPK and SSNM, and two applications in FFP. Error bars: Standard error.

Table 1. Partial economic analysis of SSNM and FFP in Isabela, Bukidnon, and Nueva Ecija/Tarlac during 2007 DS and 2007 WS.

	Isabela				Bukidnon				Nueva Ecija/Tarlac	
	2007 DS *n* =		2007 WS *n* =		2007 DS *n* =		2007 WS *n* =		2007 DS *n* =	
	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM
Yield (t ha^{-1})	7.0	9.0	8.4	8.7	6.3	7.3	9.0	10.2	8.6	8.9
Yield difference (t ha^{-1})		2.0		0.3		1.0		1.2		0.3
Yield difference (%)		28.6		3.6		15.9		13.3		3.5
AEN	9.9	15.0			11.7	8.8			17.8	21.0
Fertilizer N (kg ha^{-1})	120	167	120	120	96	140	102	120	162	146
Fertilizer P_2O_5 (kg ha^{-1})	28	80	28	42	30	89	16	42	28	50
Fertilizer K_2O (kg ha^{-1})	28	77	28	55	29	60	28	42	14	86
Difference in N rate (%)		39		0		46		18		-10
Difference in P rate (%)		192		50		200		170		83
Difference in K rate (%)		178		96		108		48		492
Total fertilizer cost (PhP ha^{-1})	6,852	12,680	6,884	8,333	6,054	11,589	5,584	7,965	8,130	10,635
Difference in fertilizer cost (%)		85.0		21.1		91.4		42.6		30.8
Plant spacing (cm)	......................60 x 20......................				.......................60 x 20..................				.......60 x 20.......	
Plant density (plants ha^{-1})	.......................83,333.......................				........................83,333..................				.......83,333.......	
Total seed cost (PhP ha^{-1})	........................3,213.......................				.........................3,213.....................				........5,789........	
Difference in seed cost (%)	..........................0.0.........................				...........................0.0......................				..........0.0..........	
Net benefit (PhP ha^{-1})	59,935	74,107	73,903	75,454	53,733	58,198	81,203	90,822	72,081	72,575
Difference in net benefit (PhP ha^{-1})		14,172		1,551		4,466		9,619		494
Difference in net benefit (%)		23.6		2.1		8.3		11.8		

irrigated and rainfed environments. Site-specific adjustment of nutrient management guidelines and robust approaches to an improved quantitative understanding of nutrient requirements to fill the deficit between plant demand and soil indigenous nutrient supply seems crucial for achieving high yield and profit. Wider-scale evaluation of SSNM has begun, using farmer-participatory approaches at existing project sites with strong support from the Department of Agriculture - National GMA Corn Program, Bureau of Agricultural Research (DA-BAR) and Bureau of Soils and Water Management (DA-BSWM).

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Section 6

Maize Livelihoods

Maize-Poultry Value Chains and Quality Protein Maize in India: Implications for Research and Development

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Abstract. Maize ranks as the third most important grain crop in India, where it is grown on 7.8 million ha. Over 50% of the annual production of 19 million t is used as feed, primarily for poultry. The Indian agricultural research system is increasingly focusing breeding efforts on quality protein maize (QPM). Despite these investments a systematic analysis of the potential role of QPM in maize-poultry value chains is still lacking, especially identification and assessment of (i) the actors involved (breeders, seed distributors, farmers/poultry producers, retailers and consumers), (ii) the relationships among the actors, and (iii) the interventions needed to facilitate further growth of these value chains. To address these issues, the authors used a qualitative market-mapping approach involving stakeholder consultation in contrasting regions, backed up by a literature review. The constraints to uptake of QPM identified include: shortage of seed; lack of knowledge of QPM within the poultry industry; yields of QPM tending to be lower than that of normal maize hybrids; and methionine is often the limiting amino acid in maize-based poultry feed, the content of which is only slightly higher in QPM than in regular maize. Further research is needed to produce QPM varieties that outperform normal maize ones and to work more closely with the poultry industry so that QPM value chains become more demand- than supply-driven.

Key words: Maize-poultry value chains, quality protein maize (QPM), smallholder farmers, contract farming.

Introduction

Maize is the third most important grain crop in India with output growing rapidly in recent decades. Maize has multiple uses in India, including food, feed and industrial uses. Over 50% of the annual production of 19 million t is used as feed, primarily for poultry. Quality protein maize (QPM) contains enhanced levels of two essential amino acids, lysine and tryptophan, and it may offer a less expensive source of high protein animal feed. This may be of interest to the poultry industry, as feed is the single largest cost item in poultry production in India, accounting for 55-64% of variable costs (Landes *et al.* 2004). However, a systematic analysis of QPM in India's maize-poultry sector is still lacking.

This paper reports on a qualitative study of maize-poultry value chains in India. The research focused on industrial poultry producers. In April 2007, field visits were organized to study the maize and poultry sectors in the states of Uttar Pradesh, Bihar, West Bengal and Andhra Pradesh. The authors used a qualitative market-mapping methodology based on Hellin *et al.* (2005) to analyze maize-poultry value chains. This research methodology was backed up by a review of literature. Through semistructured interviews, the authors identified and assessed (i) the actors involved in these value chains (breeders, seed distributors, farmers/poultry producers, retailers and consumers); (ii) the relationships among the actors; (iii) the interventions needed to enhance the uptake of QPM in India; and (iv) the research and policy interventions needed to facilitate further growth of the poultry sector.

Maize and the Poultry Industry

Maize sector

The maize area in India now averages 7.8 million ha, producing 19 million t per annum. Figure 1 shows changes in maize area, production and yields from 1960 to 2004. Maize area increased 73% from 4.5 million ha in 1962. This growth took place mainly in the 1960s and since the mid-1990s. Yields have nearly doubled (up 95%) from 1.0 t ha^{-1} in 1962. Yield increase was the main source of growth in maize production during the 1980s and 1990s. Area and yield increases have resulted in a 3.4 fold increase in maize production from 4.3 million t in 1962.

Until the late 1980s, 70% of maize production was consumed directly as food, with 30% going to feed and industrial use in equal proportions (Singh and Pal 1992). Since the 1990s there has been an increase in the quantity of maize used as feed, whereas nonfeed uses (including food and industrial use) have remained static. The increased use of maize as feed is closely associated with the rapid growth in the Indian poultry sector. This growth is being driven by rising incomes and a rapidly expanding middle class, together with the emergence of vertically integrated poultry producers. These factors have reduced consumer prices by lowering production and marketing costs. Maize (energy) and soybean meal (protein) are the dominant feed ingredients in broiler rations, with integrated operators typically using 55-65% maize (Landes *et al.* 2004). Continued growth of poultry production offers prospects for accelerating maize production and uptake of QPM.

Most of the maize in India is grown under rainfed conditions, which contributes to significant year-to-year variation in production. About a fifth of the maize area is irrigated (MoA 2005). The limited reliance on irrigation is a reflection of the fact that 90% of the maize area is cultivated during the main *kharif*/monsoon cropping season (Table 1). The monsoon crop is often sown after the onset of the monsoon in June/July and is harvested in September. During the winter or *rabi* season, maize is sown usually in November and harvested in March (Joshi *et al.* 2005). Maize yields in the *rabi*/winter season are 62% higher than in the monsoon season (Table 1), reflecting more favorable growing conditions, including increased reliance on irrigation, higher input use, cooler temperatures and fewer pest and disease problems (Singh and Morris 1997). The higher winter yields imply that the winter season contributes 15% of India's maize production against an area share of 10% (Table 1).

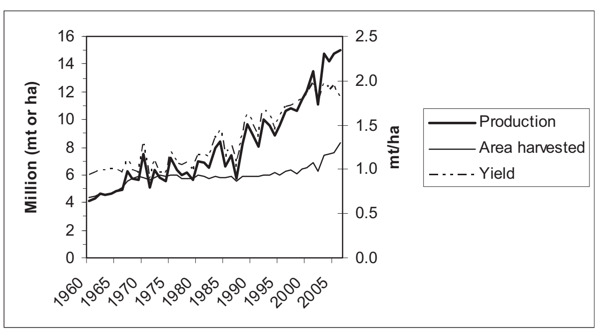

Figure 1. Maize production indicators in India (1960-2006). Source: USDA PS&D database, April 2007.

Table 1. Season-wise maize production indicators (average 1999-2004). Source: MoA 2005.

	Kharif (rainy season)	Rabi (postrainy season)	Annual
Area (10^6 ha)	6.0	0.7	6.7
Production (10^6 t)	10.6	1.9	12.5
Yield (t ha^{-1})	1.76	2.85	1.87

Two-thirds of the maize area and production in India is concentrated in six states: Andhra Pradesh (AP), Karnataka, Madhya Pradesh (MP), Rajasthan, Uttar Pradesh (UP) and Bihar. The latter four states are traditional maize-growing states located in a horizontal belt across north-central India. Andhra Pradesh and Karnataka are nontraditional maize-growing states in southern India. Production in these two states is predominantly for commercial purposes (Joshi *et al.* 2005). The Directorate of Maize Research (DMR) promotes and coordinates maize research throughout India (Joshi *et al.* 2005). This principal public maize research entity now focuses breeding efforts on high-yielding single-cross hybrids including QPM. Improved varieties have transformed maize production in India. On average, hybrid yield levels are 2-3 times higher than those of local varieties and 1.5 times higher than those of composites. The increased seed cost of hybrids is offset by increased productivity, at least in the more favorable nontraditional environments.

Both the central government (through the National Seeds Corporation, NSC) and some state governments (through the State Seeds Corporations, SSC) produce maize seed (Morris *et al.* 1998). Seed production tends to be carried out by contracted farmers. Government agencies provide farmers with parent seed, and the maize seed subsequently produced is sold to farmers directly from government warehouses or through dealers. Seed laws were liberalized in the late 1980s and since then private investment in maize research has risen sharply. Private sector seed production is concentrated in the southern states of Andhra Pradesh and Karnataka.

The poultry sector

India is now the third largest egg producer and fifth largest poultry meat producer in the world (Mitra and Bose 2005). Within India, climatic extremes make poultry production more management- and capital-intensive in the North. Hence, poultry production is concentrated in southern India. The Indian poultry industry employs about two million people, and contributes 1.1% to the national income. Growth in the poultry sector has been dominated by the large-scale commercial private sector, which controls roughly 80% of the total poultry production in the country (Joshi *et al.* 2003). Commercial broilers and eggs are often produced by separate enterprises that use specialized broiler and layer breeds. Vertically-integrated supply chains often encompass activities such as hatching of eggs, breeding of day-old chicks, production of broilers, processing of feed for chicks and broilers; and the selling of live birds and processed meat and ready-to-eat chicken items.

Poultry research is conducted in both the public and private sectors. In the private sector the research focus is on pure-line breeding of layers and broilers, and development of compound feed and vaccines. Various poultry equipment such as incubators, hatchers, and farm equipment are also manufactured by the private sector. Research in poultry has paid off: higher productivity levels are being achieved as a result of investment in poultry equipment, quality feed and pharmaceuticals. Currently, poultry meat constitutes approximately 25% of the total meat production in the country (Mitra and Bose 2005). However, in India only about 2-3% of the total poultry meat is sold as processed meat. There are two main reasons for this: consumers' preference for live chicken and inadequate infrastructure such as refrigerated transport. However, the situation is changing with international retailers opening food outlets to cater for an increasingly affluent population.

The link between the maize and poultry sectors is through poultry feed. Of India's total annual maize production of 19 million t, 48-55% is destined for poultry feed. For broiler rations, maize accounts for most of the energy in the feed ration, and soybean meal provides most of the protein. Broiler rations on average contain 60-65% maize, 28-30% soybean meal, and 2-3% oil. There are substitutes for both the energy and protein ingredients in poultry feed. The most common maize substitutes for energy are broken rice, millet and wheat. And for soybean meal, the common substitutes are fish meal, sunflower meal (decorticated) and peanut meal. Recently, the supplementation of feeds with commercially produced and relatively cheap synthetic amino acids has become common. Feed accounts for 70–75% of the total operational cost of poultry production. Therefore, improving feed efficiency is important in maximizing profitability (Singh *et al.* 2002). Reducing feed costs might also include the use of QPM.

Quality Protein Maize and Poultry

Humans and monogastric animals cannot synthesize nine of the amino acids found in proteins. These 'essential amino acids' must therefore be acquired through the diet. Two of the essential amino acids are lysine and tryptophan. The proportion of lysine and tryptophan is higher in QPM, approximately 75% and 83% respectively. There is growing interest in QPM as feed for chicken, and a number of QPM varieties have been released in India: Shakti-1 (a QPM composite); Shaktiman-1 (a three-way hybrid) and Shaktiman-2 (a single cross hybrid) and HQPM1.

The more challenging researchable constraint is that yields of QPM in India tend to be lower than that of normal maize hybrids. Ongoing research may well lead to the development of higher-yielding QPM varieties but other challenges remain. A recessive gene controls QPM's high lysine levels. Maize is an open-pollinated crop and hence this gene can be easily lost through cross-pollination. So there is a need to isolate (in space or time) or ensure blanket coverage of QPM source areas. Furthermore, some QPM varieties are more vulnerable to insects and toxin-producing molds although other QPM varieties have been found that have some in-built resistance to some molds.

Maize is primarily used for energy and not for protein in poultry feed in India. QPM may be a suitable substitute for some of the protein sources used in feed, such as soybean meal and synthetic amino acids, which are more expensive than maize. However, synthetic lysine is relatively cheap, and few in the poultry industry have heard of QPM. In addition, it is unclear whether QPM is an economically viable alternative to synthetically produced amino acids not least because while lysine is the first limiting amino acid in maize-based pig feed, for poultry feed it is methionine, the content of which is only slightly higher in QPM than in regular maize. Methionine is only available in synthetic form. Another issue is one of traceability. While some maize breeders can distinguish QPM from nonQPM maize, it is not easy for the lay person. QPM entering the maize-poultry value chain would have to be kept separate from nonQPM maize, thus adding to the costs of the product.

Research Priorities

Collaborative research

Poultry meat consumption and exports are predicted to grow in India (Manning and Baines 2004). Concomitant with growth in the poultry sector, the maize sector in India is poised to expand considerably. DMR is focusing some of its energies on QPM and yet several hurdles have to be cleared, not least that in general yields of QPM tend to be lower than that of normal hybrid maize. Demand for QPM from the poultry industry also needs to be stimulated. If a demand for QPM existed, then the private sector would likely (policy environment permitting) engage at different levels of the value chain (seed production, maize grain production, etc). A first step could be to initiate collaborative research between maize breeders and poultry companies to determine whether QPM contributes to more efficient production of broilers and whether it is cost-effective.

Policy changes

Growth in demand for maize is likely to outpace gains in domestic production. Variable domestic production, expanding feed use, and tariff and quota restrictions on maize imports could combine to constrain growth in both the poultry and egg industries, raise consumer prices and slow consumption. Decisions may have to be made to reduce tariff and quota restrictions on Indian maize imports in order to reduce feed prices for poultry and egg producers by allowing maize to be imported more freely at the world price (Landes *et al.* 2004). Another policy area that may need to be addressed is the impediments to contract farming. Historically, agricultural marketing in India has been subject to a large number of regulations and some state governments have the sole authority to establish and manage wholesale markets, effectively prohibiting contract farming. The regulations discourage private sector participation in developing markets and lead to inefficiencies (Joshi *et al.* 2003). Greater private sector activity in seed production of improved maize and expansion of the maize area in India would be encouraged were states to relax some of these regulations and allow farmers to market their produce more freely (World Bank 2007).

Conclusions

The poultry industry in India is growing, spurring the demand for maize. The maize-poultry value chains harbour a number of researchable constraints that need to be addressed especially if the Indian Government continues to place strong emphasis on QPM. These include ensuring that yields of QPM varieties are on par with normal maize hybrids and working with the poultry industry to confirm the value of using QPM in poultry feed and hence ensure that QPM value chains become more demand- than supply-driven.

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Adoption of Improved Maize Production Technology: A Case Study of Sukranagar Outreach Research Site, Chitwan, Nepal

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Abstract. Maize is a major food crop that is directly related to food security and income-generating opportunities. The main objective of this study by a multidisciplinary team was to assess the extent of adoption of maize production technology in Nepal. Both primary and secondary data were utilized for this study. Primary data was collected through a household survey and by employing RRA/PRA techniques. A total of 79 farm households with an average family size of 6.5 persons were selected randomly for the household survey. The cultivated area of the surveyed households was 83 ha with an average farm size of 1.05 ha. Farmers reported that the average yield of summer maize had increased by 14% compared to baseline data whereas that of winter maize had increased by 80%. The increase in yield was mainly due to the improved quality of seed, management, technology and timely replacement of seed. The area under improved maize varieties such as Rampur Composite, Arun 2 and Gaurav hybrid had increased because of the supply of source seed by the National Maize Research Program (NMRP), Rampur. Farmers were more attracted to winter maize as a cash crop because it fetched an attractive price as a source of seed for spring and summer planting. Winter maize was an income-generating activity because of its favorable price in the market and its use as livestock feed. There is a need to disseminate adequate packages of maize technology for enhancing production and productivity to bridge the gap between yields in the research stations and in farmers' fields. One of the important impacts of the onfarm outreach research program was enhancement of farming skills and knowledge.

Key words: Enhancing food security, income-generation, multidisciplinary team, yield.

Introduction

Maize is the second most important staple crop after rice in terms of area and production in Nepal. It is cultivated under different agroecological domains in the hill and *Terai* regions of the country. Primarily, maize is used for human food and cash income and as feed and fodder for livestock. It is cultivated on 870 401 ha with an average yield of 2091 kg ha^{-1} (MOAC 2007). About 69% of maize production is produced in the hill region where maize surpasses rice as the staple food. About 20% is produced in the *Terai* and the rest in the high hills. The demand for maize has been increasing substantially for food and feed purposes, especially in the poultry industry. A large proportion of maize production is fed to animals in accessible areas in Kathmandu, Chitwan, Nawalparasi and Makawanpur districts where poultry and dairy industries have been established.

The National Maize Research Program (NMRP) has released 19 improved maize varieties and others are in the pipeline (NARC 2008). Some varieties like Rampur Composite, Arun2 and Arun 4 have been widely adopted and can be grown from the *Terai* region to the middle range of hills. In addition, eight other varieties, ie, Arun 4, Mankamana-2, Pop44-C10, Pool-21, BA-93, Hill Pool (yellow & white), Pool-15E, Pool-17E and ZM 621, are in the pipeline (Paudyal *et al.* 2001). It has been acknowledged that adoption of improved maize varieties is limited due to the nonavailability of quality seed in time. Improved seed produced by research stations are very limited in quantity and not easily available to farmers. The Agri-business and Trade Promotion Multi-Purpose Cooperation Ltd. (ABTRACO 2004) has reported that about 33% and 58% of the households in the small and medium farm-size categories, respectively, adopted improved maize technologies and only 28% of the households in the marginal category in the Deurali AER (agroecological research) site in Palpa district. The most promising varieties adopted by farmers in this study were Manakamana-3, Rampur Composite, Rampur Yellow and Ganesh-3.

The study also assessed the constraints of researchers,extension workers, policymakers and planners.

It is expected that this study will contribute toward formulating a strong rural livelihood system based on maize production for resource-poor farmers and the *dalit* (socially underprivileged) community in the study area.

Main Objective

The main objective of the study was to assess the extent of adoption of maize production technology at onfarm outreach research (OR) sites under the command of the NMRP, Rampur, where various types of research-generated technologies are demonstrated and disseminated to farmers.

Specific objectives

1. Understand the level of adoption and impact of improved maize technology on farm households in the OR sites
2. Identify the constraints to adopting improved maize varieties
3. Examine the status of maize-based technology in improving food security
4. Identify gender roles in maize production
5. Suggest areas of intervention to enhance maize production, productivity and to improve the livelihoods of farmers

Methodology

For this study, we selected the Sukranagar OR site in the command area of NMRP, Rampur. Both primary and secondary data were used to analyze the situation. Relevant data on maize production, adoption and varieties were collected from existing literature and ongoing research programs. Primary data were collected through a household survey using RRA/PRA tools. A standard questionnaire was prepared to collect information from individual households on their socioeconomic condition, farming systems and livelihoods. Other relevant information was collected from various sources. A total of 79 households were selected randomly from the study site. The study was conducted by a multidisciplinary team that included a socioeconomist, an agronomist and a plant pathologist. Field data obtained through the household survey was analyzed using SPPS. The household survey was carried out in September 2005.

Site description

Sukranagar in Chitwan district has been an OR site under NMRP, Rampur since 1992. It is 20 km from Bharatpur, ie, the district headquarters, at an altitude of 228 m above mean sea level. It represents the rainfed lower wetland conditions typical of the inner *Terai* region. The major cropping patterns are rice-wheat, rice-lentil, rice-fallow-spring maize, rice-buckwheat-maize in the lower wetlands and maize-rapeseed, serseem-rapeseed and maize-millet in the rainfed uplands. The annual rainfall at the OR site is 1600-2000 mm, the maximum rainfall occurring during June, July and August. The average maximum temperature is 30°C from May to September and minimum 17°C in January (Site Description Survey 1997).

The NARC’s National Maize Research Program, Rampur has been significantly contributing to Nepal’s goal of national poverty alleviation since 1999. The overall goal of the project is to improve the food security and livelihoods of farm families by increasing the productivity and sustainability of maize-based cropping systems in the hill regions of Nepal and develop a sustained capacity within the NMRP to generate maize technologies. Its objectives include:

- Developing new technologies for maize farmers; and
- Facilitating dissemination of new technologies through extension and input delivery channels.

Current activities of the OR program at Sukranagar

Different activities along with prioritization of researchable issues are being carried out in collaboration with the DADO and local NGOs. In all 14 experiments have been carried out at the OR site involving maize-based cropping patterns with a full package of technologies. In addition, activities such as farmers’ field trips, training programs, farmers’ day, etc. have been carried out. Technology verification and dissemination processes are ongoing and feedback from farmers is taken into consideration. A hybrid Gaurav has been developed by NMRP, Rampur and adopted by private seed growers and distributed to local farmers and agriculture enterprises.

Results and Discussion

Community-Based Maize Seed Production program (CBMSP)

The CBMSP approach at the OR site is aimed at producing quality seed within the farm community through participatory research involving farmers, extension personnel and researchers. The main goal of this approach is to maintain varietal purity at the farmers' level, which would lead to enhanced production and productivity and thereby generate income for the farmer. Our collaborating group observed that the CBMSP at the Sukranagar study site was more effective and efficient than the practices ofindividual farmers.

The major information gathered at the site is summarized below:

- Name of group: Namuna Beej Bridhi Khrishak Samuha, Sukranagar
- Number of members : 36-41 (male and female)
- Varieties and area: Rampur Composite in 15 ha; Arun 2 in 5 ha
- Seed production: 1.6 t ha^{-1}
- Farmers' reported yield: >2 t ha^{-1} grain and 1.5 t ha^{-1} seed
- Seed sales: About 75% sold for seed purpose. Local consumption 75% and other districts 25%.
- Perception of the varieties: Rampur Composite has a big-size cob and Arun 2 is an early variety and suitable for seed purposes

Socioeconomic features

The total population of the 79 surveyed households was 515 with an average family size of 6.5 persons. The highest average family size belonged to Scheduled Caste, Gurung and Puri households: 8.2, 7.7 and 7.7 persons, respectively. The lowest average family size belonged to Chhetri, Newar and Brahmin households: 5.0, 5.0 and 6.1 persons, respectively. Brahmins (47%) were the largest ethnic group followed by Tharus (30%) (Table 1).

Agricultural landholding

The owned cultivated area of the surveyed households was 82.95 ha at an average farm size of 1.05 ha. The average landholding of owned farmland, *khet* (lowland, for rice) and *bari* (upland, for maize and vegetables) was 0.11 ha, 0.69 ha and 0.24 ha, respectively. There was no system of rented-out land, and about 0.56 ha of land was distributed among farmers in rented-in land tenancy. Being a *Terai* area, the distribution of owned farmsteads, *khet* and *bari* land was about 11%, 66% and 23%, respectively (Figure 1).

The Tharu caste is an indigenous farming caste in this *Terai* area with an average *khet* farm size of 0.88 ha. The average total farm size of Brahmins, Chhetris, Schedule Castes, Newars, Puris, Tamnags and Gurungs was 1.04 ha, 0.86 ha, 0.90 ha, 0.30 ha, 0.64 ha, 0.95 ha and 0.80 ha, respectively. The T-test analysis for *bari* and *khet* land between the Brahmin and Tharu communities was not significant (Table 2). It showed that both these communities share landholding proportionately.

Table 1. Ethnic composition of surveyed households.

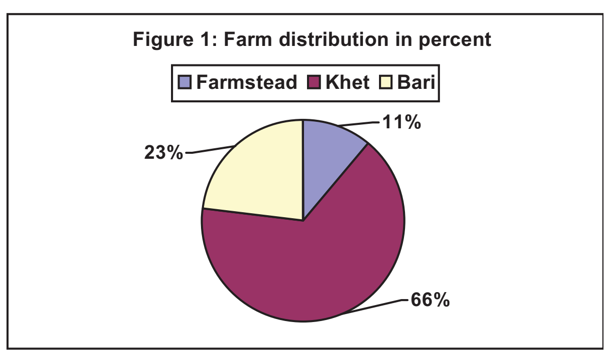
Ethnicity	No. of households (n = 79)	Population (n = 515)	Family size
Brahmin	36 (47)[1]	219 (42)	6.1
Chhetri	3 (4)	15 (3)	5.0
Tharu	24 (30)	148 (29)	6.2
Schedule Caste	2 (2)	33 (6)	8.2
Newar	1 (1)	5 (1)	5.0
Puri	3 (4)	23 (4)	7.7
Tamang	4 (5)	26 (5)	6.5
Gurung	6 (7)	46 (10)	7.7
Overall	79 (100)	515 (100)	6.5

1. The figures in parentheses are percentages.

Table 2. Average landholding (ha) by ethnicity.

Ethnicity	*Bari* (n = 18.58 ha)	*Khet* (n = 55.07 ha)	Total land (82.61 ha)
Brahmin	0.25 ns[1] (36)[2]	0.66 ns (33)	1.04 (36)
Chhetri	0.06 (3)	0.56 (3)	0.86 (3)
Tharu	0.16 ns (24)	0.88 ns (24)	1.12 (24)
Scheduled Caste	0.17 (2)	0.67 (2)	0.90 (2)
Newar	-	0.23 (1)	0.30 (1)
Puri	1.39 (3)	0.12 (3)	1.64 (3)
Tamang	0.18 (4)	0.64 (4)	0.95 (4)
Gurung	0.03 (6)	0.63 (6)	0.80 (6)
Overall	0.24 (79)	0.70 (79)	1.05 (79)

1. Figures in parentheses refer to the frequency of households.
2. NS = Not significantly different at 5% level.



Land distribution pattern

The distribution of land was inconsistent among farmers. About 43% of the total land belonged to medium-size arm households comprising 34% of the total households. Only 6% of the total land was in the possession of marginal households which comprised 19% of the surveyed households (Table 3). This makes for a very high man:land ratio. The number of farmers belonging to each land group varied significantly.

Table 3. Land distribution based on farm size (ha).

Land classification (ha)	Proportion of households	HH %	Proportion in land	% of proportion
Marginal (<0.5)	15* (-8.98)	19	5.28	6
Small (0.5-1)	32* (-10.07)	40	24.11	29
Medium (1-2)	27* (-6.14)	34	35.80	43
Large (2 or more)	5* (-2.56)	6	17.83	21
Overall	79 (100)	100	82.95	100

*Figures in parentheses refer to the paired t-test value at P <0.05 significant level.

Area and productivity

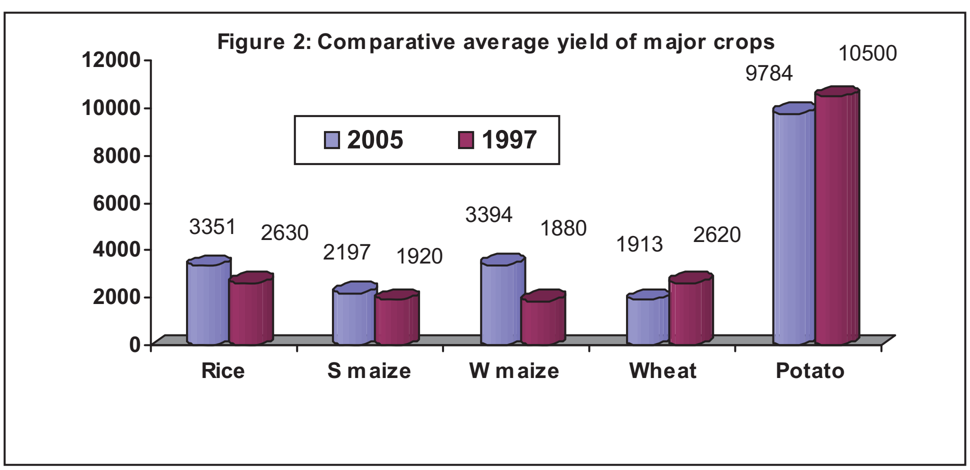
The study indicated a high potential for adoption of improved varieties of maize. This may be mainly because of the impact of NMRP, Rampur and the CBMSP program. The average yield of summer maize increased by 14% and that of winter maize by 80% as compared to baseline data (Table 4). The increase in crop yield was mainly due to improved quality seed, management, technology and timely replacement of seed. The average yield of wheat and potato decreased by 27% and 7% respectively as compared to baseline data, which was probably due to lack of improved seed, decline in soil fertility, lack of improved cultivation practices, lack of improved technology and management and lack of seed replacement.

The average yields of rice, summer maize, winter maize, wheat and potato were 3351 kg ha^{-1}, 2197 kg ha^{-1}, 3394 kg ha^{-1} and 9784 kg ha^{-1}, respectively (Figure 2). Brahmin and Tharu farmers had the highest average yield of summer and winter maize. The average yield of summer maize obtained by Brahmin, Tharu and Gurung groups was 2512 kg ha^{-1}, 2280 kg ha^{-1} and 2118 kg ha^{-1}, respectively.

Table 4. Area, production and yield of major crops and annual income.

Crop	Area (ha)	Production (kg)	Yield (kg ha^{-1}) Survey year	Baseline**	Change (%)	Average annual income (Rs)
Rice	62.48 (78[1])	209 352 (78)	3351	2630	27	12 764* (48)
Summer maize	42.10 (72)	92 436 (72)	2197	1920	14	7898* (61)
Winter maize	5.73 (12)	19 250 (12)	3394	1880	80	12 515* (10)
Wheat	5.70 (34)	10 906 (34)	1913	2620	- 27	2707* (10)
Potato	2.64 (31)	25 830 (31)	9784	10 500	- 7	7560* (15)

1. Figures in parentheses refer to the frequency of households.
* Significantly different at 0.05% level.
** Source: Site description survey of OR command area under ARS, Rampur, 1997.



Four improved varieties of maize, Rampur Composite, Arun 2, Khumal Yellow and Gaurav hybrid, had been introduced here after the initiation of the OR program. Before that, the surveyed households used to cultivate six improved maize varieties: Rampur Composite, Hetuda Composite, Rampur Yellow, Khumal Yellow, Arun 1 and Arun 2. Farmers selected those improved varieties that fit into their cropping system. The area under Rampur Composite increased by 57% to 26.63 ha after the OR program was launched. Similarly, the increase in the yield of Rampur Composite was 118 kg ha^{-1} which was a 5% increase. A similar trend of yield increment was noted for the hybrid variety as well (Table 5).

The area under the improved maize varieties Rampur Composite, Arun 2 and Gaurav increased because of the supply of source seed by NMRP, Rampur as part of the CBMSP. This program was highly effective in disseminating the preferred varieties among farmers. Farmers were more attracted to winter maize as a cash crop because of the attractive price it offered as a source of seed for spring and summer planting. Winter maize was treated as an income-generating activity because of the favorable price, market and livestock feed.

Production trend

Farmers' perceptions on the trends in maize productivity were highly significant because of the adoption of improved maize varieties and management practices. About 87% of the surveyed households said maize productivity had increased after the initiation of the OR program.

Table 5. Area and average yield (kg ha^{-1}) of different maize varieties.

Variety	Before OR[1] program		After OR program	
	Area (ha)	Yield (kg ha^{-1})	Area (ha)	Yield (kg ha^{-1})
Local	21.33	1276	-	-
Rampur Composite	16.90	2306	26.63	2424
Hetuda Composite	0.67	2100	-	-
Rampur Yellow	8.53	1614	-	-
Khumal Yellow	0.17	2100	0.33	2100
Hybrid	0.20	6000	1.37	6660
Arun 1	0.07	1500	-	-
Arun 2	-	1800	6.10	3117

1. OR = Outreach research.

Table 6. Production costs and returns of maize variety Rampur Composite, 2005.

Cost/returns	Unit	Rampur Composite		
		Quantity	Rs $unit^{-1}$	Value (Rs)
1 Human labor	Man days	57		3880
1.1 Male		20	80	1600
1.2 Female		38	60	2280
2 Animal power	Frequency	5	250	1250
2.1 Tractor	Minutes	149	10	1490
Seed	Kg	34	25	850
4 Fertilizers	Kg			902
1.1 DAP		9	22	198
1.2 Urea		41	16	656
1.3 Potash		3	16	48
5 FYM	Kg	15563	0.5	7781
6 Pesticides	Rs	-	-	50
Total cost	Rs	-	-	16 203
7 Grain yield	Kg			
7.1 Maize products		2424	9	21 816
7.2 Byproducts		4848	0.5	2424
8 Gross return	Rs			24 240
9 Net benefit	Rs			8037
10 Benefit:cost ratio				1.5

Cost of production

The costs of maize production given in Table 6 are only illustrative. A financial analysis of the variety Rampur Composite showed that net return was Rs. 8037 ha^{-1} and the cost:benefit ratio 1.5.

The major items of cost included human labor for land preparation, weeding, harvesting and threshing, which accounted for 24% of the cost of production while farmyard manure (FYM) cost 48%. Other costs such as animal power and tractor (17%), seed (5%) and fertilizers (6%) were equally important for maize cultivation. The average gross return and net return from maize was Rs 24 240 and Rs. 8037, respectively. The low benefit:cost ratio (1.5) and net return could be attributed to the high cost of FYM application and the low price of maize. Most of the farmers in the surveyed site continued to grow maize as a subsistence crop rather than as a commercial crop.

Income from major crops

The average annual income from rice, summer maize, winter maize, wheat and potato were Rs. 12 764, Rs. 7898, Rs. 12 515, Rs. 2707 and Rs. 7560, respectively (1 US$ ~ 62.00 NRs in 2005 during the study period, Figure 3). Rice and winter maize were sources of high income, the latter because of its use as seed. Crop areas corresponding to annual income significantly differed at the 5% level of paired t-test value.

Reasons for selecting improved maize varieties

Farmers' responses indicated that grain yield (58%) was the most important attribute governing their choice of improved maize variety, followed by diseases/pest resistance (56%) and drought tolerance (48%) (Table 7). The least preference was given to grain color (21%) and grain size (29%).

Livelihoods improvement

Irrespective of ethnicity, farmers perceived improvement in their livelihoods as a result of their participation in the OR program by adopting improved maize production technologies.

However, Brahmins followed by Tharus derived more benefits from the program than the other groups (Table 8). Use of maize as food, feed, fuel and for income generation was greater in these communities.

Biophysical and socioeconomic constraints

From respondent replies, it was evident that nonavailability of irrigation (25%), lack of technical knowhow (22%), lack of disease and pest control measures (17%) and nonavailability of improved seed (14%) were the major biophysical constraints to maize production. The most significant socioeconomic constraints for adopting

Table 7. Attributes preferred by farmers in selecting improved maize varieties.

Plant attribute	Preference %	T-test value
Plant height	17	6.99*
Disease/pest resistance	56	16.29*
Drought tolerance	48	17.03*
Grain size	29	9.66*
Grain color	21	12.63*
Food quality	43	18.19*
Grain yield	58	11.06*

*Significant at $P < 0.05$ level.

Figure 3: Average income from major crops

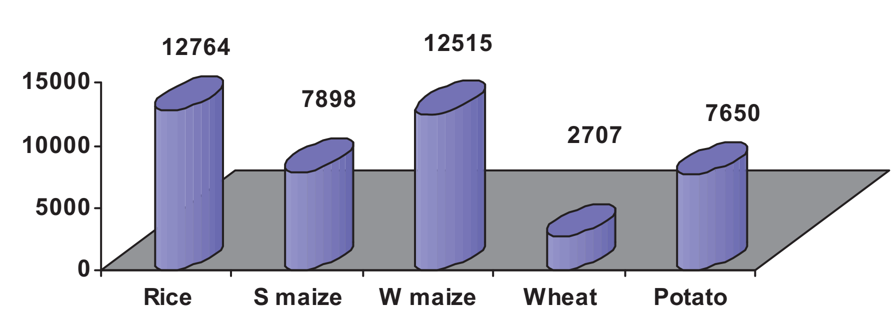


Table 8. Farmers' perception of improvement of their livelihood through participation of the OR program.

Ethnicity	Purpose (%)						
	Food	Feed	Grass	Seed	Fuel	Income	All
Brahmin	42	57	71	50	50	55	31
Chhetri	9	5	-	10	-	6	-
Tharu	39	19	14	30	50	26	38
Newar	-	5	14	-	-	3	-
Puri	-	-	-	-	-	-	23
Tamang	3	5	-	10	-	-	-
Gurung	6	9	-	-	-	10	8
Overall	100 (n=33)	100 (n=21)	100 (n=7)	100 (n=10)	100 (n=2)	100 (n=31)	100 (n=13)

Table 9. Households' perceptions on biophysical and socioeconomic constraints.

Bio-physical problems	% of response n=72	Socioeconomic problems	% of response n=72
Unavailability imp. Seed	14	Small landholdings	19
Unavailability of fertilizers	3	Labor shortage	3
Unavailability of irrigation	25	Cash shortage	13
Diseases and pests	17	Unavailability of credit	7
Poor soil	5	Technical information	26
Weeds problems	1	Low market price	19
Technical know-how	22	High risk	8
Low yield	1	Expensive technology	1
Hailstone	3	Unaware of price	1
Water-logging	5	Low income compared to alternative crops	1
Parrot problems	3		

improved maize varieties were nonavailability of technical information (26%), small landholdings and low market price (19%) and cash shortage (13%) (Table 9).

Gender

With regard to decision-making on farming activities, men seemed to have a greater say than women. However, the involvement of women showed an increasing trend. Since the launch of the OR program, 19% of the women were involved in decision-making related to seed selection activity in the surveyed households (Table 10).

Table 10. Farmers' responses on decision-making on maize cultivation.

Activities	Decision-making by gender (%)		
	Male	Female	Both
Variety selection	50 (36[1])	7 (5)	43 (31)
Seed selection	24 (18)	19 (14)	56 (41)
Fertilizer use	53 (37)	7 (5)	39 (27)
Buying and selling	51 (38)	9 (7)	40 (30)
Pesticide use	76 (41)	11 (6)	13 (7)
Postharvest operations	14 (10)	12 (9)	74 (54)

1. Figures in parentheses are the frequency of households.

Conclusions and Recommendations

Maize is directly related to food security and income-generating opportunities in Nepal. Our study revealed that adoption of improved maize varieties is increasing due to the implementation of OR activities. The National Maize Research Program, Rampur has supported technology generation, verification and dissemination through the Community-Based Maize Seed Production Program to make seed of improved, high-yielding varieties available tofarmers. There is a need to provide sufficient packages of maize technology to enhance production and productivity because there is still a big gap between research yield and farmers' yield. A complete package of practices needs to be developed to improve food security and to generate income opportunities that are directly related to poverty reduction. There is a need to promote and strengthen participatory research programs on technology generation and scale them up in the field with a strong focus on the community-based seed production program. The study showed that the OR site has been helping farmers to adopt new maize technology for enhancing productivity. However, to make the program's success sustainable, development and promotion of relevant technology is essential in close partnership with farmers, extension personnel and other stakeholders including maize agroindustrialists.

The study indicates that winter maize is a source of high income because of its use as seed for the summer crop. One of the important impacts of the OR program was enhancement of skills, knowledge and technical know-how of farmers. The overall response of different communities showed that irrespective of ethnicity, farmers achieved improvement of their livelihoods through participation in the OR program.

Acknowledgment

We are grateful to Dr. D.P. Sherchan, chief of HMRP, Rampur, and the support staff of HMRP for their encouragement in carrying out this study. We acknowledge the cooperation extended to use by the ORD. We also thank the farmers of the study site for their participation in the household survey.

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Feasibility of OPV Srikandi Maize for Overcoming Productivity and Food Security Problems in East Nusa Tenggara, Indonesia

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Abstract. Maize is the staple food in East Nusa Tenggara (ENT) province of Indonesia. Productivity is only 1-2 t ha^{-1} as a result of poor input management. This study assessed the feasibility of new maize cultivation technology in an experiment with 30 farmers on 15 ha in South Timor Tengah district of ENT during the 2007/2008 rainy season. We compared the farmers' existing technology with new technology. The data were subjected to partial budget analysis including return:cost (R:C) ratio and benefit:cost (B:C) ratio calculation. Maize productivity under the improved package [which included open-pollinated variety (OPV) Srikandi maize and recommendations relating to fertilizers and planting distance] was 3.4 t ha^{-1}. In contrast, under farmers' existing practices it was only 1.7 t ha^{-1}. TFarmers' income under the improved package was Rp. 4.8 million and the R:C ratio 3.28. Farmers' practices fetched an income of only Rp. 2.4 million. The applied maize technology was thus found to be worth disseminating on a wider scale in ENT.

Key words: Technology feasibility, dryland, maize, East Nusa Tenggara

Introduction

The East Nusa Tenggara province (NTT) of Indonesia has a predominantly dryland agroecosystem that is greatly influenced by the *muson* winds. The four-month rainy season begins in December and ends in March, and the eight-month dry season begins in April and ends in November. Rainfall is not spread evenly. During the rainy season, the highest rainfall (2000-3000 mm per year) falls in the western part of Flores Island, the western part of Sumba Island and the central part of Timor Island. The lowest rainfall (1500 mm per year) occurs in Timor, Flores, Sumba and Alor. The highest radiation (98%) takes place in October and the lowest (50%) in January (http://www.bkpmd-ntt.go.id/id/profil.html).

The province has potential for maize and bean production. The target for maize plantation in 2005/2006 was about 12 000 ha but only about 6000 ha was realized (Agricultural ENT Province 2004). This influenced the availability of food.

It is estimated that 89% of the people of ENT are farmers, 79% of them being dryland farmers with maize as their prominent crop. Land, soil and climatic conditions are the main determinants of agriculture in this province. The El Nino phenomenon, frequent drought, pests and diseases make ENT vulnerable to food scarcity.

Maize productivity is generally low at 1-2 t ha^{-1}. This is because of the use of traditional cultivation technologies with little attention being paid to to the variety used, seed, fertilizer and control of pest and diseases.

The open-pollinated variety (OPV) Srikandi, a good source of carbohydrate with high content of protein (especially lysine and tryptophan), could substitute local maize as the staple crop and consequently lead to an improvement in the people's nutrition. Compared with other excellent varieties such as OPV Lamuru, the lysine and tryptophan content of Srikandi (Azrai 2004) is almost double (protein 10.44%, lysine 0.410% and tryptophan 0.087%). Lysine and tryptophan are two of 10 essential amino acids are needed by the human body. The need for lysine and tryptophan is normally met by consuming meat and beans, but ENT farmers sell their beans and livestock to buy food for their carbohydrate needs. Therefore, any development strategy for this province must include maize with high productivity, drought tolerance and high protein content.

Objective

The aim of this study was to identify maize cultivation technologies that will enhance productivity and farmers' income as well as secure the food security of the dryland farmers ofEast Nusa Tenggara.

Methodology

Research location

Our research was conducted in farmers' fields in Tobu village in Tobu subdistrict in South Central Timor Regency (TTS)during the 2007/2008 rainy season.

Research approach

We adopted the approach of on-farm participatory research for this study. The study was conducted in farmers' fields with the active involvement of farmers, field officers and researchers. The introduced technologies were the OPV maize variety Srikandi and recommendations on fertilizers and plant spacing (Table 1). Our study was based on the principle of group empowerment of farmers to accelerate adoption of technology. The study involved a comparison of results achieved by participating farmers implementing improved technology (cooperator farmers) and and a control group of farmers using local technology (noncooperator farmers).

Data analysis

The analysis of income was done as perDowney and Erickson (1985).

$$I = \sum (y.Py) - \sum (x_i.Px_i)$$

Where
I = Income
Y = Output

Table 1. Farmers' existing practices and introducted technologies.

Technology	Farmers' existing practices	Introduced technologies
Crop	Maize	Maize
Variety	Local	White Srikandi
Plant spacing	Irregular; 4-5 seeds per hole	100 x 40 cm
Time of planting	Beginning of wet season	Beginning of wet season
Fertilizer	No fertilizer	Urea 200 kg ha^{-1} + SP36 100 kg ha^{-1} (fertilization I: ½ dosage urea at 7-10 dap + SP36; fertilization II: ½ dosage urea 25-30 dap)
Weeding	Manual	Manual

Px_i = Input cost
Py = Output cost
X_i = input (i = 1,2,3….n)

The analysis was conducted by using partial budget analysis. The R:C ratio (*return:cost ratio*) was calculated as per Soekartawi (1995). Mathematically, it was calculated as follows:

$$a = \frac{R}{C}$$

R = Py.Y
C = FC + VC
a = {(Py.Y) / (FC + VC)}

Explanation:
a = R/C ratio
R = Revenue
C = Cost
Py = Price of output
Y = Output
FC = Fixed cost
VC = Variable cost

If a>1: reasonable
a<1: not reasonable
a=1: equal/breakeven (no loss)

Results and Discussion

Feasibility of maize cultivation technology

The new maize technology did increase productivity. Productivity under new technologies was 3.404 t ha^{-1} and under farmers' practices only 1.737 t ha^{-1} (Table 2).

The productivity of farmers who persisted with their conventional practices (noncooperator farmers) was lower than that of cooperator farmers who applied the new technologies. Srikandi maize, besides having high productivity, had lower plant height than the local maize

Table 2. Maize plant height, diameter and population under existing farmers' practices[1] and new technologies.

Technology	Farmers' existing practices	Introduced technologies
Plant height (cm)	203.30	184.37
Plant diameter (cm)	2.68	2.70
Plant population per ha	43 000	62 500
Productivity (t ha^{-1})	1.737	3.438

1. All cooperator farmers used OPV Srikandi and noncooperator farmers used a local OPV variety.

cultivar. This makes it suitable for ENT province where unpredictable climate particularly rainfall and wind causes lodging in the local maize cultivar (average height 2 m). As a result, pollination does not take place perfectly which in turn causes loss of production. The plant diameter of Srikandi maize is bigger than local maize. This gives a physiological advantage compared to local maize which tends to be damaged by wind when maize cobs start growing.

Srikandi maize is suitable for development in higher areas because it has high productivity, drought tolerance and higher protein content than local varieties. Azrai (2004) reported that white Srikandi maize has 0.410% lysine content and 0.087% tryptophan, which are needed for human nutrition.

Consumption of Srikandi maize grain (0.5 kg day^{-1} per capita) will render about 2.400 mg day^{-1} (0.48% x 0.5 kg) of lysine. Srikandi maize could be the answer to nutrition problems in this province (Hosang 2006).

Both cooperator and noncooperator farmers expressed interest in growing Srikandi maize because:

- The results were quite good in spite of constraints such as water stress and strong winds which affect local maize yields by up to 50%.
- The color of Srikandi maize grain is white, which is similar to that of local maize.
- The husk cover of Srikandi is closed which makes it tolerant to *S. zeamais* and enables storage for a longer period.
- If Srikandi maize is consumed before maturity, its taste is sweeter than that of local maize.

Table 3. Economic analysis of maize cultivation with introduced technologies in comparison with conventional technology.

Component	Introduced pattern	Existing pattern
Productivity (kg ha^{-1})	3438	1737
Input cost (Rp. ha^{-1})	1 693 932	718 750
Postharvest cost (Rp. ha^{-1})	405 000	405 000
Total cost (Rp. ha^{-1})	2 098 932	1 123 750
Benefit (Rp. ha^{-1})	6 876 000	3 474 000
Revenue (Rp. ha^{-1})	4 777 068	2 350 250
B:C Ratio	3.28	3.09
R:C Ratio	2.28	2.09
MBCR	3.49	

Feasibility analysis

The feasibility analysis of cooperator farmers' cultivation with introduced technologies and noncooperator farmers' persistence with local technology during the 2007/2008 rainy season is presented in Table 3.

Productivity of maize under the introduced technologies was 3438 kg ha^{-1} in comparison with 1737 kg ha^{-1} under farrmers' conventional practices. The reasons for this big difference in productivity were: (1) control farmers' use of local varieties with low productivity; (2) higher plant population achieved under the introduced package of technologies; and (3) the conventional farmers' practice of using no fertilizer.

The profits made by cooperator farmers varied from Rp. 4 656 000 to Rp. 9 404 000 ha^{-1} with a mean of Rp. 6 876 000 ha^{-1}. In contrast, the profits of noncooperator farmers ranged from Rp. 1 824 000 to Rp. 5 024 000 ha^{-1} with a mean of Rp. 3 474 000 ha^{-1}. Apart from lower productivity, the costs of production of noncooperator farmers were higher (average Rp. 1 123 750 ha^{-1}) in comparison with those incurred by cooperator farmers (Rp. 2 098 932 ha^{-1}). Further, the noncooperator farmers tended to use seed of the local variety continuously for years. Given the fact that rainfall during the 2007/2008 rainy season was lower than during the 2006/2007 rainy season, local maize yielded 50% poorer results than in the previous year. Apart from its other advantages, Srikandi maize was faster harvested (by about 20 days) than the local variety.

The economic analysis shows that the B:C ratio for maize under the new pattern of practices was 3.28 and the R:C ratio 2.28.In other words, each investment of 1 rupiah would result in an income 3.28 times higher. The MBCR for the new pattern worked out to 3.49 in comparison with the existing pattern.

Conclusions

- Development of the new cultivation technology of OPV Srikandi maize is feasible in the drylands of East Nusa Tenggara given the B:C ratio of 3.48 and R:C ratio of 2.28. Farmers' income increased from Rp. 2 098 932 ha^{-1} to Rp. 6 876 000 ha^{-1}, with an MBCR value of 3.49.
- Farmers were satisfied with Srikandi maize because it gave them higher productivity and enabled an earlier harvest (by 20 days) than their local variety.

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Strategy for Maize Farming Systems Development of Subsistence/Semicommercial Farmers in Indonesia

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Abstract. Subsistence/semicommercial farmers have been slow to adopt new technologies of maize cultivation in East Nusa Tenggara (ENT) province of Indonesia although they were potentially profitable and compatible with local sociocultural conditions. Uptake was hampered by the predominantly food security orientation of subsistence/semicommercial agriculture and the high inputs required by the new technologies (ie, superior maize seed, fertilizer and pesticides). Farmers are constrained by the lack of cash to buy inputs or the nonavailability of inputs at the subdistrict/village level. As a consequence, new technologies could not be extended, and trained farmers tended to revert back to traditional practices. In our study, three participating farmer groups planted 15 ha of maize in South Timor Tengah district in ENT province in 2007-2008. Problems were overcome by close guidance of the groups and establishment of a technology clinic at the village level. The clinic is managed by extension workers and the farmer group (Gapoktan). The clinic provides farmers with agricultural information and ensures input supply (fertilizer and pesticides) by buying them from agricultural shops at the district/province level. Farmers can take inputs on credit from the technology clinic and repay them in kind with maize grain after harvest. The technology clinic also processes maize, sells produce during the early rainy season and helps build maize seed units at the village level. This strategy shows promise in ensuring the continuity of maize farming and has the potential to be extended to other villages.

Key words: Maize, adopt, subsistence/semicommercial farmers, technology clinic

Introduction

Maize is the staple food in East Nusa Tenggara (ENT) province of Indonesia, particularly in the rural areas where 70% of the people of the province live (BPS NTT 2004). However, maize production is very limited although attempts have been made to improve cultivation technology. Productivity at the farm level remains at 1.5-2.0 t ha^{-1}. It could be increased to 2.3-4.8 t ha^{-1} (Bobihoe *et al*. 1999), and a productivity level of 3.4-6.7 t ha^{-1} has been reported under research conditions in ENT (Subandi 1999).

External interventions to increase productivity have been attempted, but adoption of maize cultivation technology by farmers in this province has been very slow, mainly because maize cultivation is primarily oriented toward food security (Yusuf and de Rosari 2001). The Indonesian government has taken up many social and economic initiatives as part of PELITA III (a development plan, phase III) to address this problem, but change in farmers' attitude has been difficult to achieve. Farmers have tended to return to old technologies after the completion of such projects (Lidjang 1995).

Farming in this mainly dryland province remains at a subsistence or semicommercial level (Subandi *et al*. 1997) with a predominant orientation toward food security (Sumarno and Bamualim 1999). So technology information and innovation initiatives must be accordingly designed. There is need for institution building to strive for sustainable technology doption.

The maize technologies used by farmers in this province are very simple; use of production inputs such as fertilizer and pesticides is limited. As a result, land fertility continues to diminish (Murdolelono and Beding 2006).

Subsistence/semicommercial farmers are mainly constrained by low natural resources, uncertain weather during the rainy season, use of inadequate technology and limited capital ownership. They need technology and capital access. Their concerns could be addressed by strengthening farmer groups.

The purpose of our research was to identify a suitable agribusiness model for subsistence/semicommercial maize farmers in ENT.

Materials and Methods

Our study was conducted on three farmer groups in Tobu village in Mollo Utara subdistrict of South Timor Tengah district in ENT during2007-2008.

The innovations sought to be introduced were both technological and institutional. The new technologies included improved varieties, recommendations on plant spacing and fertilizer application and seed production techniques. The institutional initiatives included strengthening of farmer group capital, management of input supply and seed production (Table 1)

The initiative involved operation of technology clinics by extension workers and farmer groups (Gapoktan). The functions of the clinics were to take technological information and input access to farmers. The clinics lend inputs to farmers who then repay them in kind in the form of a part of their maize production. The clinics buy inputs (fertilizer and pesticides) from shops in the subdistrict or district and maize seed from seed production units operated by farmer groups.

Data analysis

Maize production data were collected by measurement. Other data were collected by observation and Rapid Rural Appraisal (Table 2)

Table 1. Existing and improved cultivation techniques and institutional arrangements used by farmers in study villages in ENT province.

Innovation	Existing technology	Technology improvement
A. Maize cultivation Technology		
· Varieties	Local OPVs	White Srikandi OPVs
· Plant spacing	Not uniform; 80-125 × 80-125 cm; 4-5 seeds per hole	100 × 40 cm; 2-3 seeds per hole
· Fertilizer	Not used	N 90 kg ha^{-1} + K_2O_5 36 kg ha^{-1}
· Seed production	Selection from farmers' own maize production	Maize seed production procedure
B. Farmers' institutions		
· Strengthening farmers' group capital	None	Farmers get out maize product equal than inputs price
· Management of input supply	None	Supply of inputs by technology clinics and loaned to farmers againt postharvest repayment in grain
· Management of seed production	None	Farmers produce seed of excellent quality

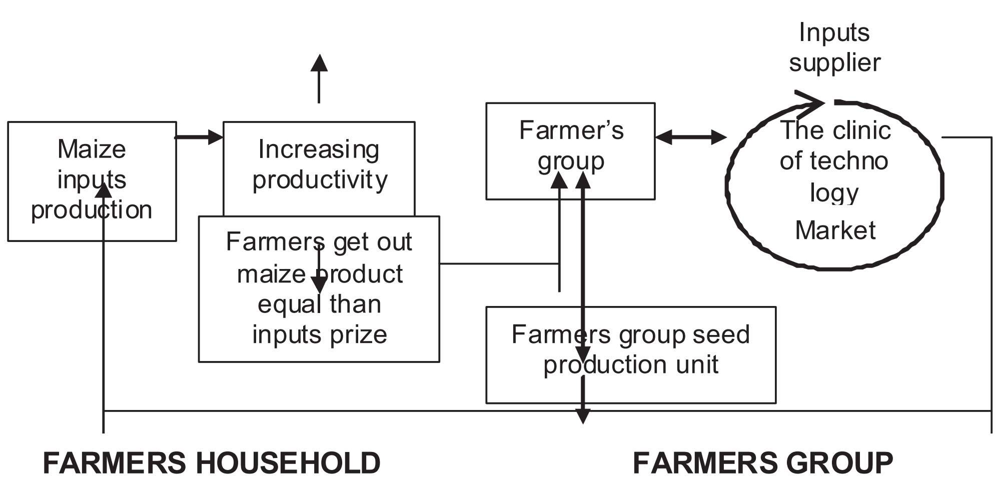

Figure 1. Design for improving farmers' institutions for energy cycle.

Table 2. Data collection and data analysis method.

Innovation	Data collection	Analysis technique
Maize cultivation		
Maize production	In the same year: 9 samples of existing maize cultivation and 9 samples of improved maize cultivation	T-test
	Previous year: measurement of maize production storage before introduction of innovation (exiting technology) and after introduction on the same land	Wilcoxon signed test
Farmers' response	Farmers' response to technology introduction	Descriptive
Institutional innovation		
Strengthening of farmers' group capital	Farmers' response to revolving system	Descriptive
Management of input supply	Farmers' response to input supply system	Descriptive
Management of seed production	Farmers' response to maize seed supply system	Descriptive Observation

Results and Discussion

Maize cultivation

Our research showed that plant height and diameter of the local open-pollinated variety (OPV) and the OPV Srikandi were not significant in the T-test for equality of means at 5%. However, there was significance in respect of amount of plant and productivity. Amount of plant under farmers' conventional practices was 13.25 holes per 10 m^2 and 17.56 holes per 10 m^2 under the newly introduced technology package. Other results showed that the new package of technologies could increase maize productivity from 1753 kg ha^{-1} to 3438 kg ha^{-1} (Table 3).

Srikandi is 105-day and the local varieties are of 120-day duration. The shorter of Srikandi holds an advantage for Timorese farmers because the rainfall period there lasts only 3-4 months. The other advantages of Srikandi are the high protein content ie, lysine 0.410% and tryptophan 0.087% (Azrai 2004).

The study's technological interventions increased farmers' maize storage by 173.2% per household. The average maize storage before our intervention was 80.2 kg per household. This rose to 219.1 kg per household after new practices were introduced (Table 4). These production figures do not include the maize grain deducted by farmers to pay for the inputs borrowed from the technology clinics. Improved production was a surprise for the participating farmers and increased their motivation to adopt maize cultivation.

Farmers' human resources in this area were very low with years of education averaging 4.2 years. This factor does influence acceptance of technology.

Table 3. Comparison of maize plant height, diameter, amount of plant per 10 m^2 and productivity under farmers' existing practices and under newly introduced technology.

Variable	Existing practices	Introduction practices	Notation
Plant height (cm)	149.62	149.78	ns[1]
Tree diameter (cm)	2.42	2.41	ns
Amount of plant (holes per $10m^2$)	13.25	17.56	**
Productivity (kg ha^{-1})	1.753	3.438	**

[1.] ns: not significant under t-test for equality of means at 5%.
** very significant under t-test for equality of means at 1%.

Table 4. Farm households' maize production before and after technological intervention.

Item	Sample size	Household production (kg)**)
Before intervention	10	80.2
After intervention	10	219.1

** Very significant under 2-tailed Wilcoxon signed ranks test.

Seed production

Maize seed production initiatives were taken up in partnership with farmer groups as part of this study. Seed production was conducted on 0.15 ha plots using Srikandi as the foundation seed. Seed production added up to 315 kg (productivity of 2100 kg ha^{-1}). These seed were sold at low prices to farmers in the farmer group as well as to others. Before this seed production initiative, farmers used to useown seed of local maize cultivars, generally selecting bigger maize grain as prospective seed.

To promote adoption of new varieties, improved seed units must be within reach of farmers. However, this was not the case in the study area. Maize seed had to be brought from the capital city of the province, which is about 150 km away. The distance adds to the cost of maize seed. The purpose of setting up seed production units at the farmer level was to ensure easy supplies to farmers and also to ensure that proper procedures of seed production were followed. The main problem affecting seed production in the study area was availability of irrigation. Seed production must be done during the dry season, which requires adequate irrigation. As a consequence, seed production needs much labor, mainly for providing irrigation to the seed plots.

Institutional innovation

Generally, subsistence/semicommercial farmers live far from the city. Their mobility and communication access is hampered by poor road infrastructure. Since government and church organizations are relatively more accessible to farmers, their help has been sought for dissemination of agricultural information in the study area. The weekly traditional market held every Saturday provides another opportunity to disseminate technology information as farmers tend to congregate there. It is therefore ideal to set up technology clinics near traditional markets.

Subsistence/semicommercial farmers are constrained by (a) low natural resources and unsuitable agricultural cultivation practices which lead to low productivity; (b) uncertain weather, mainly during the rainy season, causing uncertainties in agricultural production; (c) limited access to capital and credit; and (d) lack of agricultural knowledge. Lack of access to capital weakens farmers' bargaining position and forces them to sell their produce unprofitably.

Organizing subsistence/semicommercial farmers into farmer groups is difficult. The groups are often not based on a firm foundation and therefore break down easily. Generally, farmer groups are built by extension workers as part of a government project and not at the farmers' own initiative. For effective functioning, farmer groups need to be strengthened with capital and by building production units which can make use of the farmers' own labor.

Farmers' maize production can be increased by introducing new cultivation technologies. Farmers need technology inputs (improved varieties, fertilizer, pesticides, etc), and to buy them they need capital and credit assistance. As part of our study, loan assistance was extended to farmers through farmer groups. Direct loan assistance by government to farmers has often led to repayment defaults. Extending such assistance indirectly through farmer groups was felt to be the better option because farmers are discouraged from defaulting on repayment by the likelihood of social sanctions.

Strengthening farmer's group capital strategies can be done in two ways. The first is to increase maize production for consumption, and the second is to increase maize production for consumption as well as home industry purposes.

In the first strategy, the service provider gives production inputs as as loan assistance to the farmer group. Members of the farmer group pay for these inputs in the form of maize grain after the harvest. The farmer's group sells the maize grain and buys inputs for the members' use in the next season. This model guarantees supply of inputs for production. It needs a partnership with input suppliers, say, an input distributor or shop. Maize seed is supplied to farmers from the farmer group's seed production unit at the village level.

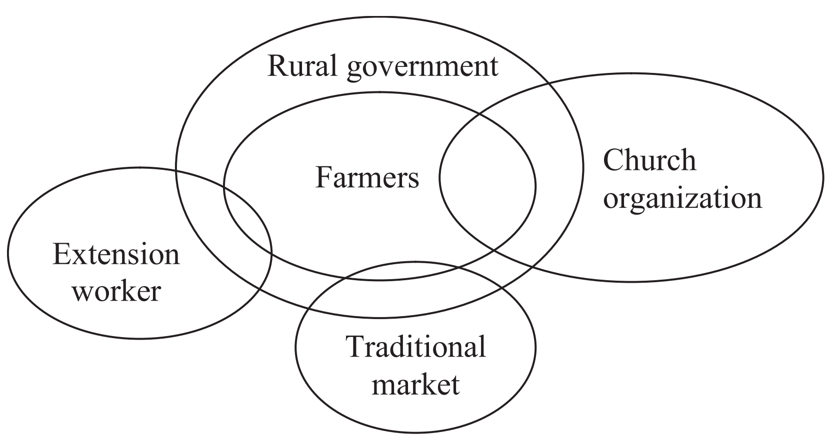

Figure 2. Venn diagram showing the relationships betwen institutions in the rural areas of Indonesia.

In the second strategy, the service provider gives production inputs as loan assistance to the farmer group. Besides, the service provider also gives loan assistance to the group to process maize production. The objective of this strategy is to strengthen farmer group capital and give wage payment to the farmer group members for the work done by them.

Management of input supply

There are no agricultural inputs shops in the study area. The nearest shops were one located in the district capital about 50 km away and another in the provincial capital about 200 km away. Such distances add to the cost of inputs for subsistence farmers.

The combination of high cost of inputs, limited knowledge agricultural technology and low capital constrain farmers from adopting improved technology. Setting up of an agricultural technology clinic was envisaged to help solve this problem, particularly that of input supply to farmers.

The clinic is not profit-oriented. Farmers are invited to visit the clinic and interact with extension workers and read agricultural information of use to them. The clinic's policy is to allow farmers to visit the clinic of their own volition. Extension workers are at hand to supply any information required by the farmers

The clinic has three divisions: the input supply division provides a linkage between farmers and input suppliers; the marketing division provides a linkage with commodity buyers; and the technology content division provides the required information to farmer.

Conclusions

The technology clinic has proven to be a useful institutional innovation. Extension workers and the farmers group manage the clinic which provides agricultural information to farmers and facilitates input supply by buying them from shops and supplying them to farmers. Farmers can take the inputs on credit from the clinic and repay it in in kind after the harvest. The technology clinic also processes maize grain and sells it in the early rainy season. It also helped build a maize seed unit at the village level. This strategy shows promise in ensuring the continuity of maize farming and has potential to be extended to other villages.

Acknowledgement

The authors express their sincere appreciation for all of support divided.

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Partnering with Farmers to Improve Maize Production and Livelihoods in the Hills of Nepal

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Abstract. Nepal is primarily an agrarian country: 88% of the population lives in the rural areas and 80% of the adult rural population is engaged in agriculture. The country is among the poorest in the world, with an annual per capita GDP of less than US$300. Maize is the most important food crop in the hills, where it is grown mainly by small-scale, resource-poor farmers. The crop is currently cultivated on approximately 0.87 m ha with an average yield of 2.09 t ha^{-1}. About 78% of this is grown in the hills. The Hill Maize Research Project (HMRP) funded by the Swiss Agency for Development and Cooperation (SDC), has been working in partnership and participatory mode with the Government of Nepal and nongovernmental organizations with the aim of developing, identifying, validating and disseminating maize technologies for the benefit of poor farmers in the hills. The project balances basic and adaptive research and emphasizes gender equity and social inclusion, multiplying seed of varieties selected by farmers in participatory variety selection trials, community-based seed production and other participatory approaches. The project's results show that the amount of seed and the number of farmer groups engaged in seed production have increased. Quality seed of new, improved varieties alone can increase maize productivity by at least 20%. Farmers can obtain significant net profits by intercropping cash crops with maize. Sowing two maize plants per hill is better for vegetable intercropping and reducing women's work. Integrated plant nutrient systems are suitable for sustainable maize production. Farmers who adopted HMRP technologies have significantly improved their food security, with greater involvement of poor women and excluded groups. This paper discusses the participatory research methodologies that have been used to increase food security and improve the livelihoods of poor maize farmers in the hills of Nepal.

Key words: Partnering, maize, livelihoods, participatory, profitability, PVS

Strategic Importance of Maize in the Hills of Nepal

After rice, maize is Nepal's most important cereal, both in terms of area under cultivation and total production. The crop is currently cultivated on approximately 0.87 million ha with an average yield of 2.09 t ha^{-1} (MoAC 2006). In the hills, where 78% of the maize in Nepal is grown, it is the most important cereal commodity and is used almost exclusively as food. In these chronically food-deficit hills, maize is the most important food crop, grown by poor and disadvantaged small farmers. The importance of this crop to food security can best be summarized by a proverb commonly used in the hills: "If there is no maize, there is nothing to eat." Maize is grown on sloping land that is partially terraced but not bunded, called *bari* land. It is grown during the summer months under rainfed conditions. The farm size is generally less than 1 ha, which must sustain a household of 6-8 persons (Ransom *et al.* 2003).

In most maize-growing areas in the hills, farmers have only limited access to improved seed, information on new technologies and markets. Planners and policymakers often mention the lack of appropriate maize technologies as one reason for low maize productivity in the country. However, Hill Maize Research Project (HMRP) studies by independent consultants (Mathema and Gurung 2006) have shown that Phases I and II of the project generated maize technologies suitable for various socioeconomic categories of hill farmers in different agroecologies and environments, and that resource-poor farmers have benefited, but that there remains an urgent need to disseminate these technologies to the more remote areas of the country, essentially "reaching the unreached," as far and soon as possible.

Similarly, evidence has shown that quality seed of new, improved maize varieties alone can increase maize productivity by at least 20%. Yet the seed replacement rate by farmers in Nepal is less than 1%. This highlights the need to strengthen demand for and accessible supply of seed of farmer-preferred improved maize varieties in the hills. It also highlights the importance of the Community-Based Seed Production (CBSP) together with a strong seed multiplication program and better seed marketing networks. A well-coordinated technology (eg, variety) evaluation and dissemination approach is still needed, and this needs to be institutionalized to ensure sustainability of maize production in the hills.

The Hill Maize Research Project, funded by the Swiss Agency for Development and Cooperation (SDC), was initiated in 1999 with the objective of increasing the food security of farm families in the hills by raising the productivity and sustainability of maize-based cropping systems. It was conceptualized as a research project directed at developing and facilitating the dissemination of technologies for small-scale maize farmers in the hills. Currently in the first year of Phase III, the project's goal for its third Phase is to help farm families in the hills of Nepal, especially of poor (food availability for less than 11 months from their own produce and income of less than 1 US$ a day) and disadvantaged groups (DAGs) to have improved food security and livelihoods. Among its many achievements, HMRP has identified farmer-preferred maize varieties through farmer-participatory research, and has strengthened seed systems (particularly community-based seed production schemes) that enhance access by resource-poor, marginalized farmers to these varieties.

Farmer-Scientist Partnerships for Technology Development, Dissemination and Adoption

Two options have generally been used in the past to upscale the development, dissemination and adoption of agricultural technologies in developing countries. The first is to strengthen the public and private sectors through vigorous policy planning and implementation (Joshi *et al.* 2005). The second relies on the principles of 'self help is the best help' and 'seeing is believing'. This approach requires an organized effort for the promotion of a new participatory model that can provide a more efficient flow of information to farmers and help them in becoming selfreliant.

Participatory research and development has been reported as an efficient approach in upscaling new agricultural technologies (Joshi *et al.* 2003; Ortiz-Ferrara *et al.* 2001; Ortiz-Ferrara *et al.* 2007; Tiwari *et al.* 2005; Witcombe *et al.* 2001). It is capable of solving farmers' problems that very often are not realized due to the vast diversity in farmers' fields. Participatory research has been used to complement the ongoing research to help farmers (Witcombe *et al.* 2003; Witcombe and Virk 2001). It is well known that early crop improvement started when farmers began selecting varieties of different crops based on their own knowledge. They developed not only their own varieties but learned to preserve the material for future generations. Over time, they also developed other useful technologies related to crop protection, production, seed preservation and utilization of crop produce.

Partners of HMRP have been collaborating with farmers in promoting improved maize varieties in farmers' fields. Participation by farmers, scientists, extension specialists, NGOs and the private sector is fostered in participatory plant variety selection (PVS) and participatory agronomic practice assessment. The 'Mother-Baby' concept that has been successfully used in maize in Africa has proved to be an efficient approach in developing and disseminating new varieties and agronomic interventions through close farmer collaboration. This methodology is described in Figure 1. A large number of Mother-Baby trials are grown in different villages for providing better options to farmers. The Mother trial consists of about 8-12 elite maize lines or recently released varieties. This includes the most popular check variety grown by farmers at each site. A number of Baby trials, consisting of one of the elite cultivars in the Mother trial plus a local check, are grown around the Mother trial. Germplasm in the Mother and Baby trials is assessed in a collaborative and consultative mode by farmers. Quantitative feedback is analyzed and used to find out farmers' preferences. Seed of farmer-selected genotypes are tested in larger fields side by side with the farmers' local varieties. These varieties are grown under the recommended agronomic interventions and the traditional method of cultivation used by farmers in the area. Seed of the farmer-selected varieties are multiplied and distributed to collaborating farmers. Farmers are trained on seed production. These varieties in turn are spread from farmer to farmer and through collaborating community-based organizations (CBOs), the private sector and extension partners.

Making Seed of Improved Varieties Accessible to Farmers

Seed of farmer-preferred maize varieties have been produced through the concept of community-based seed production (CBSP). Communities of farmers, especially farmers in very remote areas in the hills, are involved in producing seed of lines identified through the Mother-Baby trials the year before. With the collaboration of extension personnel (DoA), NGOs, CBOs, NARC and other partners such as Agrovets (semiprivate entrepreneurs involved in seed and agricultural business in the country), farmers get involved in producing large amounts of seed

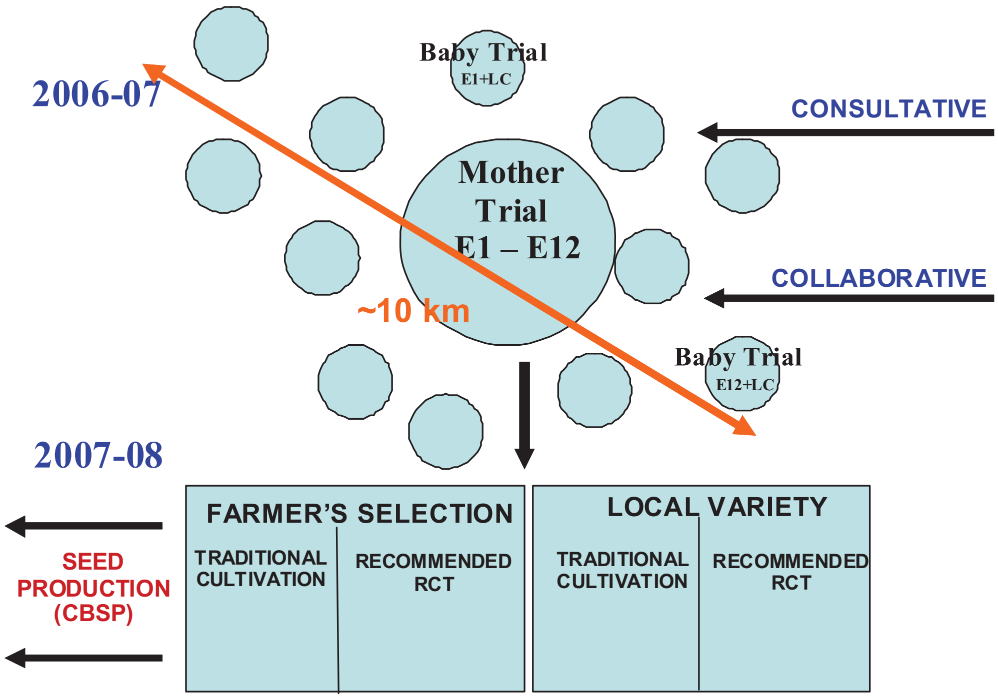

Figure 1. The 'Mother-Baby' concept used for participatory technology generation and dissemination (E = Entry; LC = Local check; RCT = Resource conservation technology; CBSP = Community-based seed production).

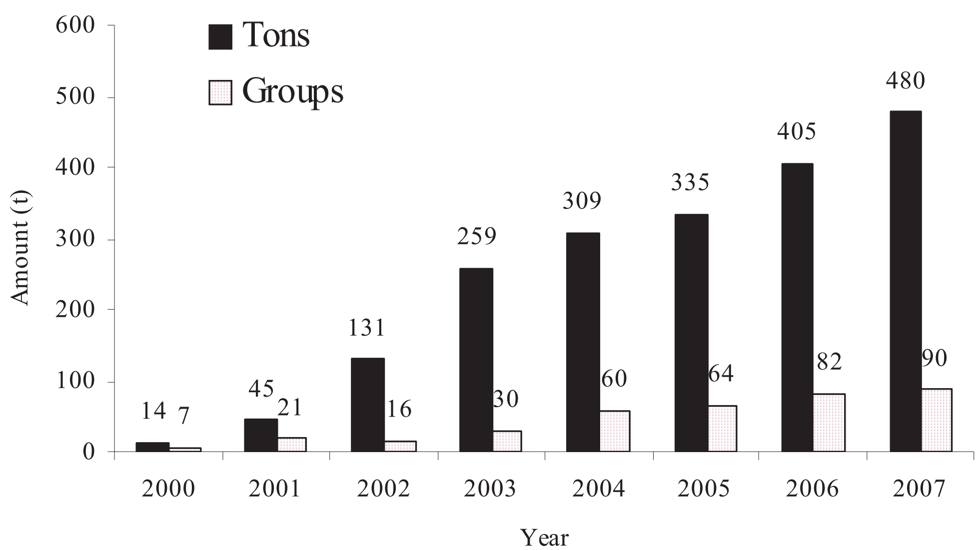

Figure 2. Amount of seed (t) produced through community-based seed production (CBSP) programs by different farmer groups, 2000-2007.

for dissemination in the specific area of work. They receive training on seed production, are provided (on a shared-cost basis) with small infrastructure for seed storage (metal bins, seed shelves, moisture meters, weighing balances, jute sacks, etc), and are frequently involved in farmer-trader orientation programs. Such interaction programs have helped in identifying marketing opportunities for farmer-produced seed.

Figure 2 describes the progress made over the past eight years by the community of farmers involved in seed production. The amount of seed increased from 14 t in

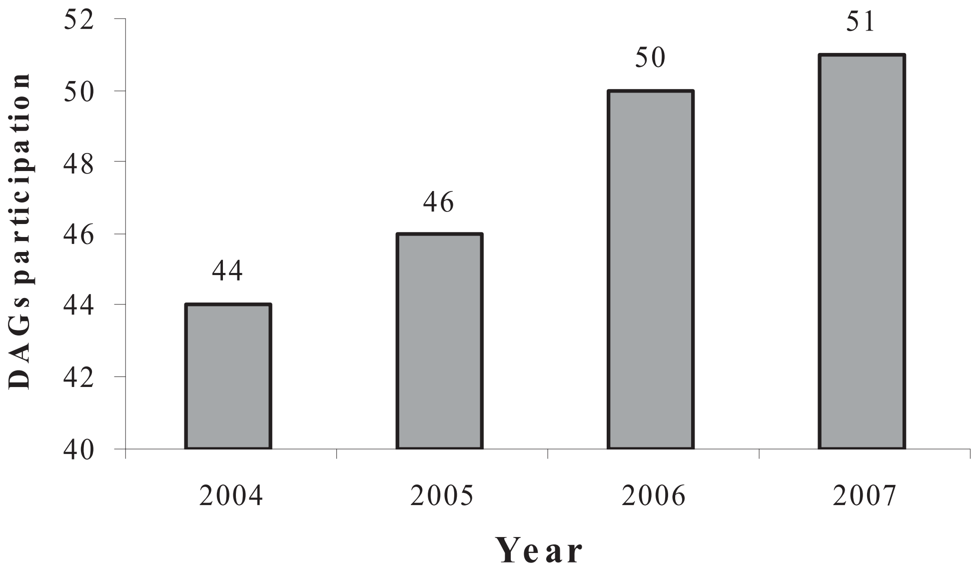

Figure 3. Trends in participation of disadvantaged groups (DAGs) in farmer-participatory activities, 2004-07.

2000 to 480 t in 2007. The group of farmers involved in these activities has also increased from 7 in the year 2000 to about 90 in 2007.

Promoting Social Inclusion, Increasing Food Security and Income Generation

Social inclusion

Working with farmers in a participatory mode has been a successful and rewarding experience. Farmers in the hills of Nepal, especially those from disadvantaged groups (DAGs), have been exposed to new technologies. This group of farmers includes *dalits*, *janajatis*, poor women and other excluded groups. They have had the opportunity to judge new varietal options and efficient agronomic interventions such as the intercropping of maize with several cash crops.

Figure 3 shows the progress made on social inclusion over the past four years. More than 20 000 farmers, on average about 48% of them belonging to DAGs, have been directly involved in participatory technology development and dissemination activities.

Food security

Results from an impact assessment study conducted by independent consultants (Mathema and Gurung 2006) revealed a positive impact on food security due to the adoption of improved technologies by participating farmers. Figure 4 shows the progress made in food security at the household level from 2002 to 2006. Of the four self-sufficiency categories used in the study, people from the less-food-availability (produced from their own) category have moved to more-food-availability category, this as a result of HMRP interventions.

Income generation

Besides higher grain yield, farmers of participating PVS villages have also brought changes in their income by growing and intercropping improved maize varieties. The data presented in Table 1 indicate that due to the cultivation of modern, farmer-preferred maize varieties, as well as the use of recommended production technologies such as the intercropping of maize with several cash crops, their income has increased. The maximum gross benefit per hectare ranged from US$ 5339 with the combination of maize + ginger, to US$ 888 with maize + groundnut. These technologies have helped resource-poor farmers improve their livelihoods and food security.

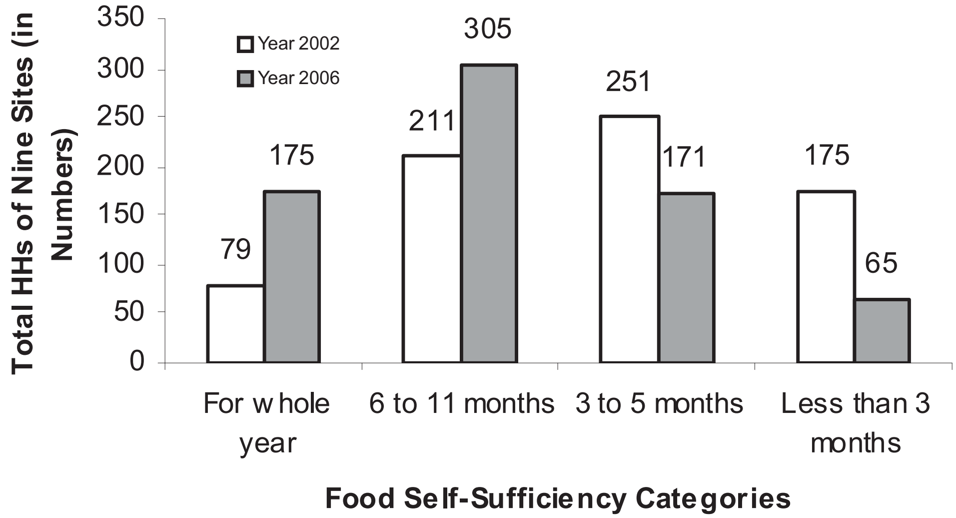

Figure 4. Shift in the food security pattern at the household (HH) level from 2002 to 2006, N = 716 (Source: Mathema and Gurung 2006).

Table 1. Profitability of various intercropping combinations between maize and vegetables and oilseed crops in the hills of Nepal.

Intercropping combination	Maximum gross benefit ha^{-1} (US$)
Maize + Ginger	5339
Maize + Tomato	2145
Maize + Soybean	1295
Maize + Cauliflower	3193
Maize + Brinjal	2214
Maize + Radish	2078
Maize + Cowpea	1120
Maize + Groundnut	886

Conclusions

Maize is a strategically important crop for the millions of resource-poor farmers and other people living in the hills of Nepal. Transfer of technology, seed systems and the lack of marketing opportunities are important constraints for low maize production and poor livelihoods in the hills. The PVS Mother-Baby approach described in this paper has been an effective strategy to promote the dissemination and adoption of new maize technologies by resource-poor farmers. The impact of the adoption of new maize technologies has already improved the food security and livelihoods of thousands of households in the hills, especially of those belonging to DAGs. Partnership with farmers has been a key factor in achieving these results.

Acknowledgment

We would like to thank the many farmers, scientists and extension researchers in Nepal who participated in PVS activities. We also thank the several NGOs and other private sector partners who collaborated in obtaining these results. These activities were conducted with the financial support of the Swiss Agency for Development and Cooperation (SDC) whom we sincerely thank.

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Do the New Maize Varieties Benefit the Poor More in Nepal?

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Abstract. Nepal is divided by various social exclusions, including discriminations that are ethnic-, gender-, caste- and class-based (eg, landlord-tenant relationships). Partly as a result of these inequalities, rich farmers have typically benefited more from interventions. The Hill Maize Research Project (HMRP) emphasizes partnership and participatory approaches and offers new maize varieties to more than 5000 farm families annually through partners to improve the food security of poor and marginalized families in the hilly areas of Nepal for whom maize is a staple food. Surveys were commissioned to measure the extent to which the new varieties have contributed to food security. A total of 230 households from seven locations across the mid-hills representing different socioeconomic, ethnic and gender divides were randomly surveyed to assess the changes in household food security. Use of new varieties either through participatory varietal selection (PVS) or community-based seed production (CBSP) has helped to increase production by more than 50% over existing local varieties (2.4 t ha^{-1}). Farmers adopted new varieties with greater yield potential, and these gains were also extended to food-deficit households from disadvantaged groups. Yield advantages were more commonly reported by female- (59%) over male-headed hosueholds (45%); dalit (61%) and janajati (56%) over privileged households (51%); and poor (C category = 62%, B = 53%) over elite households (A = 40%)[1]. There was significant improvement in food security irrespective of gender, ethnicity and socioeconomic class distinctions. Women- and men-headed households shared the same benefit of food security increase (23%); dalit and janajati households had higher food availability at the household level over privileged castes; similarly, food-deficit (C, B) over food-surplus households (A). This is indicative of the contribution of a targeted approach that is more inclusive and thereby contradicts earlier findings that maize research and development efforts only benefit the richer households.

Key words: Participatory varietal selection, gender, food security, livelihoods

Introduction

Maize is a major staple crop in Nepal, currently contributing 25% of domestic food production, grown on nearly 0.87 million ha with a productivity of a little over 2.1 t ha^{-1} (MoAC 2007). Its importance of maize has increased as it has to some extent replaced wheat in the *Terai* and lower hills of Nepal. Trends in maize production in recent years indicate a high growth, mostly due to the use of seed of improved varieties although it is limited to some geographical areas.

Various ethnic compositions, gender- and caste-based discriminations, landlord-tenant relationships are some of the social exclusions seen in Nepal. They are considered as a bottleneck to equitable development. This has in the past resulted in more benefits of development efforts accruing to the better-off farmers than to poor and marginal people. The Hill Maize Research Project (HMRP) emphasizes a participatory approach and offeres new maize varieties to thousands of (more than 5000) farm families annually through partners to improve the food security of poor and marginalized families in the hills.

The HMRP, a collaborative venture between the Ministry of Agriculture and Cooperatives (MoAC) and CIMMYT, funded by the Swish Agency for Development and Cooperation (SDC), works in the hills in partnership with likeminded but diverse types of national research and development groups that have food security and livelihood improvement of the rural poor in the hills as one of their major objectives. These partners are engaged in maize technology development and dissemination based on the needs of marginal small-scale farmers living in the remote hills.

The major activities carried out with these partners in the last seven years have included participatory varietal selection [Mother-Baby (MB) trials, Informal Research and Development (IRD), etc] and community-based seed production (CBSP). Dissemination of improved maize varieties is critically dependent on the timely availability and affordability of quality seed of preferred varieties. The genetic composition of maize plants grown from farm-saved seed tends to change considerably year after year, and quality deteriorates if precautions are not taken during selection seed. Thus, production of quality seed of farmer-preferred varieties in the remote hills has been a focused activity of HMRP in the last few years.

Field-level training is an intrinsic part of these activities. It helps to create awareness about new maize technologies among the stakeholders and at the same time entrepreneurship of farmers is encouraged. The MB trials and IRDs were implemented to create demand and CBSP to ensure supply, thus helping to promote new variety adoption by farmers. It is a well-recognized and established perception that adoption of improved seed can significantly contribute to increased food security at the household level (Ransom *et al.* 2003; Tiwari *et al.* 2007, Tiwari *et al.* 2008).

Although a few studies have been conducted to measure the impact of HMRP efforts and have shown an attractive positive benefit, quantification of the improvement in food security at the household level has been lacking. Our study was designed to assess whether the new maize varieties promoted through various channels of HMRP have contributed to food security improvement of the rural poor. However, measurement of food security and livelihood improvement is a complex issue (Morris *et al.* 1999). The benefit associated with higher grain yield has been reported. The other benefits not reflected in grain yield gain like grain colour preferences, cooking quality, quality of fodder, etc have not been addressed although these are extremely important for the adoption of improved varieties by resource-poor farmers. Thus, this report summarizes the findings of a case study that focused on the gain in maize yield by poor farmers of the mid-hills.

Materials and Methods

The study was designed to assess whether poor and disadvantaged groups (DAGs) had benefitted from new maize varieties. The study was carried out in seven locations across the mid-hills where HMRP partners have been working with farmers for the past few years. A total of 25-30 maize farmers from each location (a total of 230) who were participating in one or more of the HMRP-supported activities using new seeds either from MB or IRD or CBSP were selected for the survey. The selection of locations was based on the relative importance of maize in the diet, new maize varietal interventions in the area, level of cooperation by farmers, etc. A set of survey questionnaires designed to measure the extent to which new varieties had contributed to food security was administered to each household. The information sought from the farmers included any change in production per unit area since the use of new seed irrespective of the variety and the type of activity that the particular farmer was involved in. After each interview the completed questionnaire was reviewed for accuracy and completeness. The data on production per unit area were then converted into metric units for further analysis. Although it was mainly determined by the family size, farmers were also asked whether there had been any progress/change in food availability as a result of the use of new variety. Data on family members was not recorded.

The data were disaggregated by food security class (A, B or C); ethnicity (Bahun, Chhetri, Thakuri, Newar, considered as privileged groups, were treated as one class BCTN; and dalits and janajatis); and gender (women and men). For analysis and interpretation purposes they were considered as a treatment.

Measuring the benefits of maize varietal intervention was exceedingly difficult, because food security is a complex issue and it is nearly impossible to separate the relative influence of maize from that of other associated factors. However, farmers were asked to make estimates of benefits from new varieties in comparison to the respective local cultivars that they had used. So our results were based on farmers' direct recalled experiences. By making comparisons for each disaggregated group of data, we were able to calculate the percentage yield benefit. In assessing the benefits associated with the use of new maize varieties, it is important to examine not only the nature and size of the benefits, but also their distribution among different groups of farmers within the population.

Statistical analysis was performed for survey data across sites. Quantitative data were analyzed by computing analysis of variance (ANOVA) using GenStat (Discovery 2008) for each set of data like variety, ethnicity, food security and gender. The LSD was computed to compare treatments (Snedecor and Cochran 1973).

Results and Discussion

Variety effect

The results revealed that irrespective of gender, ethnicity and socioeconomic class, a varietal effect was detected ($P<0.001$). Improved varieties yielded significantly more (3.7 t ha^{-1}) (Table 1), as was expected. Consequently,

Table 1. Performance of new seed of improved varieties compare with that of local varieties (t ha^{-1}) for different categories of farmers and contribution to food availability (months).

		Variety (V) mean grain yield (t ha^{-1})			Food availability (months)			
		Local	Improved	Mean	Before	After	Mean	%
Gender (G)	Men	2.43	3.54	2.98	7.4	9.1	8.2	23
	Women	2.38	3.79	3.09	6.4	7.9	7.1	23
	Mean	2.39	3.70		6.7	8.3		
	Prob.[1] -				Prob. Period -			
	V		<0.001		P		<0.001	
	G		0.340		G		<0.001	
	Int. (V × G)		0.156		Int.(P × G)		0.713	
	LSD$_{0.05\%}$ -				LSD$_{0.05\%}$			
	V		0.203		P		0.474	
	G		0.212		G		0.495	
	Int. (V × G)		0.299		Int.(P × G)		0.700	
Ethnicity (E)	Dalit	2.26	3.66	2.96	5.1	6.4	5.7	26
	Janajati	2.46	3.84	3.15	5.8	7.6	6.7	30
	BCTN	2.43	3.69	3.06	7.5	9.2	8.1	21
	Prob.				Prob. Period -			
	E		0.532		P		<0.001	
	Int. (V × E)		0.827		E		<0.001	
					Int.(P × E)		0.827	
	LSD$_{0.05\%}$ -				LSD$_{0.05\%}$			
	E		0.314		P		0.438	
	Int. (V × E)		0.444		E		0.676	
					Int.(P × G)		0.956	
SE class (SE)	A	2.64	3.70	3.17	10.8	12.3	11.5	13
	B	2.31	3.55	2.96	7.0	8.9	7.9	26
	C	2.37	3.84	3.10	4.7	6.1	5.4	29
	Prob.				Prob. Period -			
	SE		0.164		P		<0.001	
	Int. (V × SE)		0.319		SE		<0.001	
					Int.(P × SE)		0.295	
	LSD$_{0.05\%}$ -				LSD$_{0.05\%}$			
	SE		0.282		P		0.265	
	Int. (V × SE)		0.399		E		0.369	
					Int.(P × G)		0.522	

1. Prob. = probability; Period (P) = before and after; SE = Socioeconomic category/class.

by the use of new seed of improved varieties there was a significant improvement in food availability at the household level (P<0.001).

Gender effect

The Gender effect was not significant (P = 0.340), nor was interaction (variety × gender) (P = 0.156) (Table 1). Despite the improved maize variety appearing to be gender-neutral (ie, nonsignificant effect of variety with respect to gender), a gender effect on food availability was detected (P<0.001). This discrepancy is surprising because there was no obvious reason why men-headed households should have greater food availability (9.1 months) than women-headed households (7.9 months) although using the same category of new seed of improved varieties. Factors other than improved varieties like men farmers cultivating improved maize in a larger area than women farmers, family size, etc might have influenced this result (data not presented). However, women and men share the same level of benefit of higher maize production gain (23%). Nonsignificant interaction effects in both cases indicate

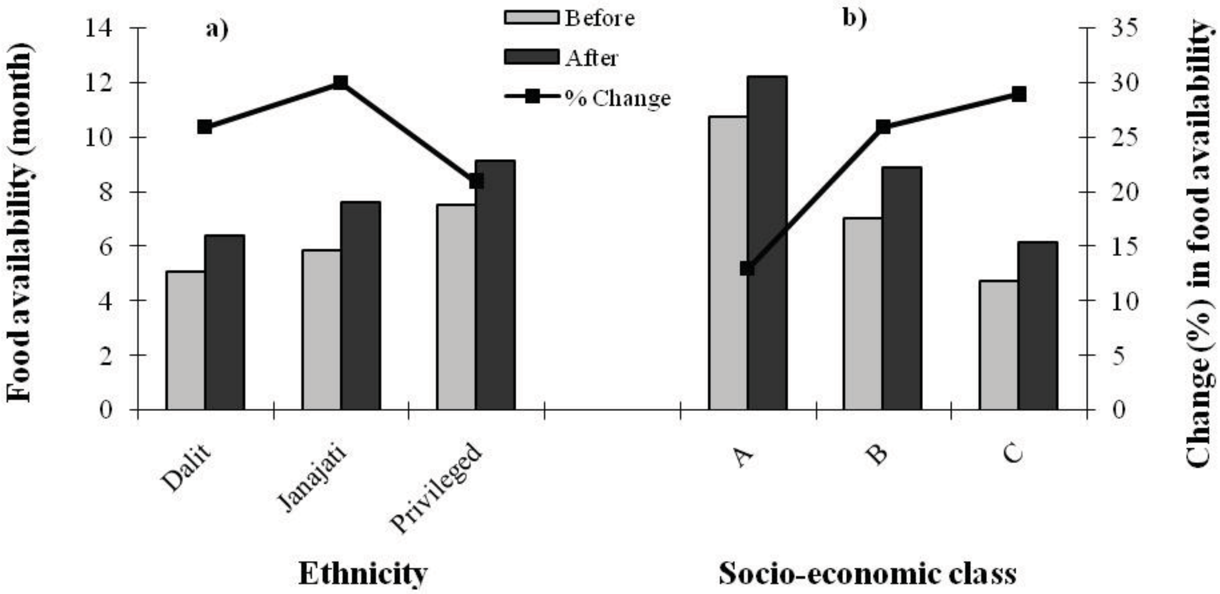

Figure 1. Targeted approach favouring discriminated groups (dalits and janajatis) (a) and food-deficit (B, C) households (b) over privileged and rich (A). A, B and C = Food availability 12 months or more, more than 6 months to less than 11 months, and less than 6 months from their own produce, respectively.

that both improved and local varieties responded in the same fashion in terms of the grain yield and its consequent effects on food availability months irrespective of gender.

Ethnicity effect

The ethnicity effect was also not significant (P = 0.532), neither was variety × ethnicity interaction (P = 0.827) (Table 1) in the performance of new varieties. The ethnicity effect on food availability was, however, detected (P<0.001), and there was significantly increased food availability (P<0.001) as a result of new varietal intervention. A higher degree of food availability or benefit was enjoyed by janajati households (30%) followed by dalits (26%). The BCTN ethnic group (the so-called privileged class) received the lowest increase (21%), and hence was the least benefitted. The lowest increase in yield for BCTN households might be due to the fact that the privileged class had already enjoyed the benefit of new maize varieties.

Socioeconomic category effect

The socioeconomic (SE)_effect on the performance of new varieties was also not significant (P = 0.164), neither was interaction (variety × ethnicity) (P = 0.319). The SE effect on food availability was detected (P<0.001), and there was significantly increased food availability (P<0.001) as a result of new varietal intervention. Category A farmers had significantly more food availability than the B and C categories, as was expected. However, the higher degree of benefit was realized by category C (29%) followed by B (26%) and A (13%).

These results confirm earlier findings and show that HMRP's efforts have had an enormous impact on household food security. This has resulted in increasing the national-level productivity (MoAC 2007). This was attributed to three major reasons:

- Maize variety dissemination through various partners was remarkably high in the past 3-4 years and the use of new seed produced within the community was significantly high in recent years. HMRP has been distributing more than 500 t of improved seed annually by means of PVS, IRD, FAT, CBSP across the hills favouring the poor and the DAGs.
- High rates of cross-pollination mean that the attribution of yield increases in farmers' fields to the specific introduction of improved seeds. Evidence suggests that in some cases use of contaminated seeds (crossed with improved varieties in the surrounding fields) significantly outperform the local varieties that farmers were growing previously.
- Weather conditions for maize production have been better and relatively favorable.

Despite the encouraging progress that has been achieved, considerable challenges remain to be addressed if new varieties are to reach the poorest of the poor. Over 90% of the maize area in the hills is still planted with farm-saved seed with variable quality. In many cases, farmers continue to use farm-saved seeds not because they are not aware of new seeds or that the seed is nonavailable; rather, the problem is that these farmers are located in remote hills and are not well integrated into the market economy.

Particularly important is whether the new maize varieties have been accessible and benefiting to women as well as to men; dalits and janajatis as well as to BCTN groups and A and B category as well as to C category farmers. Accessibility of new varieties for women is important because they represent a relatively disadvantaged group within society and also because women make household-level decisions on maize production. Similarly, accessibility of improved varieties for dalits and janajatis, and B and C category farmers is more important because maize is the staple food for them and they have been deprived ofaccess to new technologies in the past.

Conclusions

The study revealed that a partnership and participatory approach can be extremely successful. It provides convincing evidence that it is highly effective in benefiting the poor more. However, access to new seeds remains a prerequisite so as to bring about a maize production revolution in the remote hills. A more systematic effort from all concerned is required for wider impact, and the Government should play a leading role in this.

Acknowledgement

This publication is an output of a collaborative initiative between the Nepal Agricultural Research Council (NARC) and the International Maize and Wheat Improvement Center (CIMMYT) funded by the Swiss Agency for Development and Cooperation (SDC) through the Hill Maize Research Project (HMRP). The views expressed in this paper are not necessarily those of these agencies. We are thankful to farmers of the mid-hills. The staff of GOs, NGOs and CBOs are thankfully acknowledged.

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Prospects of Maize Farming Development in the Rainfed Lowlands of Indonesia

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ICERI - Indonesia

Abstract. Parts of Indonesia such as Sulawesi Island have potential for development of maize farming. Maize cropping has recently been increasing rapidly at 20-30% per annum, particularly in the lowlands of the country. Given an impetus, maize can compete with rice production. So more emphasis should be placed on planting maize in the rainfed lowlands, especially since technology is available to enhance productivity and cost efficiency. Toward this objective, integrated maize management trials were conducted in Sidrap regency in South Sulawesi during the dry seasons of 2006 and 2007. The results indicated that planting maize OPVs (Lamuru and Srikandi Kuning-1) produced an average of 4-4.5 t ha^{-1} with a benefit of Rp. 4.6 million ha^{-1} and a return:cost ratio of 2.31. This shows the marked promise of developing maize in the rainfed lowlands.

Key words: Production, rainfed lowland, maize

Introduction

Maize is cultivated in diverse environments in Indonesia. Based on surveys of the past 20 years, Mink *et al.* (1987) reported that about 79% of Indonesia's maize cultivation takes place in the dry season. About 11% of it is in the lowlands, and 10% in rainfed lowlands. Maize cultivation in lowland areas, particularly rainfed lowland areas, has been increasing by 10-15% and 20-30% respectively, predominantly in the commercial maize production areas (Kasryno 2002). Area increase has been more rapid for dry-season maize (93.5%) than wet-season (1.5%). East Java and Lampung provinces are the predominant maize-producing areas in Indonesia. Farmers in Central Java, Yogyakarta and South Sulawesi also cultivate maize in the lowland areas where it is the most preferred crop after rice.

Development of dry-season maize in the lowland areas is a strategic policy of the Indonesian Government for several reasons: (1) there is a maize production deficit during the dry season; (2) dry-season maize grain quality is better than wet-season; and (3) farmers find dry-season maize more beneficial (ICERI 2007). The potential for maize development can be realized if farmers obtain the benefits they hope for from the crop. To achieve that purpose, we need cultivation technology that enables high productivity and cost-efficient production and yields quality produce that is competitive in the market (Zubachtirodin *et al.* 2007).

To achieve optimal maize productivity, integrated technology management (ITM) is an appropriate approach to create a synergistic effect ofthe many kinds of component technologies involved such as high-yielding varieties, seed quality, land preparation, optimal plant population, efficient fertility, pest and disease control and harvest and postharvest management (IAARD 2008).

Methodology

Our research, using a participative approach, was conducted in Sidrap regency in South Sulawesi during the dry season in September 2006 and October 2007 on 5 ha and 10 ha respectively. The coastal western region of South Sulawesi was selected for the study because (a) South Sulawesi holds promise of growth in maize production, especially in the dry season; (b) South Sulawesi is an important part of the Corn Belt Program with the objective of boosting maize production to 5 million t a year; and (c) the western part of South Sulawesi has a large expanse of rainfed lowlands which are not currently cultivated in the dry season and are not optimum for rice.

Data for our study was gathered through Rural Participatory Appraisals (RPAs) and respondent cluster surveys determined sample (purposive sampling) from farmer cooperator of 27 people. The number of respondents was determined based on 5-10% of maize farmer population or depend on homogeneity of cultivated area (Teken 1973). Primary data collection was done through respondent interviews based on a questionnaire. Secondary data was obtained from institutions such as the Agriculture Department, the Central Statistics Bureau, the Irigation Department and the Extension Central Bureau.

The survey covered all the technologies implemented through the Integrated Technology Program for rainfed lowlands, ie, land preparation, sowing, weed control, fertilization, pest and disease management, irrigation, harvest and processing, yield and marketing. The data were tabulated and subjected to descriptive analysis (Anonymous 1987).

Results and Discussion

Among the several maize varieties that have been introduced, farmers preferred Lamuru and Srikandi Kuning-1 most, mainly because they possessed superior characteristics: Lamuru is drought-tolerant while Srikandi Kuning-1 has higher protein content than normal maize. According to the Agriculture Extention Services report for 2006, there are 12 poultry farms in Sidrap regency which would need a lot of maize for their feed.

The maize technology implemented by farmers varied from incomplete application of the recommended technology to complete application by a few. The yields achieved by the farmers varied too. Cooperator farmers achieved yields of 3.20-4.94 t ha^{-1} with the variety Lamuru and 3.82 t ha^{-1} with Srikandi Kuning-1 in 2006. In 2007, the grain yield of Lamuru ranged 3.03-752 t ha^{-1} (Table 1). This was still short of the yield potential of Lamuru (7.60 t ha^{-1}) and Srikandi Kuning-1 (7.92 t ha^{-1}) (Syuryawati *et al.* 2007).

To attain higher productivity (a minimum of 6.0 t ha^{-1}) we need to improve technology at the farmers' level through perfect implementation of the recommended technology. Research results based on survey data and farmer interviews indicate that the component technologies that have to be more efficiently implemented are irrigation cover and drainage, fertilizer application and timely weed control. Rat infestation during the grain-filling stage, a factor that decreases grain yield, is facilitated by the presence of noncultivated land around the maize plots. Although dry-season rainfall is not sufficient for rice cultivation, farmers in this area still sow rice every year as a habit, even if drought is a distinct possibility. The farmers are not aware that sowing maize in these water-stress conditions would be more beneficial than rice.

The constraints cited above can be overcome through use of adequate resources and by making use of market opportunities. Land isavailable in this area and water resources such s dams or wells with pump to irrigate maize can be harnessed. We found that poultry farms were ready to buy maize grain in large amounts; so marketing would be no problem in this area. Conditions such as these will support development of maize cropping on a large scale here.

Table 1 presents an analysis of the production costs and returns of cooperator farmers in Sidrap regency who adopted the integrated plant management approach in 2006 and 2007.

Analysis of costs and returns showed that for an average yield of 4.09 t ha^{-1} for Lamuru) and 3.82 t ha^{-1} for Srikandi Kuning-1), the production value obtained in 2006 by farmers was Rp. 6 544 000 ha^{-1} and Rp. 6 112 000 ha^{-1} respectively at a grain price of Rp. 1600 kg^{-1}. In 2007, an average production of 4.53 t ha^{-1} fetched a return of Rp. 8 154 000 ha^{-1} at a grain price of Rp.1700 kg^{-1}. The R:C ratio achieved by these cooperator farmers was >1 in the two years of the study (2.12-2.31), indicating that maize farming could be beneficial provided the farmers implemented the recommended production technology. Although productivity was less than optimum, the prospects are conducive for development of maize farming in this area.

Table 1. Production costs and returns[1] of Lamuru and Srikandi Kuning-1 maize varieties in the rainfed lowlands of Sidrap, South Sulawesi, 2006-2007.

Item	Variety		
	Lamuru		Srikandi Kuning-1
	2006	2007	2006
I. Production (Rp. ha^{-1})	6 544 000	8 154 000	6 112 000
II. Production costs (Rp. ha^{-1})			
- Material[2]	1 665 000	2 130 000	1,665 000
- Labor[3]	1 226 300	1 400 000	1 214 800
Total	2 891 300	3 530 000	2 879 800
III. Income (Rp. ha^{-1})	3 652 700	4 624 000	3 232 200
IV. R:C ratio	2.26	2.31	2.12

1. Maize grain value at harvest: Rp. 1600 kg^{-1} (2006) and Rp. 1700 kg^{-1} (2007).
2. Material: seed, fertilizer (urea, Phonska, KCl), herbicide, pesticide, gasoline.
3. Labour: Land preparation/drainage, sowing, fertilization, weed control, pest and disease control, irrigation and harvest.

The following five component technologies need to be implemented in farmers' lands forintegrated plant management of maize production:

1. High-yielding, composite/open-pollinated varieties (OPVs) that are suitable for the land and its environment, cropping system, and local farmer preferences in lowland and rainfed lowland areas.
2. Quality seed (purity/certificate and germination capacity >95%, seed treatment with metalaxil 2 g per kg of seed to control downy mildew and carbofuran applied 3-5 granules per plant hole to control ants and seedling fly).
3. Plant population/density between 66 600-70 000, with plant spacing of 75 cm × 40 cm at 2 plants per hole or 75 cm × 20 cm at 1 plant per hole in the wet season, and plant spacing 70 cm × 40 cm at 2 plants per hole or 70 cm × 20 cm at 1 plant per hole in the dry season.
4. Nitrogen fertilization (urea) based on the plant growth phase and leaf colour chart (LCC). P and K fertilization based on the soil nutrient status agree with laboratory analysis or local recommendation. Organic matter or manure 1.5-3.0 t ha^{-1} as seed cover to overcome nonfertile land especially in acid soils.
5. Water channel construction (for cropping on dryland in the wet season) or distribution channel (especially cropping on lowland in the dry season).

If the five technology components are simultaneously implemented, it would increase maize production and make it more efficient.

Conclusions

- There is limited interest in cultivating maize in the dry season. Farmers still prefer rice even if there is limited water during the dry season.
- Maize productivity in farmers' fields is less than the potential. Average maize yields reached 4.1 t ha^{-1} for the Lamuru variety and 3.8 t ha^{-1} for Srikandi Kuning-1. Maize grain yields could be raised through enhanced crop management.
- The returns from maize are substantial: for eg, the Lamuru variety returned Rp. 3.7-4.6 million ha^{-1} and Srikandi Kuning-1 Rp. 3.2 million ha^{-1} with return:cost ratios >1 (2.12-2.31). Maize farming is thus a promising option in the rainfed lowlands.

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Impact of Single-Cross Hybrids and Prospects of Maize in Peninsular India

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Introduction

There has been a tremendous increase in the area, production and productivity of maize in Andhra Pradesh state of India. Maize area registered a three-fold increase from 260 000 ha in 1973-74 to 840 000 ha in 2005-06 while production has gone up from 300 000 t to 2 400 000 t. There has been a quantum jump in productivity from 1.4 t ha^{-1} in 1974-75 to 3.4 t ha^{-1} in 2005-06, and the state ranks first in the country in productivity. Nearly 81% of the maize area in Andhra Pradesh is in the Telangana region of the state but in the last decade maize cultivation has spread to the coastal districts of the state on the strength of assured yields, low biotic and abiotic stresses and remunerative market prices. The productivity increase in the last two decades can be attributed to cultivation of promising single-cross hybrids under assured irrigation particularly in winter. The low yields two decades ago were mainly due to the cultivation of open-pollinated varieties (OPVs) and F_2 seed wherever the farmers were unable to purchase F_1 seed. Low production and productivity in some years during the past decade have been mainly due to drought and erratic rainfall during flowering and postflowering stages.

Total maize area sown in Andhra Pradesh	248 270 ha
Major districts	Area sown (ha)
Krishna	10 169
Guntur	55 947
East Godavari	6815
West Godavari	28 934
Warangal	19 720
Karimnagar	72 860

Strategies

- Development of long-, medium- and short-duration single-cross hybrids
- Development of high-yielding quality protein maize (QPM) single-cross hybrids
- Development of super sweet corn (sh-2) single-cross hybrids
- Development of popcorn single-cross hybrids with high popping quality
- Development of single-cross hybrids with high oil content (6.0%)
- Development of single-cross baby corn hybrids

Maize Crop Outlook in India

Demand-supply gap (2000/01 to 2006/07)

– Growth rate of demand	5.85%
– Growth rate of production	3.97%

For the financial year 2007-08, ending Mar 31, 2008, corn prices were up almost 38% over the previous year.

Yearend Prices of Maize Grain in India

- March 2005 Rs.5580 t^{-1}
- March 2006 Rs.5890 t^{-1} (5.27% over Mar 2005)
- March 2007 Rs.7258 t^{-1} (18.75% over Mar 2006)
- March 2008 Rs.9500 t^{-1} (23.5% over Mar 2007)

- In the next few years until 2012/13, assuming that demand for maize increases by a very conservative estimate of 5.83%, and production increases by an average of 4%, the gap in demand is going to widen.
- This, assuming that demand from the poultry sector grows at 6.32% (2000-2013) and demand from other sectors grows at 5%.
- About 20% of maize produced in the USA has been diverted to ethanol production in 2007. This has escalated the demand for maize at the global level.

Maize Market Size in India (t)

Karnataka	15 000
Andhra Pradesh	12 000
Bihar	10 000
Maharashtra	8000
Tamil Nadu	3000
Punjab	3500
Madhya Pradesh, Rajasthan, Gujarat, Haryana, Uttar Pradesh	20 000

Maize Market (season-wise)

Main season (June-July)	75%
Winter (Oct-Nov)	25%

Maize Market (segment-wise)

Late-maturity	60%
Medium	15%
Early	15%

Impact of Maize

- Maize in peninsular India has a great impact on the livelihoods of rural farmers and urban consumers.
- It is the staple food of people.
- It is a quality feed for animals
- It is a basic raw material for production of starch, oil, protein, alcohols, beverages, food sweeteners and biofuel

Utilization Pattern in India

Maize is used in India as human food (28%), animal feed (11%), poultry feed (48%) and industrial products (12%). Around 40 maize starch-based industries are located in peninsular India.

Impact of Maize on Livelihoods of Farmers

Maize is a poor man's crop. It is by and large grown in rainfed areas mostly by marginal farmers. Increase in yield and consequently net returns can improve the livelihood security of these farmers.

Maize Hybrid Seed Production in India

About 97% of India's maize seed production is in the peninsular states. About 80% of it is in Andhra Pradesh.

- Andhra Pradesh — 130 000 acres (winter season)
- Karnataka — 20 000 acres (main season)
- Tamil Nadu — 8000 acres (winter season)
- Gujarat — 2000 acres (main season)

Maize Hybrid Seed Production in Andhra Pradesh

About 130 000 acres are under seed production in major seed-producing districts in Andhra Pradesh.

- Eluru — 85 000 acres
- Warangal, Karimnagar — 20 000 acres
- Khammam — 20 000 acres
- Nizamabad — 5000 acres

Maize Hybrid Seed Production by Seed Companies

- Monsanto — 28 000 acres
- Pioneer — 18 000 acres
- Syngenta — 12 000 acres
- Bioseeds — 11 000 acres
- Others — 61 000 acres

Role of Maize in Furthering Farmers' Livelihoods

High-yielding hybrids increase output and thereby income levels. Increased income levels provide economic surplus for a higher standard of living. Economic sufficiency paves the way for adopting new farm technologies/agronomic practices thereby making corn cropping profitable. Neighboring farmers follow suit. It leads to a win-win situation for farmers and the seed industry. The seed village concept is followed by public and private sector seed companies in villages in Eluru, Karimnagar, Warangal and Khammam in the corn seed production belt in Andhra Pradesh.

Benefits to Farmers from Seed Production

1. Guaranteed buy-back of produce by seed companies.
2. Exposure to modern agronomic practices.
3. Farmers are weaned away from traditional to streamlined enterprise.
4. Infrastructure development like roads, transportation, power availability, etc.
5. Social benefits such as building of schools for farmers' children.
6. Health camps bring health care to the doorsteps of farmers

7. Employment for a large number of skilled/semiskilled and nonskilled workers in supervision, sowing, detasseling, weeding, spraying, harvesting, etc.
8. Scope for ancillary enterprise development such as transport operations, godown and container facility services, etc.
9. Awareness of safety consciousness among seed farmers and workers because they are training in the handling of machinery and/or chemicals.

Maize in Poultry Industry

- India is the third largest egg producer in the world.
- It is the fifth largest poultry meat producer in the world.
- The Indian poultry industry employs about two million people.
- The poultry industry is more concentrated in peninsular India.
- The private sector controls roughly 80% of the poultry production.
- Poultry feed provides the link between the maize and poultry sectors.
- Maize accounts for most of the energy in feed.
- 7-8 million t of maize is required for poultry feed every year.
- That is over 50% of total annual maize production.
- The poultry industry has a buy-back arrangement with maize farmers.
- Maize farmers have an assured market and a reasonably reliable income.

Role of Self-Help Groups (SHGs)

Self-help groups (SHGs) play an important role in the marketing and procurement of maize seed, thereby generating income for themselves and for maize farmers. The SHGs in Andhra Pradesh procured (helped the state government buy produce from farmers) 862 000 quintals of maize worth about Rs. 46.55 crore (10.34 million US$), through 90 procurement centers in just three months in the main cropping season of 2005 (*Deccan Chronicle*, March 3 2006). In seven districts of Telangana, SHGs facilitated the purchase of 40% of the total 2 185 000 quintals of maize bought by the Andhra Pradesh Marketing Federation, earning Rs. 4 650 000 as commission in the process. The farmers were offered a minimum support price of Rs. 540 per quintal of maize.

Conclusions

The genetic potential of maize can be better realized through single-cross hybrids. It is an appropriate technology that deserves to be promoted on a large scale. There is a need for

- identification of productive seed parents as females to minimize the cost of seed production;
- identification of a suitable male parent with dense tassel with longevity of pollen shedding;
- early-maturing single-cross hybrids with tolerance for drought/excess water;
- seed technology research for new products;
- agronomic packages for rainfed situations;
- strengthening research on hybrid technology for nonfavourable environments, including more emphasis on seed production in marginal areas;
- total yield increased resulting from genetic and nongenetic causes. Estimates of gains from genetic causes range from 0.33 q ha^{-1} to 1.94 q ha^{-1} per year; and
- development of stress-tolerant germplasm.

Acceleration of Seed Distribution through Community-Based Seed Production in East Nusa Tenggara, Indonesia

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Abstract. Maize yields are relatively low in East Nusa Tenggara province of Indonesia. In 2003, the average yield here was 2.26 t ha^{-1} while it was 3.20 t ha^{-1} at the national level. In 2004, the average yield increased to 2.35 t ha^{-1}, while the national average rose to 3.40 t ha^{-1}. Planted area in the province increased from 246 599 ha in 2003 to 279 403 ha in 2004. The main reason for the low productivity is the predominant use of local varieties with nonlabelled seed (74%), with regenerated seed of open-pollinated high-yielding varieties accounting for a 26% share and hybrids only 0.3%. The variety Metro – introduced in 1950 – is still widely grown, as is Pukis, a variety that is preferred as a traditional staple food. Productivity is also limited by low and uncertain rainfall. To increase maize productivity in NTT there is a need to provide new seed of high-yielding varieties which are suitable to the environment and preferred by farmers. Community-based seed production should be introduced and implemented especially in remote areas in order to facilatate farmers' access to high-quality seed at an acceptable price. As part of such an initiative, seed multiplied in Nun Kurus village of Kupang district in 2006 and 2007 was subsequently adopted by farmers in the village and sold in other areas at an acceptable price. The seed growers' income was Rp. 15.4 million ha^{-1}, with an R:C ratio of 3.20 and a break-even of Rp.1642 kg^{-1} for stock seed production.

Key words: Acceleration, seed distribution, maize, high-yielding OPVs

Introduction

In 1985–1986, local varieties accounted for 73% of the maize area in Indonesia. In 1992-1993, this fell to 56%, and to 43% in 2005. On the other hand, the share of improved varieties has increased with 35.36% accounted for by hybrids and 21.71% taken by new OPVs (Directorate of Food Crop Production 2006).

Maize is a staple food for farmers in East Nusa Tenggara province of Indonesia. The average yield is low. It was 2.26 t ha^{-1} in 2003 and increased to 2.35 t ha^{-1} in 2004 compared to the national average of 3.40 t ha^{-1}. Maize planted area increased from 246 599 ha in 2003 to 279 403 ha in 2004. Productivity is low because of the predominant use of nonlabelled seed of local varieties (74.23%) with regenerated seed of open-pollinated high-yielding varieties accounting for 25.50% and hybrids only 0.27% (Extension Service 2003). In 2005, the share of local varieties fell to 51.04% with high-yielding OPVs going up to 48.87% buthybrids still only 0.09% (www.deptan.go.id 2005). Low and uncertain rainfall is a major cause of the predominance of local varieties with early crop duration in this province. Most of the maize crop, ie, 246 599 ha, is cultivated in drylands with short-duration local cultivars and under low rainfall conditions (www.deptan.go.id 2003), which leads to low productivity.

ICERI has conducted field presentations and other activities to disseminate seed of new high-yielding varieties. In the upland areas of East Nusa Tenggara, farmers produced only 1-2 t ha^{-1} using local varieties while farmers who grew cv Lamuru (a new open-pollinated variety) produced 4-5 t ha^{-1} using similar technology (Saenong *et al.* 2005).

Therefore, there is a need to establish a system of seed distribution of high-yielding varieties in the rural areas to provide an adequate quantity of quality seed at an acceptable price in a timely manner to farmers.

Methodology

The first steps of this initiative included: (1) evaluate seed performance, identify problems of seed supply and assess farmers' perception of new varieties; (2) take an inventory of the available resources, ie, land, facilities and human resources; (3) notw how the farmers solved their difficulties regardingaccess the seed; (4) generate support from the community, seed growers and institutions; (5) prepare guidelines for seed production, processing, storage and distribution at the community level; (6) determine an appropriate technology for community-based seed production; (7) determine the demand for seed at the community level; (8) create linkages among BPTPs, extension services, seed certification and quality control agencies (BPSB), ICERI and nonformal institutions to support the sustainability of seed distribution; and (9) record the progress and problems faced by farmers during the experiment.

The objectives of our study were: (1) to introduce community-based seed production of new OPVs that are suitable for to the agroecological zone, and sociological conditions and farmers' preferences; and (2) to accelerate distribution of high quality seed of such varieties in adequate quantities and at affordable prices in the rural areas of East Nusa Tenggara.

Two series of experiments were conducted in Nun Kurus village in the district of Kupang in 2006 and 2007 in collaboration with the Tirosa Farmers Group at the village level, the Assessment Institute for Agriculture Technology (AIAT) and the Institute of Seed Certification and Quality Control at the provincial level, the Extension Service at the provincial and district level, private sector companies and nongovernmental organizations (NGOs). Grain yield , return:cost ratio, income generation, rate of distribution and farmers' response to the new varieties were recorded.

Table 1. Yield of graded aned nongraded seed of Lamuru and Srikandi Putih-1 maize varieties in Nun Kurus village in East Kupang subdistrict in East Nusa Tenggara province of Indonesia, late dry season, 2007.

Variety/site	Nongraded seed weight at 11% moisture content (t ha^{-1})	Graded seed (t ha^{-1})
Lamuru, Site 1	1.80	0.50
Lamuru, Site 2	4.00	2.00
Srikandi Putih-1,Site 3	0.65	1.5

Results and Discussion

Open-pollinated variety Lamuru was preferred by farmers since they believed that it is suitable for direct consumption. Soon after harvest, farmers dry the cobs outside their houses in the branches of trees or using a very simple drying rack (Fig. 1a). In several villages farmers store the nonhusked cobs in their kitchens for seed and food purposes. Seed remain viable with good germination for up to one year. The grain remain good enough for direct consumption for up to two years, free of maize weevil (Fig. 1b).

In the first year of our study (2006), seed growers obtained a yield of 4.25 t ha^{-1} after followed the guidelines on seed production, processing and grading. About 55% (3050 kg) of the seed produced was distributed within the community and the rest was sent out to other villages. Farmers have a tradition of exchanging maize cobs with other farmers when they have a new variety. The seed produced as part of our initiative was distributed to several districts: Kupang (400 kg), Alor (50 kg), TTS (50 kg), the Extension Service (550 kg), and East Kupang subdistrict (150 kg).

In the second year (2007), the seed growers of Nun Kurus village produced seed of Lamuru and Srikandi Putih-1 varieties at three sites in the village. The amount of graded seed obtained was low due to late planting.There was high

Figure 1a. Maize cobs being dried after harvest. (b) Traditional seed storage in the kitchen of a house in village inEast Nusa Tenggara province of Indonesia.

humidity during the grain drying process, as a conseuence of which there was a loss ofgrain.

Conclusions

The seed that was produced in 2006 and 2007 through Community-Based Seed Production in Nun Kurus village in East Kupang district of East Nusa Tenggara was well-adopted by farmers in the village and sold in other areas at an acceptable price.

The seed growers received a benefit of Rp.10 476 000 ha^{-1} at a return:cost ratio (R:C) of 2.9, and a breakeven of Rp. 3065 kg^{-1} of seed.

Farmers prefer to persist with their traditional method of seed storage in their kitchens.

Community-Based Seed Production in the rural areas can be extended to other village where farmers still find it difficult to access seed of high-yielding varieties suitable to their agroclimatic zone and meet their special preferences.

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The Effect of Seed Size on Seed Viability of Maize Varieties Lamuru and Srikandi Kuning-1

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Abstract. New varieties, open-pollinated (OPVs) or hybrid, can significantly increase productivity. Hybrid varieties do yield higher compared to OPVs. However, due to the high price of hybrid seeds, OPVs are preferred in strategies for increasing maize productivity, especially for poor farmers. Although productivity of OPVs has been remarkably high, some farmers still complain about their nonuniform seed size. This paper reviews experiments conducted in Maros, South Sulawesi and West Nusa Tenggara in 2004, 2005 and 2006, respectively, using cvs. Lamuru and Srikandi Kuning-1. The results showed that graded seed using a screen perforator of 8 mm and 10 mm diameter produced high seed germination rates at 12 months storage. These rates were 98% for Lamuru and 94.6% for Srikandi Kuning-1 compared to rates attained by graded seeds using a screen perforator of 7 mm. On the other hand, the germination rate of seeds that were graded using a 7 mm screen perforator dropped to 86.67% for Lamuru but remained high (94.67%) for Srikandi Kuning-1. These results indicate that the screen diameter for grading of Lamuru seed should be not less than 8 mm, and 7 mm for Srikandi Kuning-1.

Key words: Seed quality, seed viability, OPVs, germination and seed storage

Introduction

Farmers' preferences for seed quality mostly concern rapid growth and uniform stand during germination. However, the seed size of maize, especially of open-pollinated varieties (OPVs), varies from the bottom to the top of the cob. Seeds at the bottom and the top are generally smaller compared to those in the middle of the cob. Smaller seed produce stems of smaller diameter and shorter root length than medium and large seeds. As a consequence, under less than optimum conditions such as drought, plants produced from smaller seeder do not survive. Larger seed in a seed lot produce bigger cotyledons and twice the net photosynthetic rate than smaller seed.

An experiment on seed size from different parts of the cob conducted by Arief *et al*. (2004) showed that there were no significant differences in terms of storability between different one-thirds of the maize cob but there was a tendency for seed from the base and the top of the cob to produce smaller stems and taller plants compared to seed from the middle 3/5ths of the cob. Furthermore, this method needs more labor and time allocation. On the other hand, seeds from the middle of the cob produced more vigorous plants compared to those from the base and the top of the cob (Saenong 1982). Priestley (1986) also reported that smaller seed of the same cultivar were less storable than medium and larger seed. Gardner *et al*. (1991) also reported that larger seed have adequate food to support a higher photosynthetic rate than smaller size. Using a maize seeder is also difficult when seed are not uniformly sized because the perforator of the seeder needs to be adjusted according to the size of the seed to allow a uniform number of seeds to be dispersed. The problem for small seed growers to provide a uniform seed size remains how they screen their seed effectively and efficiently in terms of time and labor allocation to minimize the cost and optimizing the seed yield.

Methodology

The first experiments were carried out in 2004 with seed production in Sambelia village in western Nusa Tenggara Province. The seed were kept in Maros for one year (from 2004 to 2005). The screens used were: e"10 mm, 8-10 mm, 6-8 mm, without seed grading and farmers' practice. The varieties used were Lamuru and Srikandi Kuning-1. Seed germination and vigor were tested every two months starting before storage (0 month) up to 12 months of storage.

Seed production in the first experiment was carried out in Sambelia village in the district of east Lombok in Nusa Tenggara Province in 2005, while seed quality was evaluated in the Maros seed laboratory in 2005 using the OPV Lamuru. The treatments consisted of three seed sizes: big seed of diameter $\geq$10 mm (S1); medium-sized seed of diameter 8-10 mm (S2); and small seed of diameter 6-8 mm (S3). Seeds were kept in an airtight container (high-density polyethylene bag) 5 kg per bag and then kept in a silo or wooden box at 28-32°C for 12 months. Testing of seed viability and vigor were conducted every two months using ISTA (2004).

Results and Discussion

The results showed that up to 12 months of storage at room temperature, seed graded using a screen of e”8 mm diameter produced a high germination rate of 94.7% for Lamuru and 98% for Srikandi Kuning-1. On the other hand, using a perforator of 7-8 mm diameter produced a lower seed germination rate of 86.7% for Lamuru but 94.7% for Srikandi Kuning-1. Therefore big seed of Lamuru need to be screened with a perforator of e”8 mm diameter, but seed of Srikandi Kuning-1 need to be screened at e”7-8 mm to provide good seed quality. All seeds that passed through the screen with a perforator of 7 mm should not be used for sowing. The farmers' practice had good results. It showed that the highest germination was 96% for Lamuru and 94.7% for Srikandi Kuning-1 (Table 1). However, it was time-consuming and needed more labor because they it was needed to cut the cob 1/5 at the bottom and top of the cob. Furthermore, the net seed yield obtained with farmers' technology was lower than that obtained using a perforator with a machine (air screen cleaner). In terms of storability, small seed from the same seed lot were less storable than medium and big seeds. An experiment conducted by Saenong (1982) showed that seed from the middle part of the cob produced more vigorous plants that seed from the bottom and top of the cop. Nongraded seed produced a low germination percentage at 12 months of storage, especially Srikandi Kuning-1 (80%).

Based on speed of germination as a measure of seed vigor, the germination speed of seed in initial seed storage (before storage) varied from 30.3% to 32.7% per day for Lamuru at the perforator size of ³8 mm, reduced to 24.7% per day to 24.7% per day (Table 2). A similar trend was obtained for Srikandi Kuning-1. The lowest germination speed was obtained for nongraded seed of both Lamuru

Table 1. Average seed germination of cvs. Lamuru and Srikandi Kuning-1 at storage period of 0-12 months at Maros, Indonesia, 2004-2005.

Seed Grading	Seed weigh (100) (g)	Initial Seed moisture (%)	Germination capacity (%) Storage Periods (Months)						
			0	2	4	6	8	10	12
cv Lamuru									
Seed size ≥8 mm	29.57	9.63	100.0 a	99.3a	100.0a	98.67a	96.67ab	96.00a	94.67ab
Seedsize.7-8 mm	24	9.87	98.7a	100.0a	99.3ab	99.33a	92,00bcd	97.33a	86.67bc
Non graded seed	28.47	9.43	99.3 a	98.7a	98.7ab	95.33ab	90.67 cd	95.33a	86.67bc
Farmers practice	28.73	9.63	99.3a	92.0b	93.3bc	98.67a	94.67abc	96.67a	96.00 a
cv Srikandi Kuning-1									
Seed size ≥8 mm	27.13	9.2	100.0a	98.7a	96.7ab	96.7ab	96.7ab	97.3 a	98.0 a
Size 7-8mm	23	9.1	99.3a	100.0a	98.0ab	96.0ab	98.7 a	94.0 a	94.7 ab
Non graded seed	26	9.27	96.3a	93.3b	96.7ab	92.0b	86.7 d	80.0b	80.0 c
Farmers practice	26.17	9.03	97.3a	97.3a	89.3 c	97.3ab	96.7 ab	92.0 a	94.7 ab

Table 2. Average germination speed of maize cvs. Lamuru and Srikandi Kuning-1 at storage periods from 0 to 12 months under 28-32°C temperature, Maros, Indonesia, 2004-2005.

Seed Grading	Seed weigh (100) (g)	Initial Seed moisture (%)	Germination capacity (%) Storage Periods (Months)						
			0	2	4	6	8	10	12
cv Lamuru									
Seed size ≥8 mm	29.57	9.63	100.0 a	99.3a	100.0a	98.67a	96.67ab	96.00a	94.67ab
Seedsize.7-8 mm	24	9.87	98.7a	100.0a	99.3ab	99.33a	92,00bcd	97.33a	86.67bc
Non graded seed	28.47	9.43	99.3 a	98.7a	98.7ab	95.33ab	90.67 cd	95.33a	86.67bc
Farmers practice	28.73	9.63	99.3a	92.0b	93.3bc	98.67a	94.67abc	96.67a	96.00 a
cv Srikandi Kuning-1									
Seed size ≥8 mm	27.13	9.2	100.0a	98.7a	96.7ab	96.7ab	96.7ab	97.3 a	98.0 a
Size 7-8mm	23	9.1	99.3a	100.0a	98.0ab	96.0ab	98.7 a	94.0 a	94.7 ab
Non graded seed	26	9.27	96.3a	93.3b	96.7ab	92.0b	86.7 d	80.0b	80.0 c
Farmers practice	26.17	9.03	97.3a	97.3a	89.3 c	97.3ab	96.7 ab	92.0 a	94.7 ab

and Srikandi Kuning-1: 24.4% per day for Srikandi Kuning-1 and 24.7% per day for Lamuru (Table 2). In this connection, Abd-El-Rahman and Bourdu (1986) reported that the growth rate of seedlings increased with larger seed and round-shaped seed than thinner seed shape. Seed size has good correlation with seed density. High-density seed have adequate food reserves of protein and mitochondria which support energy production (ATP) which aids rapid growth and development (Prapto 2006). The results of this experiment showed that seed of ^{3}8 mm Were more storable in terms of germination percentage and speed of germination compared to seeds of 7-8 mm size.

Conclusion

Seed grading of open-pollinated varieties of maize is necessary to produce good seed quality with a high rate of germination and vigor (germination speed). Use of the screen depends on the general size of the seed. Using an e” 8mm screen usually does work for many OPVs, but for small-seeded varieties it is suggested to screen seed with a screen of 7-8 mm.

The farmers' technique of seed grading by cutting off one-third at the base and the top of the cob does produces high seed quality. However, it is not recommended because its produces less seed yield. Therefore, for community-based seed production, it is recommended to use a small air-screen cleaner combined with a grader with a proper perforator.

Nongraded seed and smaller seed reduced seed quality, especially when stored for at least eight months under room storage conditions (25-32^{o}C).

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Farmers' Participation to Increase N-efficiency and Farmers' Income

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Abstract. Karo regency, characterized by highland terrain, is the most important maize production area in North Sumatra. The maize area here is about 50 182 ha. This study was conducted during March-June 2007 at Tigabinanga in Karo regency at a site located 600-700 m above sea level. The experiment was arranged in a randomized complete design consisting of two factors (varieties and nitrogen) with 3 replications. The varieties were P12, BISI 9, NK 99 and DK 3, while the nitrogen treatments were N1 = without N; N2 = 160 kg ha^{-1}; N3 = 207 kg ha^{-1}; and N4 = 250 kg ha^{-1}. Maize productivity in this region is about 7-8 t ha^{-1} but there are possibilities of increasing it to 10 t ha^{-1} by using nitrogen site-specific nutrient management (SSNM) technology. As labor shortage is a problem in this area, introduction of SSNM technology should include planter and herbicide application. The objectives of the experiment were increased productivity and efficiency of N fertilizer. The results indicate an increase of 11.5% in productivity, 25% in farmers' income and 53.6% in efficiency of N fertilizer.

Key words: Maize, SSNM, efficiency N, income increase

Introduction

Nitrogen is a major nutrient element needed by the maize crop, especially in areas where high-yielding varieties are intensively cultivated. Several experiments have been conducted to assess the nitrogen fertilizer need and response in Indonesia (Taslim 1993).

Nitrogen is a nucleate acidic amino acid compiler nutrient win an important role in the making of chlorophyll and nucleotides. It accelerates plant growth in the crop coronet and leaf area. It has an effect on all parameters related to yield. Concentration of nitrogen in the leaf plays a role in photosynthesis and the rate of biomass production. Giving of nitrogen because requirement of crop to other nutrient increased to make balance to growth rate plant which quickly (Makarim 2003).

Nitrogen is a regulator of the usage of potassium, phosphates and other nutrient elements (Mengel and Kirby 1987). Nitrogen deficiency can cause emergence of crop elevator scapula with limited root development, and leaves become light green or yellow and tend to quickly fall off (Makarim *et al.* 2003).

Poor fertilization of N causes abundant emission of the gas N_2O during ammonification, nitrification and denitrification. According to Partohardjono (1999), emission of N_2O is influenced by the form of fertilizer N. Urea tablets result in low emission of N_2O and granular urea results in high levels of the gas. Efficient use of fertilizer use leads to lower emissions of N_2O. On the other hand, according to Stevens *et al*. (1999), use of fertilizer N in abundance can increase crop damage due to pest attack and disease, lengthen crop duration and cause lodging.

Abolition of fertilizer subsidies by the government does not necessarily lead to farmers using less fertilizer because they have become accustomed to using high dosages. As a result, the operating costs of farmer tend to remain high. In this context, technology relating to optimum fertilizer use efficiency, especially of fertilizer N, is required. Site-specific nutrient management aims at fertilization as per crop requirements and increased absorption of N by the crop.

The objective of our study was to increase the efficiency of fertilization of nitrogen, increase productivity and farmers' income in Tigabinanga subdistrict at a location characterized by Inceptisols.

Materials and Method

Our research was carried out during March-June 2007 at a dryland location 600-700 m above sea level. Nutrient and soil analysis was done at the BPTP Laboratory in North Sumatra. We used a factorial completely randomized block design with two treatment factors (variety and fertilization) with three replications. The experimental varieties were P 12 (V1), BISI 9 (V2), NK 99 (V3) and DK 3 (V4). The four

Table 1. Fertilization dosage for site-specific nutrient management of maize in Tigabinanga subdistrict, Indonesia.

Location	Fertilizer	N0(kg ha^{-1})	N1(kg ha^{-1})	N2(kg ha^{-1})	N3(kg ha^{-1})
Sp. Gunung	$NP_2O_5K_2O$	07290	1607290	2077290	2507290
Simolap	$NP_2O_5K_2O$	07290	1607290	2077290	2507290
Kutabangun	$NP_2O_5K_2O$	07290	1607290	2077290	2507290

Table 2. Soil analysis results at three locations in Tigabinanga subdistrict, Indonesia.

Location	Texture Pasir %	Organic Debu %	C/N Clay	Olsen-P C P_2O_5 (ppm)	Bray 1-P N			
Sp.Gunung (0-20)	48	13	39	0.92	0.06	15	188	65
Sp. Gunung (20-40)	50	12	38	0.78	0.06	13	128	174
Simolap (0-20)	36	29	35	0.85	0.07	12	64	56
Simolap (20-40)	36	29	35	0.85	0.07	12	64	56
K.bangun (0-20)	36	23	41	0.89	0.07	13	119	146
K.bangun (20-40)	36	29	35	0.83	0.07	14	70	60
Soil texture	Clay loam to clay							
Organic C and N		Low	Wide					
P available				High				

Location	pH		Acidity		Exchangeable Bases						Base
	H2O	KCl	Al	H	Ca	Mg	K	Na	SUM	CEC	saturation
			-------------------------------- cmol+/kg -------------------								%
Sp.gunung (0-20)	5.4	4.5	0.00	0.19	3.39	1.22	0.78	0.17	5.56	8.31	8.31
Sp.gunung (20-40)	5.3	4.3	0.00	0.11	3.44	1.06	0.79	0.10	5.39	8.10	8.10
Simolap (0-20)	5.2	4.3	0.13	0.13	3.48	0.74	0.52	0.16	4.86	7.70	7.70
Simolap (20-40)	5.2	4.3	0.13	0.13	3.28	0.89	0.52	0.17	4.86	7.71	7.71
Kutabangun (0-20)	4.8	4.0	0.50	0.25	1.41	0.81	0.51	0.17	2.90	6.93	6.93
Kutabangun (20-40)	5.3	4.3	0.07	0.13	3.11	2.45	0.62	0.14	6.32	8.83	8.83
pH	Acid-neutral										
Acidity			Low								
Exchange Bases					Low-high		Med-high	Low			
CED										High	
Base saturation											Low-high

nitrogen treatments were: N0 = without nitrogen; N1= 160 kg ha^{-1} N; N2= 207 kg ha^{-1} N; N3 = 250 kg ha^{-1} N. There were 48 (4×4×3) trial units.

Fertilization (Table 1) was done at 7 HST with 30% of the dosage of urea, SP36 entirely and 50% KCl; 35% of the urea was applied at 25 HST; and 35% urea and 50% KCl at 50 HST for treatment N1. But for treatments N2 and N3 fertilization was done two times: 50% at 25 HST and 50% at 50 HST. Thinning was done at 7 days to leave 1 seed per hole.

Weed control was done manually but pest and disease control operations were done as per recommended practices. Crop husbandry was done from planting to harvest. Harvest done by selecting 6 samples from plots of 4 × 3 m to compute productivity and agronomic efficiency of N.

Observations were recorded on:

- Productivity (t ha^{-1})
- Agronomic efficiency of N = (Production with NPK - Production without N)/fertilizer amount of N)
- Profitability ratio = Net profit which/Cost which required by every treatment

Treatment influence was determined by analysis of variance (tested F) at 5% and 1% levels and continuation test DMRT.

Results

Productivity (t ha^{-1}) was highest (10.21 t ha^{-1}) in treatment N1 (Table 3) and lowest in the without-N treatment (N0) with an average of 5.95 t ha^{-1}. Statistical data analysis indicated that treatment N1 was significantly different from treatments N0, N2 and N3.

Fertilization as per SSNM (N1) had a real effect on crop productivity (10.21 t ha$^{-1)}$, and proved to be better than farmers' fertilization practices by 11.5% compared with the FFP2 treatment (9.03 t ha^{-1}). This supports the findings of Sanchez (1993) that nitrogen is important as a chlorophyll compiler with an important role in photosynthesis and growth of bar and leaves.

Agronomic efficiency of N (Table 4) was highest in the SSNM treatment (N1) with 26.59 kg grain per kg N and lowest for the FFP 1 treatment (N2) with 11.96 kg grain per kg N. Data analysis indicated that the SSNM treatment was significantly different from treatments FFP 1 (N2) and FFP 2 (N3).

Results presented in Table 4 show that agronomic efficiency of N (AEN) for the SSNM treatment included in low category (25 kg per kg). Agronomic efficiency N was highest for the SSNM treatment at 26.59 kg grain per kg N and lowest for FFP 1 at 11.96 kg grain per kg N with increasing of efficiency agronomy 53.6% mean in increasing of 26 result kg require N counted 1 kilogram. This supports the findings of Witt (2000) expressing agronomy efficiency excelsior a in location hence progressively a few the application of given by fertilizations is crop for yielding optimal production. For treatments FFP 1 and FFP 2 AEN each = 11.96 and 12.32 kg grain per kg N (low), this thing is as according to the application of fertilization and amount of fertilizers given by farmers unmatched to productions are yielded. SSNM treatment kg grain per kg N (low), this

Table 3. Maize productivity (t ha^{-1}) under site-specific nutrient management (SSNM) in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P 12)	V2(BISI 9)	V3(NK 99)	V4 (DK 3)	Average
N0 (control)	6.20	6.00	5.60	6.00	5.95d[1]
N1 (SSNM)	10.30	10.10	10.02	10.40	10.21a
N2 (FFP[2] 1)	8.50	8.45	8.10	8.20	8.31c
N3 (FFP 2)	9.16	9.00	8.78	9.18	9.03b
Average	8.54a	8.39a	8.13a	8.45a	

1. Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
2. FFP = Farmers' fertilizer practice.

Table 4. Agronomic efficiency of N (kg grain per kg N) under site-specific nutrient management (SSNM) for maize in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P12)	V2 (BISI 9)	V3 (NK 99)	V4 (DK 3)	Average
N0 (control)	0	0	0	0	0
N1 (SSNM)	25.63	25.63	27.63	27.50	26.59a[1]
N2 (FFP[2] 1)	12.08	13.04	12.08	10.63	11.96b
N3 (FFP 2)	11.84	12.00	12.72	12.72	12.32b
Average	16.51a	16.89a	17.47a	16.95a	

1. Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
2. FFP = Farmers' fertilizer practice.

Table 5. Recommendations for N fertilization of maize crop on the basis of agronomic efficiency of N and increase in yield if fertilized by N compared with without-N treatment.

Agronomic efficiency (kg grain increase per kg N)	Low (25 kg/kg)	Medium (29 kg/kg)	High (33 kg/kg)
Yield response (t ha^{-1})	Fertilizer N rate (kg ha^{-1})		
1	40	35	30
2	80	70	60
3	120	105	90
4	160	140	120
5	200	175	150
6	240	210	180
7	Ä%	245	210
8	Ä%	Ä%	240

Ä%= select a lower yield response
Sumber: Witt (2008).

Table 6. Benefit:cost (B/C) ratio under site-specific nutrient management of maize compared to other treatments in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P12)	V2(BISI 9)	V3(NK 99)	V4(DK 3)	Average
N0 (control)	1.26	1.22	1.09	1.22	1.20d[1]
N1 (SSNM)	1.77	1.74	1.73	1.78	1.76a
N2 (FFP[2] 1)	1.40	1.39	1.32	1.35	1.37c
N3 (FFP 2)	1.53	1.50	1.46	1.53	1.51b
RATAAN	1.49a	1.46a	1.40a	1.47a	

[1.] Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
[2.] FFP = Farmers' fertilizer practice.

thing is according to the application of fertilization and amount of fertilizers given by farmers unmatched to productions yang are yielded. In the SSNM treatment fertilization was done in three applications (I = 30%; II = 35%; and III = 35%) using a dibber and providing cover. In the farmers' practices, fertilization was done in two applications (I = 50%; II= 50%) as side dressing without cover. As per research results (Garcia 2007), fertilization with the same dosage but different time and manner of application may result in a difference of 1 t ha^{-1} in yield. According to the findings of Witt (2000), the maize plant requires N during three phases of growth: early growth, flower formation and grain filling. If in this phase assessed BWD hence vegetative growth and generative of crop will be pursued so that produce yield low production.

Computatio of the benefit:cost (B/C) ratio (Table 6) indicated that the highest advantage was afforded by at the SSNM treatment with an average B/C ratio of 1.76. The lowest B/C ratio (1.20) was for the without-N treatment (N0). The SSNM treatment was statistically different from the other three treatments.

The benefit by using SSNM was equal to 25%.

Conclusion

From these research results, the recommended fertilization under site-specific nutrient management for maize in Tigabinanga subdistrict is: urea = 347.8 kg ha^{-1}; SP36 = 200 kg ha^{-1} and KCl = 150 kg ha^{-1}. Under SSNM treatment we obtained a yield increase of 11.5%, increased farmers' income by 25% and agronomy efficiency of N equal to 53.6%. Fertilization of N should be done by a dibber and then covered.

Acknowledgement

We thank Dr. Christian Witt and the Assessment Institutes for Agricultural Technology North Sumatra (AIAT) for their inputs to this research.

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Strategy of Maize Farming System Development for Subsistence/Semicommercial Farmers

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Abstract. Subsistence/semi-commercial farmers tend to be slow to adopt new technologies of maize cultivation even when they make economic sense and are compatible with their sociocultural values. Generally, that is because of the high cost of inputs (such as seed of superior varieties, fertilizer and pesticides). Also, their orientation is toward food security rather than commercial farming. Moreover, input shops at the subdistrict/village level are scarce. As a consequence, new technologies do not reach these farmers, who fall back upon existing technologies after the awareness building stage. This research involving three farmer groups and maize planted area of 15 ha was conducted in South East Timor Tengah district in East Nusa Tenggara province of Indonesia during 2007-2008 with the aim of finding an alternative strategy for maize farming by subsistence/semicommercial farmers. The research indicated that problems of technology adoption can be overcome by guiding farmers/farmer groups on the uses of new technologies over 1-2 planting seasons and building institutional support structures such as technology clinics at the village level. Extension workers and farmer groups (GAPOKTAN) can manage these technology clinics. The clinic's functions would be to disseminate information on new technologies and undertake the responsibility of supplying inputs to farmers/farmer groups. However, to ensure continuity, farmers need loan assistance. Farmers/farmer groups borrow inputs from the clinics and repay the loan in kind in the form of maize grain after harvest. The clinic also processes the grain and sells it in the early rainy season. It builds a maize seed industry in the village level and buys inputs (fertilizer and pesticides) from shops at the district/province level. This strategy could guarantee sustainability of maize farming systems and has the potential to develop maize farming in other village.

Key words: Maize, adopt, subsistence/semicommercial farmers, technology clinic

Introduction

Seventy per cent of the people of East Nusa Tenggara (ENT) in Indonesia live in rural areas and maize is their staple food (BPS NTT 2004). Maize productivity has been poor (1.5-2.0 t ha^{-1}) and subsistence farm households suffer from food shortages. However, by improving technology adoption, productivity can be increased to 2.3-4.8 t ha^{-1} (Bobihoe *et al.* 1999), or even 3.4-6.7 t ha^{-1} (Subandi 1999).

External interventions to increase productivity have been attempted, but technology adoption by farmers has been very slow. That is mainly because maize cultivation in this province is oriented toward food security and farmers tend to be satisfied with their maize productivity (Yusuf *et al.* 2001). The Indonesian government has taken up many projects such as PELITA III (Indonesian development plan phase III) but social change (farmers' attitude and adoption of new technologies) has been limited.

Extension of new technology to farmers has been attempted but adoption by farmers has been slow (Lidjang 1995). These external interventions relating to seed, fertilizer and pesticide technologies did increase farmers' agricultural productivity but there have often been relapses after the initial stages and farmers continued to produce maize for self-consumption purposes.

Farmers in dryland areas of ENT province are of the subsistence and semicommercial type (Subandi *et al.* 1997). They find it difficult to relate to maize farming as an agribusiness because of their orientation toward food security (Sumarno and Bamualim 1999). So technology information dissemination and innovation adoption efforts have to be adapted to these conditions. It needs an institution building dimension to achieve sustainable technology introduction.

Maize farmers in this province use very simple technologies. Use of production inputs such as fertilizer and pesticide is very limited. Land fertility has been gradually decreasing because of nutrient depletion (Murdolelono and Beding 2006).

Generally, poverty of subsistence/semicommercial farmers is an outcome of poor natural resources, uncertainty of climate, low levels of cultivation technology and limited capital. Dissemination of information regarding cultivation technology and capital access may address the problems of maize cultivation in this province. This could be achieved by strengthening farmer groups.

The purpose of our research was to find a maize agribusiness model for subsistence/semicommercial farmers in ENT.

Materials and Method

Field design and measurements

The study was conducted with the involvement of three farmer groups in Tobu village in Mollo Utara subdistrict of South East Timor Tengah district during 2007-2008. The innovations sought to be promoted were new maize cultivation techniques and building of farmers' institutions. The improved maize cultivation techniques included new varieties and improved plant spacing, fertilization and seed production techniques. The institutional initiatives included strengthening of farmers' group capital, management of input supplies and seed production (Table 1).

The technology clinics were managed by extension workers and farmer groups (GAPOKTAN). The clinics' functions were to disseminate information on technology and inputs to farmers. They The clinics bought the inputs (fertilizer and pesticide) from shops at the subdistrict or district level and supplied them to farmers for which the latter paid in grain after harvest. Seed was sourced from seed production unit operated by farmer groups.

Table 1. Improved cultivation techniques and farmers' institutional initiatives.

Innovation	Existing technology	Technology improvement
A. Maize cultivation		
· Varieties	Local OPVs	White Srikandi OPVs
· Plant spacing	Not uniform; 80-125 × 80-125 cm; 4-5 seeds per hole	100 × 40 cm; 2-3 seeds per hole
· Fertilizer	Not used	N 90 kg ha^{-1} + K_2O_5 36 kg ha^{-1}
· Seed production	Selection from farmers' own maize production	Maize seed production procedure
B. Improving farmers' institutions		
· Strengthening farmers' group capital	None	Farmers get out maize product equal than inputs price
· Management of input supply	None	Supply of inputs by technology clinics and loaned to farmers againt postharvest repayment in grain
· Management of seed production	None	Farmers produce quality seed

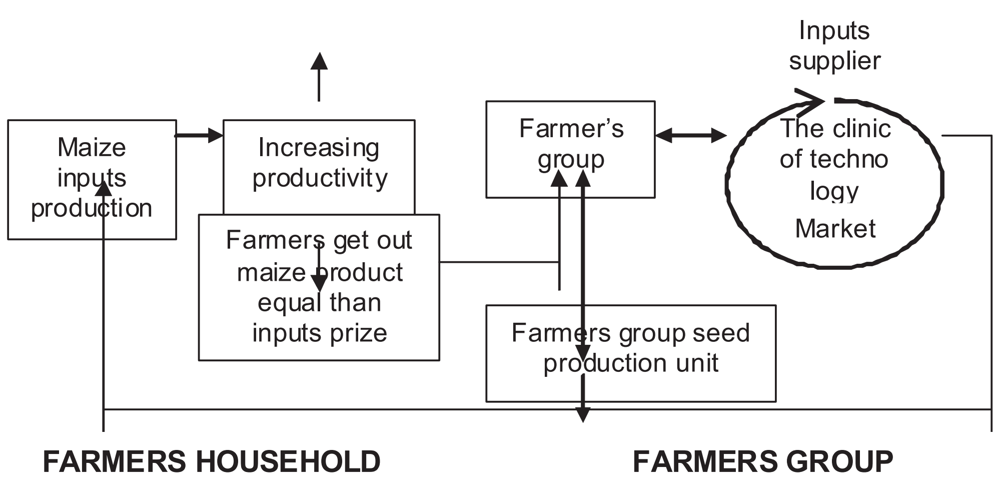

Figure 1. Strategy for improving farmers' institutions.

Table 2. Data collection and data analysis method.

Innovation	Data collection	Analysis technique
Maize cultivation		
Maize production	In the same year: 9 samples of existing maize cultivation and 9 samples of improved maize cultivation	T-test
	Previous year: measurement of maize production storage before introduction of innovation (exiting technology) and after introduction on the same land	Wilcoxon signed test
Farmers' response	Farmers' response to technology introduction	Descriptive
Institutional innovation		
Strengthening of farmers' group capital	Farmers' response to revolving system	Descriptive
Management of input supply	Farmers' response to input supply system	Descriptive
Management of seed production	Farmers' response to maize seed supply system	Descriptive Observation

Data analysis

Production data were collected by measurement and other data were collected by observation and Rapid Rural Appraisal (Table 2)

Results and Discussion

Maize cultivation

The results showed that plant height and tree diameter of local OPVs and Srikandi OPVs were not significantly different under the t-test of equality of means at 5%, but were significant for amount of plant and productivity. Amount of plant under existing technology was 13.25 holes per $10m^2$ and under introduced technology 17.56 holes per $10m^2$. Introduced technology increased maize productivity from 1.753 kg ha^{-1} to 3.438 kg ha^{-1} (Table 3).

The duration of Srikandi OPVs was 105 days and that of local varieties 120 days. The shorter duration offers an advantage to Timorese farmers because the rainfall period in this area is only 3-4 months, so farmers can plant other commodities after maize is harvested. Another advantage of Srikandi OPVs is their high content of lysine (0.410%) and tryptophan (0.087%) (Azrai 2004). The problem of poor nutrient levels in this area needs to be tackled.

Technology intervention increased farmers' maize storage by 173.2% per household. Average maize storage before intervention (previous year) was 80.2 kg per household and after intervention 219.1 kg per household (Table 4). This was net production after farmers repaid the cost of inputs to the technology clinic. The good results were a surprise to farmers and increased their motivation to adopt improved maize cultivation.

Table 3. Maize plant height, diameter, amount of plant per $10m^2$ and productivity under existing and introduced technologies.

Variable	Existing	Introduction	Notation
Plant height (cm)	149.62	149.78	ns[1]
Tree diameter (cm)	2.42	2.41	ns
Amount of plant (holes per $10m^2$)	13.25	17.56	**
Productivity (kg ha^{-1})	1.753	3.438	**

[1.] ns: not significant under t-test for equality of means at 5%.
** very significant under t-test for equality of means at 1%.

Table 4. Farmers' maize production before and after intervention.

Item	Number of sample	Farmers' production (kg per household**)
Before intervention	10	80.2
After intervention	10	219.1

** Very significant under 2-tailed Wilcoxon signed ranks test.

Farmer human resources in this area were very low. Their number of years of education ranged from 0 to 12 years and averaged 4.2 years. It influences acceptance of technology information. So to increase maize cultivation technology adoption, farmers need guidance over 2-3 times planting seasons.

Seed production

Maize seed production for this study was conducted at the farmer group level on 0.15 ha with white Srikandi OPV seed foundation. Seed production seed was 315 kg (productivity 2.100 kg seed ha^{-1}). These seed were sold at affordable rates to farmers in the farmer group and others.

Before this seed production initiative, farmers used to rely on their own seed of local OPVs. Generally, they selected the bigger grain for use as seed. Since the local OPVs had low production potential, introduction of new OPVs of maize was necessary.

To ensure that new varieties are adopted, it is necessary that the seed industry is located in proximity of farmers. Unfortunately there was no such seed industry in the study area. Maize seed had to be brought in from the provincial capital, which was about 150 km away. This only adds to the farmers' cost. Seed production at the farmers' level is a solution to this problem, but proper seed production practices ought to be followed.

The main problem affecting seed production in this area is availability of water for irrigation. Seed production must be done during the dry season when water availability is poor. So seed production must be done near rivers by the watering technique, which requires much labor.

Institutional innovation

Generally, subsistence/semicommercial farmers live far from the city under conditions of poor road infrastructure. This restricts farmers' mobility and access to communication. In such a situation, government and church organizations both can help disseminate agricultural information in this area. For instance, agricultural technology information can be disseminated at the traditional weekly market on Saturday where a large number of farmers gather. It is also imperative that the agricultural clinics be located near these traditional markets.

Generally, poverty of subsistence/semicommercial farmers is caused by (a) low level of natural resources and poor agricultural cultivation technologies resulting in low productivity; (b) climatic uncertainty, mainly during the rainy season; (c) limited capital of farmers and limited access to credit; and (d) poor knowledge of improved agricultural technologies and nonavailability of information. The lack of capital leaves farmers in a poor bargaining position forcing them to sell their commodities before maturity.

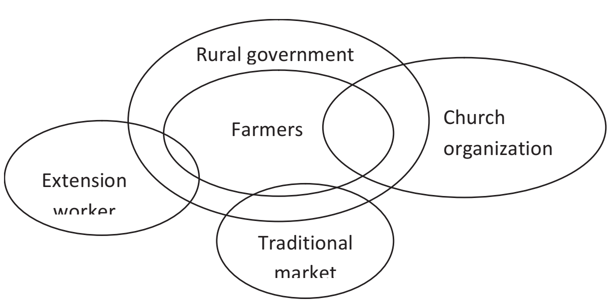

Figure 2. Venn diagram showing the farmers' relationship with different institutionals in the rural areas.

Strengthening farmer groups of subsistence/ semicommercial farmers is difficult. They break easily and are difficult to develop. Generally, farmer groups are built by extension workers for the purpose of a new government project and not upon the farmers' own initiative. To impart independence to the farmer group, we need to strengthen the group capital by building a seed production unit with the help of the farmers' own labor.

Farmer's maize production can be increased by adopting new technologies. However, they need capital to purchase the needed improved inputs (new varieties, fertilizer, pesticides and others). Loan assistance under a semigrand system was offered to farmers who participated in this research. This concept was chosen because stagnation of credit is very common in the grand system of loan assistance. Generally, if farmers default on instalment payments, the government finds it difficult to collect the debt. In the semigrand system, loan assistance is given to a farmer group. It is used to buy production inputs. The risk of stagnation of credit in this system is very limited because farmers tend to be deterred from defaults by the possibility of social sanctions.

Strengthening of farmer's group capital can be done in two ways. The first strategy is to increase maize production for consumption and the second is to increase maize production for a combination of consumption and home industry.

In the first strategy, suppliers give inputs such as seed, fertilizer and pesticides on a semigrand assistance basis to the farmer group. Members of the group access these inputs on the condition of repaying them in the form of grain after harvest. The farmer group sells the grain and with the proceeds buys inputs for next season. This model guarantees supply of inputs.

In the second strategy, the service provider supplies production inputs to the farmer group on a semigrand assistance basis. In addition, he gives assistance to the group to process the maize production. This has the opportunity of engaging the services of the farmer group members in the processing in return for payment for their labor.

Management of input supplies

The technology clinic was built at the village level because there were no input supply shops in the village. The nearest shop was located in the district capital about 50 km away and another was located in the provincial capital about 200 km away. This added to the high cost of cultivation for subsistence farmers.

The combination of high cost of inputs, limited agricultural knowledge and low capital is the reason why adoption of agricultural technology by these farmers tends to be very slow. The clinic was built to solve this problem.

The sale agricultural inputs at the technology clinic was not a profit-oriented activity, but to invite farmers to visit the clinic, interact with extension workers and read any agricultural information available at the clinic. It was a policy of the clinic that the farmers must come of their own volition to the clinic. Extension workers and clinic workers only supplied the information sought by the farmers.

The clinic management was drawn from the deputy heads of the farmer groups. The management had three divisions. The first division administered the supply of inputs, mediating between the farmers and the suppliers. The second looked after marketing by connecting farmers and commodity buyers. The third division looked after rural development planning.

Conclusions

Extension workers and farmer groups (GAPOKTAN) manage the technology clinics at the village level. Farmers/ farmer groups borrow inputs from the clinic and repay it in grain after harvest. Maize grain is processed at the clinic and sold during the early rainy season. The clinic built a maize seed industry unit at the village level and bought inputs (fertilizer and pesticides) from agricultural shops in the district/provincial capital. This strategy can guarantee sustainability of maize farming systems and has the potential for development in other villages.

Acknowledgement

The authors express their sincere appreciation for all the support provided to them.

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Maize Technology Transfer to the Poor: Lessons Learned from Community-Based Approaches of Technology Transfer to the Hill and Mountain Farmers of Nepal

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Abstract. Maize (Zea mays L.) contributes about 25% of the total food requirement of Nepal. It covers 80% (mountain region 10% and hill region 70%) of the total cultivated land in Nepal and plays a vital role in the livelihoods of hill and mountain dwellers. Nepal has a long history of maize research. Nineteen maize varieties including one hybrid have been released for cultivation so far. But production improvement is still insufficient. Technology transfer is undertaken through different community-based approaches – mainly District Seed Sufficiency Program (DISSPRO), Community-Based Seed Production (CBSP) and participatory varietal selection (PVS). Community mobilization by forming groups and cooperatives and technology transfer through demonstrations (production and results), training programs, mini-kits and visits are keys to increasing technology adoption by farmers and increasing food production. The District Seed Sufficiency Program (DISSPRO) was started in 1998 with the basic principle of encouraging and strengthening local seed production and marketing to meet local and district level seed demand. A few years later the Crop Development Directorate (CDD) through the Hill Maize Research Program (HMRP) joined hands with CIMMYT and several nongovernmental agencies to execute CBSP and PVS in maize particularly in the hill and mountain districts. Theoretically, DISSPRO, CBSP and PVS are similar at least from the community participation perspective; there are, however, important improvements in CBSP and PVS over DISSPRO. Both are more holistic, inclusive and localized. We argue that integration of the community approach of DISSPRO, the technology transfer approach of PVS and the inclusive approach of CBSP will be effective in achieving technology transfer to the poor in the future.

Key words: Maize, seed, technology, DISSPRO, PVS, CBSP

Objective of This Paper

The objective of this paper is to share the experiences of the Government of Nepal's Department of Agriculture/ Crop Development Directorate (DOA/CDD) in maize technology transfer to poor farmers and highlight the possible ways of improving the technology transfer approaches involving multiple actors: public (research and extension), civil society and private and farmer groups/ cooperatives.

Maize in Nepal

Maize is the major traditional cereal crop in the rainfed ecosystem of the hills and mountains of Nepal which cover 80% (mountain regions 10% and hill regions 70%) of the total cultivated land (MOAC 2007) in the country. It contributes about 25% of the total food requirement and plays a vital role in the livelihoods of the people living in these regions. It is cultivated for food, feed and fodder purposes on sloping *bari* land (rainfed uplands). It is grown under rainfed conditions during the summer (April-August) as a single crop or relayed with millet later in the season. In the *terai*, inner *terai,* valleys and low-lying river basin areas, maize is also grown in the winter and spring with irrigation. The *terai*/foothills (lowlands), midhills and high hill maize growing environments are depicted in the map in Figure 1. Although the map accurately represents the elevation bands, it does not indicate whether the area is cropped or if maize is grown there. More than two-thirds of the maize produced in the midhills and high hills is used for direct human consumption at the farm level, and the ratio of human consumption to total production is higher in the less accessible areas. In the *terai*, less than 50% of the maize is used for human consumption and a significant proportion goes to the market (Paudyal *et al.* 2001). Maize contributes about 6.88% of the total agricultural GDP of the nation (MOAC 2008). In 2006/2007, maize was grown on about 870 401 ha which represents 26.3% of the total area planted to cereals in Nepal. In the same period, 1 819 925 t of maize was produced, representing about 25% of Nepal's total cereal production. Out of the total cereal production in the mountains and hills, maize contributes only about 43.5%. In recent years, maize has come up as a new and promising cash crop in the *terai* region due to the growing demand from the feed industry. Maize demand has been growing by 5% annually throughout the last decade.

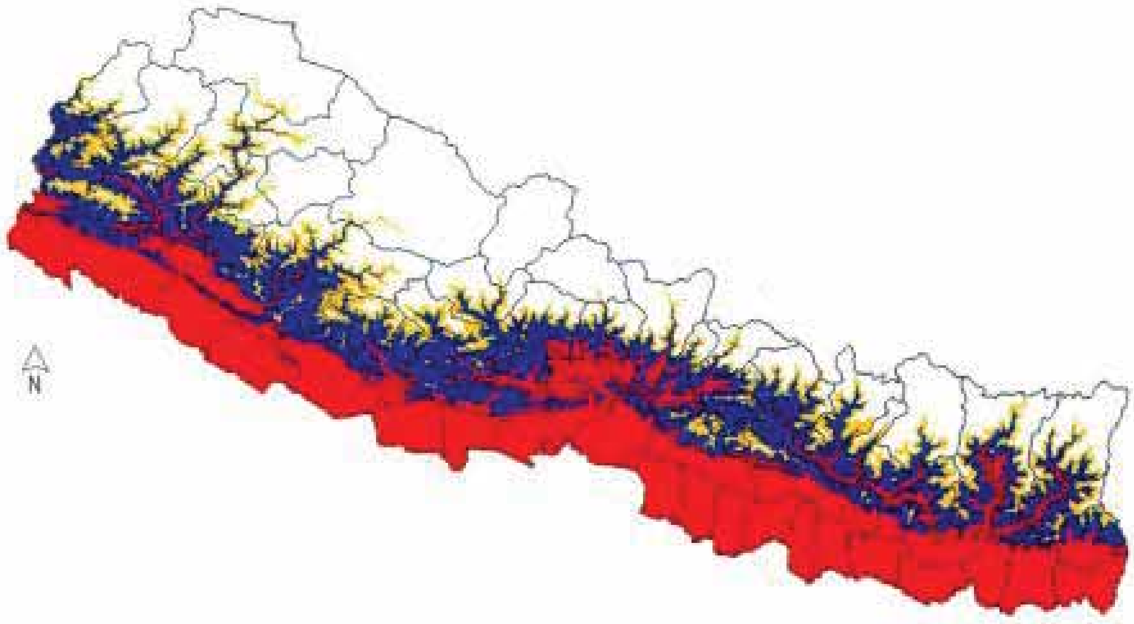
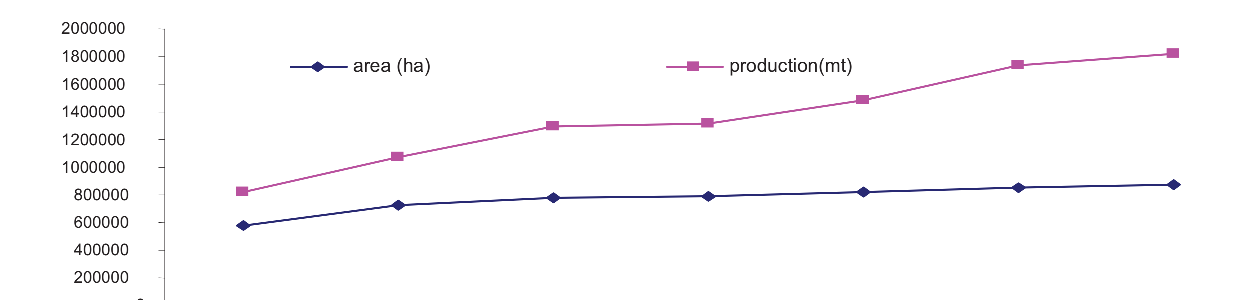


Figure 1. Distribution of lowland (red), mid-hill (blue) and high hill (yellow) maize growing environments in Nepal. Source: Paudyal *et al.* (2001).



	1984/85	1988/89	1992/93	1996/97	2000/01	2005/06	2006/07
area (ha)	578720	721870	775220	793720	824525	850947	870401
production(mt)	819850	1071610	1290500	1316840	1484112	1734417	1819925

Figure 2. Maize production trends in Nepal. Source: (MOAC 2007).

Area, Production and Productivity Trends

Although data (Fig. 2) show that there has been a constant increase in area, production and productivity of maize in Nepal, there has been very little yield improvement compared to nationwide yields some 20 years ago. This is probably due to the expansion of maize cultivation into less suitable terrain, declining soil fertility and sluggish adoption of improved management practices. Compared to 1984/85, the percentage increase in area, production and productivity of maize in 2006 was 50.4%, 121.9% and 47.5%, respectively in 2006 (MOAC 2007). Though most of the maize area (80%) lies in the hills and mountains, productivity there (hills 1.99 t ha^{-1} and mountains 1.73 t ha^{-1}) is rather less than the national average (2.09 t ha^{-1}) (Table 1 and 2).

Table 1. Area, production and productivity in the hill regions of Nepal, 2006/07.

Region	Area (ha)	Production (t)	Productivity (t ha^{-1})
Eastern Hills	141 779	271 603	1.916
Central Hills	138 098	292 542	2.118
Western Hills	208 695	473 301	2.268
Midwestern Hills	104 174	198 662	1.907
Far-western Hills	21 028	37 003	1.760
Total	613 774	1 273 111	1.993

Source: MOAC 2007.

Table 2. Area, production and productivity in the mountain regions of Nepal, 2006/07.

Region	Area (ha)	Production (t)	Productivity (t ha^{-1})
Eastern Mountains	39 065	69 448	1.778
Central Mountains	28 740	62 397	2.171
Western Mountains	691	1145	1.657
Midwestern Mountains	9252	13 690	1.480
Far-western Mountains	10 540	16 929	1.606
Total	88 288	163 609	1.738

Source: MOAC 2007.

Maize Research and Extension

Varietal research and extension activities are vital for technology generation and transfer. Sustained efforts have been made in Nepal to develop high-yielding varieties. Altogether 19 maize varieties including one hybrid have been released since 1960 (Bhatta and Bajracharya 2008). Efforts are underway to develop hybrids and quality protein maize (QPM) varieties to further improve productivity and nutrition. In extension activities taken up by the Government of Nepal (GON), technology transfer is underway through different approaches like DISSPRO, CBSP and PVS. Despite four decades of endeavor toward maize production improvement, progress has been insignificant. Although 19 maize varieties have been released so far, the annual seed replacement rate (SRR) is only 5.8% and average productivity 2.09 t ha^{-1}. Maize seed supply from the public sector is less than 1% and adoption of improved maize varieties is low. Our past experience shows that the policies, programs and approaches of followed in these research and development efforts were erroneous.

What Are Our Existing Approaches?

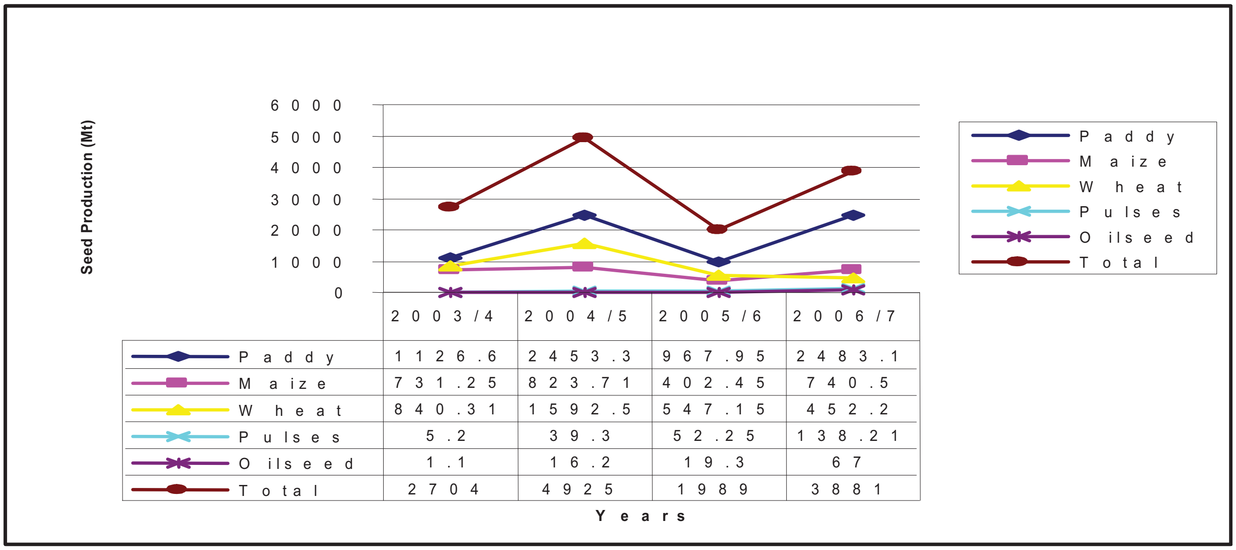
Technology transfer has been undertaken through different community-based approaches, mainly DISSPRO, CBSP and PVS. Community mobilization through formation of groups and cooperatives and technology transfer through demonstrations (production and results), training, mini-kits and visits are keys to increasing technology adoption by farmers and increasing food production. DISSPRO is a major public sector seed production program at the community/farmer level with the policy strategy of forming and strengthening of farmers groups to produce quality seed of the desired verities and in the required quantity. It has the basic principle of encouraging and strengthening local seed production and marketing to meet local and district level seed demand. This is becoming a very effective approach. DISSPRO was started in 1998. A few years later CDD through the HMRP joined hands with CIMMYT and several nongovernmental agencies to execute community-based seed production (CBSP) and participatory varietal selection (PVS) in maize, particularly in the hill and mountain districts.

District Seed Sufficiency Program (DISSPRO)

DISSPRO was envisaged in the Ninth Five Year Plan (1998/99 to 2001/03). The basic underlying principle of DISSPRO is to encourage and strengthen local seed production and marketing to meet local and district level seed demand and develop small-scale seed entrepreneurship and facilitate development of a larger seed market in the future; create awareness about quality seed among farmers; disseminate and multiply newly released varieties. Training, 25% subsidy on source seed, provision of a seed fund and a seed revolving fund of Nepali rupees 5000 and 60000 respectively for each group and provision of 200.00 Nepali rupees per ha for insect pest control are the major support programs. Formation and strengthening of farmer groups to produce quality seed of the desired varieties in the required quantity is a major policy strategy of DISSPRO. To assure supply of source seed as per demand, seed balance seed is prepared each year.

Implementation status of DISSPRO

Since the initiation of DISSPRO many districts have adopted the program by utilizing available funds and also by getting minor support (technical and financial) from the Crop Development Directorate. Provision of seed fund of Nepali rupees 5000 for subsistence groups and Nepali rupees 60000 for commercially oriented groups were major attractions of this program. In 2007, a total of 63 districts



	2003/4	2004/5	2005/6	2006/7
Paddy	1126.6	2453.3	967.95	2483.1
Maize	731.25	823.71	402.45	740.5
Wheat	840.31	1592.5	547.15	452.2
Pulses	5.2	39.3	52.25	138.21
Oilseed	1.1	16.2	19.3	67
Total	2704	4925	1989	3881

Figure 3. Seed production trends under DISSPRO, 2003-2007. Source: CDD 2007; 2008).

(out of 75) implemented the DISSPRO program. In the same year, 3881 t of quality seeds of cereals, pulses and oilseed crops were produced under the program (Fig. 3).

Lessons learned from DISSPRO

- Supply-driven (eg, identification and seed multiplication of farmer-preferred varieties)
- There is a scarcity of source seed of major cereals, especially rice, oilseed and pulses.
- The existing seed production technology dissemination system is not adequate.
- The seed quality control mechanism should be made easy and effective. The present mechanism is not sufficient and not effective.
- Participation of stakeholders is encouraging in the districts and should be increased.
- Community-based infrastructure development should be supported through government as well as private sectors/NGO efforts.
- The seed marketing and market information system is poor and needs strengthening.
- Farmer groups and farmer cooperatives must be strengthened with different support programs and capacity building activities through training in seed business skill development, entrepreneurship development and managerial skill development.
- Agro-vets and seed traders should be involved for sustainable seed marketing channel development.
- Rural youth, especially educated and unemployed youth, should be trained and involved in seed production and marketing activities to run it in a sustainable manner.

Participatory varietal selection and community-based seed production in maize

In 1999, HMRP/CIMMYT for the first time worked with the DOA/CDD to improve food security through increased maize production in the four hill and mountain districts of Nepal. In the second phase of this initiative, the number of districts was increased to 11. After successful completion of the first and second phases, the third phase of this project (2008-2010) has been implementing technology transfer to poor and socioeconomically disadvantaged communities in the hills and mountains (NMRP 2008). Participatory Technology Development (PTD) using the mother/baby trial concept and the community-based seed production program are the major technical interventions. In 2007, 105 t of maize seed was produced in the 11 districts. For the year 2008, this is expected to be at least 210 t from 25 districts.

Lesson learned from CBSP

- A cheap and successful intervention to increase food production and make it sustainable.

- Identification of location-specific maize varieties through PVS and production and supply of quality seed of farmer-preferred varieties through CBSP are promising approaches.
- Participation of multiple stakeholders in maize technology research and extension activities create an enabling environment to realize the interests of each actor in a coordinated way.
- PVS/CBSP have increased access of the poor, women and disadvantaged farmers to available technology and inputs.
- However, focus on marketing and development of community structures for seed storage and marketing is insufficient.
- Involvement of enterprising community groups in seed production, processing, storage and marketing is still weak in a majority of cases.
- Increase in maize yield by 17% was realized in the DOA/CDD project areas mainly due to use of quality seed of the improved varieties and minor changes in technology.
- Through PVS (mother-baby trial approach) farmer-preferred varieties were selected and included in the CBSP which facilitated the availability of quality seed to farmers. Rampur Composite, Manakamana 1, Manakamana 3, Deuti, Shitala varieties were released under the national variety registration and release system.

Conclusion

The DISSPRO is designed to transfer seed production technologies and produce and market quality seed at the community level. The main benefit of this approach is community participation and empowerment. It is cost-efficient. PVS on the other hand has proved to be an important approach and tool to generate and promote locally suitable technologies where farmers are involved in the whole process. CBSP has gained strength since CBSP groups are very inclusive and the support package is more complete and holistic. Considering these strengths of various approaches, it is recommended to integrate community participation, participatory technology development and community empowerment to transfer technology to the poor in future. DOA is giving priority to promote approaches of PVS and CBSP in the national extension system.

Acknowledgement

The authors are thankful to Dr. G. Ortiz Ferrara, team leader HMRP/CIMMYT; Dr. T.P. Tiwari, Agronomist, CIMMYT and are also grateful to the Director General, Department of Agriculture and the Swiss Agency for Development and Cooperation/Nepal. Similarly, we also acknowledge the contribution of Dr D.B. Gurung, Dr. D.P. Serchan and B.B. Hamal (DADO Syangja).

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Development of Parboiled Polished Corn [*Jagung Sosoh Pratanak JSP*)] as Practical Raw Material for Many Local Corn-Based Foods

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Abstract. There are many indigenous corn-based foods consumed as a carbohydrate source such as bassang in South Sulawesi and binte biluhuta in Gorontalo and kambewe in South East Sulawesi. However, the duration of processing and the method of serving are less practical. Therefore, the foods have disappeared from the common menu. Research has been carried out to solve this problem and the result was Jagung Sosoh Pratanak (JSP) or parboiled polished corn which is a practical raw material for making many corn-based foods such as bassang. JSP is produced through a series of procedures such as polishing, soaking, cooking and drying. Usually the process for making bassang takes a long time, ie, 18-24 hours, but by using JSP, the time could be reduced to 15-30 minutes. Research results relating to JSP production showed that the yield was 64.98%, rehydration time 15 minutes, water content 11.46%, fat 0.36%, protein 8.59%, carbohydrate 79.63%, ash 0.24% and crude fiber 1.1%. JSP packed in cartons, polypropylene and polyethylene could be stored for 498 days, 771 days and 899 days, or 16.6 months, 25.7 months and 30 months, respectively. Besides bassang, JSP can be used as a raw material for corn soup, corn rice and corn porridge with various serving options. By producing JSP, people's interest in corn-based foods can be revived, which would meet the objectives of food diversification programs.

Key words: Parboiled polished corn, carbohydrate source, bassang, diversification program

Introduction

Corn is a major source of carbohydrate food apart from rice. It is relatively cheap. Many kinds of corn-based traditional foods are widely known in Indonesia, such as *bassang* in South Sulawesi, *bintebiluhuta* in Gorontalo, *kambewe* in South East Sulawesi and grontol in Jawa. However, some of these traditional foods are losing place in people's menus because their preparation is perceived as impractical and their taste and appearance as nonappealing. These foods are considered as food for marginalized communities.

Bassang, which for generations was a widely consumed food in South Sulawesi, is made of corn combined with coconut milk and salt or sugar. It is served for breakfast or as a between-meal-time food by people in South Sulawesi. Its preparation takes 18-24 hours, including 8-20 hours for soaking and 2-3 hours for cooking. Survey results have shown that a lot of people in South Sulawesi still prefer bassang. It is commonly consumed in traditional markets (50%), at people's residences (20%), houses (20%) and schools (10%). *Bassang* is consumed by merchants (20%), *becak* drivers (16.7%), office staff (16.7%), housewives (16.7%), drivers (10%), laborers (6.7%) (Tawali *et al*. 2003). These results indicated that consumption is limited and confined mainly to marginalized communities. Against this background, we investigated how to make *bassang* more attractive to consume and convenient to prepare in order to revive people's interest in corn-based foods.

Materials and Methods

Raw materials and chemicals

Corn for this study was bought from the Takalar regency. All the chemicals used in this research were bought from chemical distributors in Makassar.

Method

This research was carried out in three steps. The first was production of Jagung Sosoh Pratanak (JSP); the second step involved determination of the physical and chemical profile of JSP and its yield; and the last step was prediction of the shelf life of JSP.

Measurement parameters

The parameters measured in this research were the JSP yield, water content, fat, protein, carbohydrate, ash and crude fiber (AOAC 1980) and shelf life prediction using the sorption isotherm method (Labuza 1982)

Data analysis

Research data were processed by descriptive and quantitative methods.

Results and Discussion

Production of parboiled polished corn (JSP)

Use of whole maize corn as a raw material of food is not widely known because of its use specifically as a traditional food in some regions. Processing of parboiled polished corn for this research generally consisted of the following steps: polishing, soaking, cooking (parboiling) and drying. Polishing was carried out to lose the bran and pericarp of corn using a polishing machine. Cleaned corn is dripped and then watered well to ensure the pericarp comes off. Research showed that in order to have polished corn with a high percentage of whole corn, the corn should be processed three times with the polish machine. Besides, maize corn was watered at a ratio of 1:1. This leaves the outside of corn wet and the inside in a hard and dry condition.

Soaking was done to let the product adsorb water rapidly and uniformly (Wimberly 1983). In order to make the corn tissue open and apart, amylase enzyme and $CaCl_2$ were added to the soaking water. As a consequence, some of the starch chains are broken and rendered loose so that the starch can swell optimally. The final result showed that there was a significant difference between rehydration time and soaking with and without the enzyme. Soaking was done for 24 hours. Cooking was aimed at gelatinizing the starch (Miah *et al*. 2002). Cooking of corn previously soaked in the enzyme solution was done for 2 hours. During cooking, starch gelatinization occurs, making the corn texture soft.

The soft corn was then dried until the water content is down to less than 13%. To reach this level of water content, drying is done for 7-8 hours at 50-70^{o}C using an electric drying machine. Figure 1 illustrates the method used to produce JSP. By using JSP, *bassang* can be produced in just 15-30 minutes depending on the consumer's preference regarding corn texture.

Product and nutrition profile of JSP

The rehydration time of JSP was 15 minutes. It is defined as the time needed by JSP to absorb water and become soft when recooked. The time was calculated since water was boiled until *bassang* became soft and ready to eat. Soaking in the enzyme solution is aimed at breaking the starch molecules and loosen the carbohydrate chain, thereby opening the pores. As a consequence, water rehydration was accelerated after the corn is dried.

During the processing of JSP, there was a loss of the corn matrix particularly during polishing and parboiling. The JSP yield based on the standard operational procedure was 64.98%. Yield from the polishing process was 75.86% and from the parboiling process 85.66%.

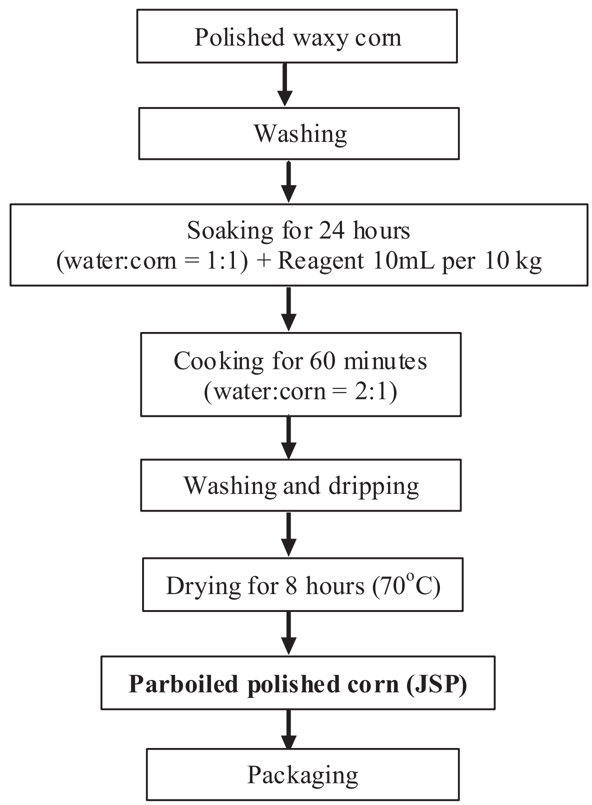

Figure 1. Processing of parboiled polished corn.

Table 1. Nutrition profile of JSP.

Component (%)	Content
Water content	11.46 ± 0.4
Fat	0.36 ± 0.01
Protein	8.59 ± 0.01
Carbohydrate	79.63 ± 0.33
Ash	0.24 ± 0.06
Crude fiber	1.10 ± 0.10

Table 2. Permeability, thickness and water vapor transmission rate (WVTR) of packaging material and the shelf life of JSP.

Material	Thickness (mm)	WVTR ($g/m^2/hr$)	Permeability ($g/mm.m^2/mmHg/hr$)	Shelf life of JSP (months)
Carton	410.00	125.088	0.305	16.6
Plastic PE	30.70	5.195	0.169	30
Plastic PP	37.00	7.303	0.197	25.7

The steps used to process from dry corn into JSP caused physical change as a consequence of polishing, cooking and drying. The complete analytical results of the JSP component are displayed in Table 1. The fat and ash content of JSP were lower because the germ (where the fat is concentrated) and pericarp are removed.

Prediction of the shelf life

Prediction of the shelf life of JSP was carried for packaging in cartons, polyethylene (PE) and polypropylene (PP). The shelf life of JSP is determined by the sorption isotherm method for each packaging material. The permeability, thickness and water vapor transmission rate (WVTR) of the packaging material are displayed in Table 2. The results showed that JSP can be stored for 16.6 months in carton packaging, 25.7 months in PP plastic and 30 months in PE plastic. The best packaging material to pack JSP in is PE plastic with a permeability value 0.169 $g/mm.m^2/mmHg/hr$. PE plastic had the lowest permeability compared to other materials. Syarief *et al*. (1989) reported that PE plastic protects the product from moisture.

Conclusions

1. The yield of JSP from standard operational procedure was 64.98%.
2. The rehydration time of JSP was 15 min with the following nutrition profile: water content 11.46%, fat 0.36%, protein 8.59%, carbohydrate 79.63%, ash 0.24% and crude fiber 1.1%.
3. The shelf life of JSP packaged in carton material, PP plastic and PE plastic was 16.6, 25.7 and 30 months, respectively.

Acknowledgement

We are thankful for the funding provided by the Indonesian Ministry of Research and Technology through Riset Unggulan Strategis Nasional-Diversifikasi Pangan Pokok (RUSNAS-DPP; National Strategic Research-Staple Food Diversification) for this research.

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Increasing Farm Income through Maize-Based Strip Intercropping Practices during the Winter Season in Nepal

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Abstract. A study was undertaken during two consecutive winter seasons (2006 and 2007) at the research farm of the National Maize Research Program (NMRP) in Rampur, Chitwan (228 masl), Nepal to assess the effect of maize varieties, row distances for strips on different intercrops and maize yield. Promising results were tested in farmers' fields in the second year of the study. Among the different intercrops evaluated, maize + potato was found to be the most productive with the highest maize grain equivalent yield (MGEY) of 9836 kg ha^{-1} followed by maize + kidney bean (7423 kg ha^{-1}). However, maize + kidney bean in lieu of sole maize gave a higher marginal rate of return (MRR) of 1.32 compared to maize + potato (1.02) when partial budgeting was performed. The Land Equivalent Ratio (LER) obtained with maize + kidney bean (1.37) was also significantly higher compared to maize + potato (1.16). Rampur Composite gave significantly higher MGEY (7937 kg ha^{-1}) compared to early-maturing Arun-2 (7328 kg ha^{-1}). The MGEY obtained with strip intercropping with 1.8 m space was significantly higher (8190 kg ha^{-1}) even with lower maize yield (3703 kg ha^{-1}) than strip intercropping with 1.5 m space (7076 kg ha^{-1}). This maize + kidney bean practice was also found beneficial in farmers' fields (B:C ratio 1.6, MRR 1.48) compared to maize + potato (B:C 1.05, MRR 0.79) and maize + lentil (B:C 1.38, MRR 0.75). The two years' experiment results indicated that growing maize + kidney bean with strip intercropping was found more productive and economic in comparison to sole maize as well as other tested intercrops in Chitwan conditions.

Key words: Strip intercropping, maize, kidney bean, LER, equivalent yield

Introduction

Maize (*Zea mays* L.) is the second most important cereal grain in Nepal and a priority staple food crop in the hill regions of the country. It is commercially grown in the *terai* (plains) area. Maize is also a major constituent of poultry feed for which demand is increasing. Similarly potato (*Solanum tuberosum* L.) is one of the main vegetable crops grown in the winter in the *terai* and mid-hill areas and in the summer in the high hill regions as well. Kidney bean/common bean (*Phaseolus vulgaris* L.) is an important legume crop grown for green vegetable and dry seed purposes in a wide range of agroclimatic conditions ranging from the *terai* (300 m amsl) to the high hills (2500 m amsl). It is a summer rainfed crop often grown in association with maize in the hills although sole cropping has been practised in recent years in the *terai*. Lentil (*Lens culinaris* M.) is equally an important winter legume crop grown as a sole or mixed intercrop with mustard in the *terai* and inner *terai* of Nepal.

Intercropping is a way to increase the diversity of farming systems. It not only produces more than two kinds of commodities at a time in the same field but also increases the amount of humus in the soil due to the decaying of crop remains. It averts the risk of crop failure and increases land-use efficiency. Maize is one of the best crops for intercropping due to its physiological nature. Crops like legumes, cereals, vegetables and spices are different intercropping options. Therefore, the potentiality of intercropping different crops with maize in the *terai* and inner *terai* has been studied with the objective of reducing poverty by improving farm production and farmers' income.

Maize being a major crop in the hilly areas, most studies on maize-based intercropping have concentrated on the hilly areas of Nepal. Mixed cropping is a traditional practice in Nepal, which may be one of the major countries where multiple cropping is practised mainly for subsistence (Bhattarai 1992). Wolfswinkel reported that each crop in an intercropping system should have adequate space to maximize cooperation and minimize competition between them. The benefits from intercropping are higher when two crops with different rooting systems, different patterns of water and nutrient demand and different above-ground habits are planted together. Water, nutrients and sunlight are thus used more efficiently, and strip intercropping may result in benefits greater than from a sole crop. Winter maize yields are better from intercropping than summer maize. Shiblee *et al.* (2000) found the highest marginal rate of return (MRR) of 598% from maize + chilli or potato or cucumber intercropping.

Materials and Method

On-station experiment

Our research included an experiment carried out during two consecutive winter seasons in 2006 and 2007 at NMRP Rampur, Chitwan (228 masl). Three different factors – two maize varieties, two row distances for the strip and three different intercrops – were evaluated in three replications. The net harvested plot was 24 m^2. The experiment was carried out in a sandy loam soil which was acidic (pH 5.5), low in organic matter (1.7%) and total nitrogen (0.10%), medium in available phosphorus (277 ka ha^{-1}) and potassium (134 ha^{-1}). Two maize varieties, Rampur Composite (full season) and Arun-2 (early-maturing) were planted on Sep 21 in 2006 and Oct 6 in 2007 and all the intercrops (potato, kidney bean and lentil) were planted 20-30 days after maize sowing. Maize was planted in paired rows (60 × 180 cm and 75 × 150 cm) resulting in 1.8 m and 1.5 m strips for intercrop planting. Intrarow spacing for maize was 40 cm with double plants per hill in the paired row (60 × 180 cm) design and a single plant and 25 cm in the other arrangement. Maize was fertilized with 120:60:40 kg ha^{-1} $N:P_2O_5:K_2O$ and common bean, potato and lentil were grown with 80:50:30, 140:100:60 and 18:46:30 kg ha^{-1} respectively. The crops were irrigated once during the maize silking stage. But two irrigations were given when no rainfall occurred in 2007. The potato crop was infected with late blight disease and four sprays of Mancozeb @ 2.5 g L^{-1} of water were applied at 10-day intervals in the later stages in both seasons. Maize (early), maize (full-season), potato, kidney bean and lentil were harvested 146, 158, 95, 130, and 142 days after planting, respectively.

On-farm experiment

Four treatments were tested in farmers' fields (considered as a replication) in both upland and lowland conditions during winter 2007. Three different intercrops (potato, kidney bean and lentil) and one sole maize crop were evaluated. The planting space available for intercrops was 1.8 m in strip and potato was planted with 60 × 25 spacing, kidney bean with 45 × 10 cm, and lentil in 25 cm × continuous. The net harvested size of each experimental unit was 48 m^2. The following activities were performed in different farmers' fields.

Results and Discussion

Maize

The combined analysis of variation and yield data are presented in Table 1 and Table 2. The good performance recorded in 2006 (Table 2) might have been due to better rainfall and early planting (Sep 21) compared to 2007. Full-season maize variety Rampur Composite produced significantly higher maize grain yield (4369 kg ha^{-1}) compared to early-maturing Arun-2 (3321 kg ha^{-1}). Maize plant population was significantly higher (41 000 ha^{-1}) with 1.8 m strip space and planting in 60 × 40 cm (two plants per hill) compared to 1.5 m strip space and planting in 75 × 25 cm (35 500 ha^{-1}). However, strip space 1.5 m yielded significantly more maize grain yield (3987 kg ha^{-1}) compared to 1.8 m strip space which might be due to the planting of two plants per hill. Maize yield did not vary significantly due to different intercrops.

Intercrops

The results for intercrop yield were the reverse of results for maize yield which was due to the shading effect for a longer duration caused by the full-season variety which was lower (2438 kg ha^{-1}) compared to the early-maturing maize variety (2757 kg ha^{-1}). Strip space 1.8 m yielded significantly more intercrop yield (3138 kg ha^{-1}) compared to the 1.5 m strip space (2057 kg ha^{-1}). The highest maze grain yield treatment yielded a lower intercrop yield and vice versa for the lower maize grain yield treatment. This indicates strong competition between maize and the intercrops. Potato was yielded the highest (6316 kg ha^{-1}) followed by kidney bean (1001 kg ha^{-1}) and lentil (476 kg ha^{-1}). The performance of potato could have been much higher but for the late blight infection in both study years. This disease reduced yield and increased the cost of cultivation.

Activities	Farmers/sites				
	1	2	3	4	5
Planting date (maize)	Sep 16	Sep 20	Oct 26	Nov 5	Nov 8
Planting date (intercrop)	Oct 18	Oct 18	Nov 15	Dec 5	Nov 30
Irrigation	Dec 2	-	Dec 31	Dec 6	Dec 6
Previous crop	Maize	Sesame	Rice	Rice	Rice

Table 1. Combined analysis of variance for maize variety, strip space and intercrops in intercropping trials during the winter seasons of 2006 and 2007.

Source of variation	Mean square			
	Maize grain yield	Intercrop yield	MGEY	LER
Year (Y)	889 284.9*	13 674 532.3**	46 134 131.5**	0.049
Maize variety (A)	19 804 413.5**	1 827 999.5*	6 670 874.5*	0.048
Strip space (B)	1 448 686.6**	21 069 137.0**	22 336 085.1**	0.200
Intercrops (C)	44 824.8	250 509 932.8**	106 444 679.9**	0.270*
AB	114 186.9	1 154 288.2	2 744 870.6	0.047
AC	5 270.7	1 093 732.5	1 750 091.9	0.106
BC	32 293.8	14 898 008.5**	15 908 157.6**	0.005
ABC	2 035.8	636 254.3	166 185.0	0.028
YABC	115 222.5	847 147.8	849 203.1	0.042
Error	154 446.7	378 280.6	984 572.9	0.071

* and ** indicate significance at p = 0.05 and 0.01 respectively.

Table 2. Mean yield (kg ha^{-1}) and land equivalent ratio (LER) of maize variety, strip space and intercrops evaluated over two years in intercropping trials (W).

Treatment	Maize grain yield (kg ha^{-1})	Intercrop yield (kg ha^{-1})	Maize GEY (kg ha^{-1})	LER
Y. Year				
2006	3956	3033	8434	1.30
2007	3734	2162	6833	1.24
A. Maize variety				
Rampur Composite	4369	2438	7937	1.24
Arun-2	3321	2757	7329	1.30
B. Strip space				
1.5 m	3987	2057	7076	1.22
1.8 m	3703	3138	8190	1.32
C. Intercrop				
Potato	3864	6316	9836	1.16
Kidney bean	3876	1001	7423	1.37
Lentil	3796	476	5640	1.27
CV %	10.2	23.7	13.0	20.9
LSD (p = 0.05)				
Y, A, B	132.4	207.1	334.3	ns
C	ns	253.6	409.2	0.11

Analysis of crop mixture

Due to variation in the yield potential of different intercrops, their market price and profitability should be considered prior to making inferences. Therefore, we calculated the maize grain equivalent yield, and performed an economic analysis. Given the good performance of maize as well as the intercrops, MGEY and LER were better in the first year of the experiment. Rampur Composite performed better in terms of MGEY than Arun-2 but the results for LER were the opposite of that. Strip space of 1.8 m resulted in significantly higher MGEY (8190 kg ha^{-1}) and LER (1.32) compared to 1.5 m strip space. The potato intercrop yielded a higher MGEY but lower LER (1.16) compared to kidney bean while lentil was found poor in producing maize and intercrop yield.

Economic analysis

The highest biological yield, and consequently total income, were obtained with the full-season maize variety, 1.8 m strip space and potato intercrop. However, kidney bean produced the highest marginal rate of return (revenue gain:additional cost ratio), of 1.32 than potato (<1.0) when partial budgeting was performed based on high-yielding sole maize among two varieties. The reason behind this result is the remarkably variable cost of cultivation of intercrops. The wide strip space and full-season variety

Table 3. Partial budgeting-based economic analysis of maize-based intercropping study in the winter season for treatments having >1.0 MRR (revenue gain: additional cost ratio) at Rampur, Chitwan, Nepal, 2006-2007.

Treatment	Revenue(NRs '000)		Cultivation cost(NRs '000)		Revenue gain: add. cost
	Total	Gain	Variable	Additional	
1. Rampur Composite; strip 1.5 m + Kidney bean	109.94	27.89	34	23	1.21
2. Rampur Composite; strip 1.8 m + kidney bean	115.12	33.07	36	25	1.32
3. Arun-2; Strip 1.8 m + potato	157.57	75.52	85	74	1.02
4. Rampur Composite (sole)	82.05	=	11		

Table 4. Performance of maize-based intercropping practice (W) in farmers' fields (5) of Chitwan, Nepal and its economic analysis (partial budgeting), 2007.

Treatment	Maize GY[1] (t ha^{-1})		Intercrop yield (t ha^{-1})		MEGY[2] (t ha^{-1})	Revenue (NRs '000)		Cost (NRs '000)		MRR[3]	B:C ratio[4]
	Mean	Range	Mean	Range		Total	Gain	Vary	Ad[5]		
Maize + potato	3.174	2.8-3.5	5.270	4.2-6.0	7.915	111	58.4	85	74	0.79	1.05
Maize + Kidney bean	3.155	2.6-3.5	1.005	0.7-1.5	6.385	89.4	36.9	36	25	1.48	1.60
Maize + lentil	3.084	2.5-3.3	0.412	0.4-0.5	4.558	63.8	11.3	26	15	0.75	1.38
Maize (sole)	3.748	2.4-5.1	0		3.749	52.5		11			1.69
CV %	21.8				15.8						
LSD (p=0.05)	ns				1.228						

1. GY = Grain yield.
2. MEGY = Maize equivalent grain yield
3. MRR = marginal rate of return (revenue gain:additional cost)
4. B:C = Benefit:cost ratio
5. Ad = Additional

treatments gave a higher MRR than the narrow strip space and early-maturing variety treatments respectively (Table 3). For calculating partial budgeting, the prevailing price of produce for maize, potato, kidney bean and lentil was considered (NRs 10, 10, 35, 40 in 2006 and 14, 12, 45, 50 in 2007 respectively) at the time of harvesting.

On-farm results

Though sole maize yielded the highest grain yield (3748 kg ha^{-1}) it was nonsignificant with other intercrop treatments. Naturally intercropping of potato produced the highest economic tuber yield than kidney bean and lentil and consequently high MGEY. But intercropping of kidney bean gave the highest economic return, ie, MRR and benefit:cost ratio (1.48 and 1.60 respectively) compared to intercropping of potato (0.79 and 1.05) and lentil (0.75 and 1.38) in farmers' fields (Table 4). The trials were conducted in both lowland and upland conditions with different dates of planting (Sep 16 to Nov 8). However, the results obtained were comparable for all planting dates and conditions.

Conclusion

Rampur Composite yielded higher MGEY (7937 kg ha^{-1}) and maize yield (4369 kg ha^{-1}) without additional cost for maize variety. Strip space 1.8 m was more profitable with higher intercrops, MGEY, higher LER and MRR than 1.5 m space. Among the tested intercrops, potato yielded higher tuber yield as well as MGEY but the potato intercrop was found less beneficial due to the higher cost of cultivation. Intercropping of kidney bean gave the second highest MGEY with higher LER (1.37) and MRR (1.32) compared to potato and lentil. Maize + kidney bean intercropping also gave the highest MRR (1.48) and B:C ratio (1.60) in farmers' fields. Therefore, growing maize + kidney bean in 1.8 m strip space was found more productive, economic and promising compared to other tested intercrops and sole maize in Chitwan conditions.

Acknowledgement

The authors would like to thank NMRP management for their kind support in conducting our research activities.

We are also thankful to NMRP's Mr. R.K. Shrestha for his sincere support in trial management and data collection. The financial support of HMRP/CIMMYT and nomination from NARC to participate in the 10th ARMW is also duly acknowledged.

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Demand for Technology Innovation and Transfer to Maize Farmers in South Sulawesi, Indonesia

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Abstract. This paper illustrates the demand for technology innovation and transfer to maize farmers in South Sulawesi Province. Data for this study was gathered through Participatory Rural Appraisals (PRA) conducted in several subdistricts of this province and also from secondary data sources. The pattern of maize varieties used in this province has been changing. In 1995 local/white varieties accounted for 40.0% of the usage, open-pollinated superior varieties 48.6% and hybrid maize varieties 14.4%. Over the next 10 years, the use of local varieties decreased to 16.2% and that of open-pollinated superior varieties to 23.4% including Quality Protein Maize (QPM) varieties. The share of hybrid maize varieties rose to 55.6% in 2007 although in 2006 it was only 29.6%. Innovation technologies have been used by some farmers. Farmer groups are using open-pollinated superior varieties and hybrid varieties. The other technology innovations adopted have been use of zero and minimum tillage, site-specific nutrient recommendations, improvement in postharvest handling technologies like threshers, drying and processing equipment. These technologies were recommended by the Indonesian Government through the Agricultural Technology Committee. Initiatives to transfer technology innovations to farmers have included demonstrations, case studies and communication development. The strategy and methods to disseminate technology innovations have included (1) direct communication to the clients; (2), learning through media like leaflets, folders, and booklets; and (3) a combination of both methods.

Key words: Maize farmers, technology innovation, transfer methods

Introduction

Maize is a strategic commodity in Indonesia because of its multiple uses as food, feed and raw material for industry. As a food commodity, maize is second in importance to rice. Suryana (2006) reports that the contribution of maize to Indonesia's economy has doubled in three years, from 2004 to 2006, growing from Rp.9.4 trillion to Rp.19.2 trillion. However, maize production is still not sufficient to meet domestic demand, and imports are needed. The Ministry of Agriculture planned to achieve maize self-sufficiency in 2007, and then to expand production for export. The targets were not achieved. Farmers hope to increase production by adopting new technologies.

Farmers of South Sulawesi used to cultivate local white varieties of maize in the upland areas for food purposes. Later they used to sow open-pollinated superior varieties in the dryland in the rainy season. Under the government-supported maize development program named BIMAS Palawija (Mass Guidance for Second Crop Intensification), farmers have been planting not only white corn and open-pollinated superior varieties, but also hybrid maize varieties in the drylands in the rainy season, and in irrigated lands after the rice crop. The Mass Guidance or *BIMAS* approach delivered both technology innovations and production resources like fertilizers and insecticides.

Innovation is defined as a new idea, practice or object that is successfully introduced into economic or social processes (Hartwich *et al.* 2007). In agriculture, innovations can include new knowledge or technologies related to primary production, processing and commercialization all of which can positively affect productivity, competitiveness and livelihoods of farmer and others. Both maize price and good seed assistance from the government can motivate farmers to adopt technological innovations and increase their production. The government's objective of increasing maize production by 2.24% per year can be attained by increasing productivity and expanding the planting area.

This assessment paper is aimed at strengthening activities to increase maize production by adoption of innovation in South Sulawesi, Indonesia.

Methodology

Data for this paper was collected through Participatory Rural Appraisals (PRAs) in several subregencies of South Sulawesi, especially the regencies of Bulukumba, Takalar, Bantaeng and Bone. Secondary data was obtained from related institutions and assessment data from AIAT of South Sulawesi.

Results and Discussion

Maize planting area, production and trends

Development of maize in South Sulawesi has been related to the other staple foods of the people of this area, especially rice. When rice used to be sown during only one season (rainy season) every year until the 1960s, farmers used to plant while local varieties of maize in the upland dry areas and in rainfed lowlands area after rice. The pattern of food consumption of rice for six months and maize or rice mixed with maize in the other six months was reflected in the management of maize.

Initiatives have been undertaken in recent decades to increase maize production and productivity in South Sulawesi. There have been changes in the composition of maize varieties used. In 1995, 40.0% of the maize varieties used were local/white varieties, 48.6% open-pollinated superior varieties and 14.4% hybrid maize varieties. In the one decade since then, the composition has changed: the share of local varieties has decreased to 16.21% and that of open-pollinated superior varieties to 23.42% including Quality Protein Maize (QPM) varieties such as Srikandi Kuning-1, while the share of hybrid maize varieties rose to 55.56% in 2007 although in 2006 it was only 29.6% (Table 1). The sharp increase in the total area of maize hybrids was mainly due to seed assistance from the government. Local white varieties used to be the staple food of people in some districts, but with the increase in rice production in South Sulawesi, the number of people using maize as the main food has decreased.

Adoption of modern varieties, including hybrid varieties and inputs, have helped increase yields.

Table 1. Distribution of maize varieties, 2006 and 2007.

Maize varieties	Year of production			
	2006		2007	
	Planted area (ha)	%	Planted area (ha)	%
Local (white, glutinous)	32 590.48	15.1	34 751.05	16.2
Superior open-pollinated including QPM	86 979.80	40.3	50 198.64	23.4
Hybrid				
F_2 or F_3 hybrid	63 885.98	29.6	119 129.88	55.6
	32 374.65	15.0	10 321.67	4.8
Total	215 831	100	214401.21	100

Source: UPTD BPSB Tanaman Pangan dan Hortikultura Propinsi Sulawesi Selatan 2007.

Production has grown 8.86% from 639 414 t to 696 084 t, and productivity 5.08% from 3.210 t ha-1 to 3.373 t ha-1.

Demand for technology innovations

Several agricultural technologies have been introduced to farmers and farmer groups in this region. Some of them have been successfully adopted such as open-pollinated superior varieties and hybrid varieties and zero and minimum tillage. Site-specific nutrient management has also been recommended but not successfully adopted by farmers yet. Improvement in postharvest handling technologies like using threshers, drying and processing has been recommended. The government's recomendations for the maize planting seasons of 2008 and 2008/2009 are summarized in Table 2.

The other innovations include growing certified seed, of both open-pollinated superior varieties and hybrid maize varieties, by farmer groups as part of the Seed Grower Base Community System and local enterprise. Farmers have adopted hybrid maize seed as a production technology innovation which could increase their production and income. Production of certified seed of open-pollinated superior varieties including Quality Protein Maize (QPM) variety Srikandi Kuning-1, and hybrid maize varieties will be developed as part of the community-based development initiative.

Adoption of technology is dependent on farmers' motivation. In the case of adoption of new crop varieties, farmers not only consider the capacity of the crop to boost productivity, but also other characteristics such as resistance to drought, flooding, pests and disease. A lack of assets such as land, education or equipment (eg, pumps), can also limit adoption. So more attention needs to be paid to technologies that require fewer assets and less expensive inputs.

Transfer of technology innovations

Innovators are agents, farmers, processors or other private sector entities who introduce and adopt innovations. Researchers and extension personnel are inventors, knowledge communicators who assist the innovator in the conduct of the innovation, which in any case may also occur without their contribution. Training farmers and bringing to them training materials that are understandable to those with low literacy can help them adopt a new technology. Cultural characteristics influence adoption in many different ways, such as preferences for certain tastes and textures.

Table 2. Summarized recomendations for development of maize in South Sulawesi Province of Indonesia for planting seasons 2008 and 2008/2009.

No.	Subject	Wet season	Dry season
1.	Land Resources		
	Upland areas	Upland rice + maize	-
		Maize	Soybean
	Irrigated lowland areas	Rice	Dry season 1 : Rice
			Dry season 2: Maize zero tillage
	Rainfed lowland areas	Rice	Maize zero tillage
	Rainfed lowland areas with pump irrigation	Rice	Maize zero tillage (dry season 1)
2.	Technological innovations		
2.1	Seed and varieties	Certified seed (commercial seed) Varieties recommended: Open-pollinated superior varieties like Lamuru, Sukmaraga, Bisma, Gumarang, Srikandi Kuning-1 (QPM) Hybrid varieties: BISI, Pioneer, Bima 1, Bima 2, Bima 3	
2.2.	Fertilizer amount	250-300 kg urea ha^{-1} 100-150 kg SP 36 ha^{-1} 50-100 kg KCL ha^{-1}	
	Fertilizer application	Application of urea 3 times: 1. At 7-10 DAS; 2. 28-20 DAS; 3. 40-45 DAS Third application based on LCC measure	
2.3.	Weed control	Manual weeding two times: 14 DAS and 30 DAS. Or using herbicides	
2.4.	Pest control	Seed treatment with fungicide Metalaxyl for downy mildew; Using resistant varieties and proper planting times; Maize stem borer controlled by insecticide if more than one group of larvae or eggs hatched per 30 plants. Cutworm or armyworm controlled if more than two larval groups from eggs hatched per 36 plants. Applied Carbofuran 3 G 10 kg ha^{-1} through tip of maize.	
2.5.	Postproduction handling	Threshing and cleaning with machine, and drying manually or with dryer.	

[1.]DAS = Days after sowing.
Source: Dinas Pertanian Tanaman Pangan dan Hotikultura Propinsi Sulawesi Selatan (2008).

Dissemination pathways, how people learn about or obtain a technology play a fundamental role in determining who will adopt a new technology. Aidar *et al.* (2002) reported that of the media used to communicate effectively, 88% learn from leaflets and only 50% from booklets. Both media could grow up of maize production by 43.3%, time efficiency by 35% and cost efficiency by about 21%. A broad range of dissemination methods were used to promote maize in South Sulawesi Province for three decades. However, there was no one “best” method of dissemination for all regions or groups of farmers within one region. So, dissemination is specific to location, ethnic group, knowledge level, attitudes and practices of farmers or farmer groups.

According to Sumarno (1997), some of the technologies developed by research were not adopted by farmers because: (1) there was a barrier of communication between the source of innovation and the farmer; (2) many innovations are developed away from the farmers’ fields without involving the participation of farmers; (3) there are problems relating to socioeconomic conditions and environmental resources; and (4) there are problems internal to farmers.

Show case of technology innovation is a dissemination method successfully used in maize because: (1) it establishes a direct relation with the client (farmers and their families); (2) it is conducted on farmers’ lands with the participation of farmers, (3) it uses multimedia, visual, verbal and printed material; (4) it can be documented and used again by other people efficiently and effectively combining with other instructional media (Aidar *et al.* 2002). A case study on zero tillage for maize in Takalar Regency (with 50 farmer respondents) showed that 50% of farmers found it easy to obtain herbicide, all of them said zero

The Effect of Seed Size on Seed Viability of Maize Varieties Lamuru and Srikandi Kuning-1

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Abstract. New varieties, open-pollinated (OPVs) or hybrid, can significantly increase productivity. Hybrid varieties do yield higher compared to OPVs. However, due to the high price of hybrid seeds, OPVs are preferred in strategies for increasing maize productivity, especially for poor farmers. Although productivity of OPVs has been remarkably high, some farmers still complain about their nonuniform seed size. This paper reviews experiments conducted in Maros, South Sulawesi and West Nusa Tenggara in 2004, 2005 and 2006, respectively, using cvs. Lamuru and Srikandi Kuning-1. The results showed that graded seed using a screen perforator of 8 mm and 10 mm diameter produced high seed germination rates at 12 months storage. These rates were 98% for Lamuru and 94.6% for Srikandi Kuning-1 compared to rates attained by graded seeds using a screen perforator of 7 mm. On the other hand, the germination rate of seeds that were graded using a 7 mm screen perforator dropped to 86.67% for Lamuru but remained high (94.67%) for Srikandi Kuning-1. These results indicate that the screen diameter for grading of Lamuru seed should be not less than 8 mm, and 7 mm for Srikandi Kuning-1.

Key words: Seed quality, seed viability, OPVs, germination and seed storage

Introduction

Farmers' preferences for seed quality mostly concern rapid growth and uniform stand during germination. However, the seed size of maize, especially of open-pollinated varieties (OPVs), varies from the bottom to the top of the cob. Seeds at the bottom and the top are generally smaller compared to those in the middle of the cob. Smaller seed produce stems of smaller diameter and shorter root length than medium and large seeds. As a consequence, under less than optimum conditions such as drought, plants produced from smaller seeder do not survive. Larger seed in a seed lot produce bigger cotyledons and twice the net photosynthetic rate than smaller seed.

An experiment on seed size from different parts of the cob conducted by Arief *et al*. (2004) showed that there were no significant differences in terms of storability between different one-thirds of the maize cob but there was a tendency for seed from the base and the top of the cob to produce smaller stems and taller plants compared to seed from the middle 3/5ths of the cob. Furthermore, this method needs more labor and time allocation. On the other hand, seeds from the middle of the cob produced more vigorous plants compared to those from the base and the top of the cob (Saenong 1982). Priestley (1986) also reported that smaller seed of the same cultivar were less storable than medium and larger seed. Gardner *et al*. (1991) also reported that larger seed have adequate food to support a higher photosynthetic rate than smaller size. Using a maize seeder is also difficult when seed are not uniformly sized because the perforator of the seeder needs to be adjusted according to the size of the seed to allow a uniform number of seeds to be dispersed. The problem for small seed growers to provide a uniform seed size remains how they screen their seed effectively and efficiently in terms of time and labor allocation to minimize the cost and optimizing the seed yield.

Methodology

The first experiments were carried out in 2004 with seed production in Sambelia village in western Nusa Tenggara Province. The seed were kept in Maros for one year (from 2004 to 2005). The screens used were: e"10 mm, 8-10 mm, 6-8 mm, without seed grading and farmers' practice. The varieties used were Lamuru and Srikandi Kuning-1. Seed germination and vigor were tested every two months starting before storage (0 month) up to 12 months of storage.

Seed production in the first experiment was carried out in Sambelia village in the district of east Lombok in Nusa Tenggara Province in 2005, while seed quality was evaluated in the Maros seed laboratory in 2005 using the OPV Lamuru. The treatments consisted of three seed sizes: big seed of diameter $\geq$10 mm (S1); medium-sized seed of diameter 8-10 mm (S2); and small seed of diameter 6-8 mm (S3). Seeds were kept in an airtight container (high-density polyethylene bag) 5 kg per bag and then kept in a silo or wooden box at 28-32°C for 12 months. Testing of seed viability and vigor were conducted every two months using ISTA(2004).

Results and Discussion

The results showed that up to 12 months of storage at room temperature, seed graded using a screen of e”8 mm diameter produced a high germination rate of 94.7% for Lamuru and 98% for Srikandi Kuning-1. On the other hand, using a perforator of 7-8 mm diameter produced a lower seed germination rate of 86.7% for Lamuru but 94.7% for Srikandi Kuning-1. Therefore big seed of Lamuru need to be screened with a perforator of e”8 mm diameter, but seed of Srikandi Kuning-1 need to be screened at e”7-8 mm to provide good seed quality. All seeds that passed through the screen with a perforator of 7 mm should not be used for sowing. The farmers' practice had good results. It showed that the highest germination was 96% for Lamuru and 94.7% for Srikandi Kuning-1 (Table 1). However, it was time-consuming and needed more labor because they it was needed to cut the cob 1/5 at the bottom and top of the cob. Furthermore, the net seed yield obtained with farmers' technology was lower than that obtained using a perforator with a machine (air screen cleaner). In terms of storability, small seed from the same seed lot were less storable than medium and big seeds. An experiment conducted by Saenong (1982) showed that seed from the middle part of the cob produced more vigorous plants that seed from the bottom and top of the cop. Nongraded seed produced a low germination percentage at 12 months of storage, especially Srikandi Kuning-1 (80%).

Based on speed of germination as a measure of seed vigor, the germination speed of seed in initial seed storage (before storage) varied from 30.3% to 32.7% per day for Lamuru at the perforator size of ^{3}8 mm, reduced to 24.7% per day to 24.7% per day (Table 2). A similar trend was obtained for Srikandi Kuning-1. The lowest germination speed was obtained for nongraded seed of both Lamuru

Table 1. Average seed germination of cvs. Lamuru and Srikandi Kuning-1 at storage period of 0-12 months at Maros, Indonesia, 2004-2005.

Seed Grading	Seed weigh (100) (g)	Initial Seed moisture (%)	Germination capacity (%) Storage Periods (Months) 0	2	4	6	8	10	12
			cv Lamuru						
Seed size ≥8 mm	29.57	9.63	100.0 a	99.3a	100.0a	98.67a	96.67ab	96.00a	94.67ab
Seedsize.7-8 mm	24	9.87	98.7a	100.0a	99.3ab	99.33a	92,00bcd	97.33a	86.67bc
Non graded seed	28.47	9.43	99.3 a	98.7a	98.7ab	95.33ab	90.67 cd	95.33a	86.67bc
Farmers practice	28.73	9.63	99.3a	92.0b	93.3bc	98.67a	94.67abc	96.67a	96.00 a
			cv Srikandi Kuning-1						
Seed size ≥8 mm	27.13	9.2	100.0a	98.7a	96.7ab	96.7ab	96.7ab	97.3 a	98.0 a
Size 7-8mm	23	9.1	99.3a	100.0a	98.0ab	96.0ab	98.7 a	94.0 a	94.7 ab
Non graded seed	26	9.27	96.3a	93.3b	96.7ab	92.0b	86.7 d	80.0b	80.0 c
Farmers practice	26.17	9.03	97.3a	97.3a	89.3 c	97.3ab	96.7 ab	92.0 a	94.7 ab

Table 2. Average germination speed of maize cvs. Lamuru and Srikandi Kuning-1 at storage periods from 0 to 12 months under 28-32^{o}C temperature, Maros, Indonesia, 2004-2005.

Seed Grading	Seed weigh (100) (g)	Initial Seed moisture (%)	Germination capacity (%) Storage Periods (Months) 0	2	4	6	8	10	12
			cv Lamuru						
Seed size ≥8 mm	29.57	9.63	100.0 a	99.3a	100.0a	98.67a	96.67ab	96.00a	94.67ab
Seedsize.7-8 mm	24	9.87	98.7a	100.0a	99.3ab	99.33a	92,00bcd	97.33a	86.67bc
Non graded seed	28.47	9.43	99.3 a	98.7a	98.7ab	95.33ab	90.67 cd	95.33a	86.67bc
Farmers practice	28.73	9.63	99.3a	92.0b	93.3bc	98.67a	94.67abc	96.67a	96.00 a
			cv Srikandi Kuning-1						
Seed size ≥8 mm	27.13	9.2	100.0a	98.7a	96.7ab	96.7ab	96.7ab	97.3 a	98.0 a
Size 7-8mm	23	9.1	99.3a	100.0a	98.0ab	96.0ab	98.7 a	94.0 a	94.7 ab
Non graded seed	26	9.27	96.3a	93.3b	96.7ab	92.0b	86.7 d	80.0b	80.0 c
Farmers practice	26.17	9.03	97.3a	97.3a	89.3 c	97.3ab	96.7 ab	92.0 a	94.7 ab

and Srikandi Kuning-1: 24.4% per day for Srikandi Kuning-1 and 24.7% per day for Lamuru (Table 2). In this connection, Abd-El-Rahman and Bourdu (1986) reported that the growth rate of seedlings increased with larger seed and round-shaped seed than thinner seed shape. Seed size has good correlation with seed density. High-density seed have adequate food reserves of protein and mitochondria which support energy production (ATP) which aids rapid growth and development (Prapto 2006). The results of this experiment showed that seed of ³8 mm Were more storable in terms of germination percentage and speed of germination compared to seeds of 7-8 mm size.

Conclusion

Seed grading of open-pollinated varieties of maize is necessary to produce good seed quality with a high rate of germination and vigor (germination speed). Use of the screen depends on the general size of the seed. Using an e" 8mm screen usually does work for many OPVs, but for small-seeded varieties it is suggested to screen seed with a screen of 7-8 mm.

The farmers' technique of seed grading by cutting off one-third at the base and the top of the cob does produces high seed quality. However, it is not recommended because its produces less seed yield. Therefore, for community-based seed production, it is recommended to use a small air-screen cleaner combined with a grader with a proper perforator.

Nongraded seed and smaller seed reduced seed quality, especially when stored for at least eight months under room storage conditions (25-32^{o}C).

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Farmers' Participation to Increase N-efficiency and Farmers' Income

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Abstract. Karo regency, characterized by highland terrain, is the most important maize production area in North Sumatra. The maize area here is about 50 182 ha. This study was conducted during March-June 2007 at Tigabinanga in Karo regency at a site located 600-700 m above sea level. The experiment was arranged in a randomized complete design consisting of two factors (varieties and nitrogen) with 3 replications. The varieties were P12, BISI 9, NK 99 and DK 3, while the nitrogen treatments were N1 = without N; N2 = 160 kg ha^{-1}; N3 = 207 kg ha^{-1}; and N4 = 250 kg ha^{-1}. Maize productivity in this region is about 7-8 t ha^{-1} but there are possibilities of increasing it to 10 t ha^{-1} by using nitrogen site-specific nutrient management (SSNM) technology. As labor shortage is a problem in this area, introduction of SSNM technology should include planter and herbicide application. The objectives of the experiment were increased productivity and efficiency of N fertilizer. The results indicate an increase of 11.5% in productivity, 25% in farmers' income and 53.6% in efficiency of N fertilizer.

Key words: Maize, SSNM, efficiency N, income increase

Introduction

Nitrogen is a major nutrient element needed by the maize crop, especially in areas where high-yielding varieties are intensively cultivated. Several experiments have been conducted to assess the nitrogen fertilizer need and response in Indonesia (Taslim 1993).

Nitrogen is a nucleate acidic amino acid compiler nutrient win an important role in the making of chlorophyll and nucleotides. It accelerates plant growth in the crop coronet and leaf area. It has an effect on all parameters related to yield. Concentration of nitrogen in the leaf plays a role in photosynthesis and the rate of biomass production. Giving of nitrogen because requirement of crop to other nutrient increased to make balance to growth rate plant which quickly (Makarim 2003).

Nitrogen is a regulator of the usage of potassium, phosphates and other nutrient elements (Mengel and Kirby 1987). Nitrogen deficiency can cause emergence of crop elevator scapula with limited root development, and leaves become light green or yellow and tend to quickly fall off (Makarim *et al.* 2003).

Poor fertilization of N causes abundant emission of the gas N_2O during ammonification, nitrification and denitrification. According to Partohardjono (1999), emission of N_2O is influenced by the form of fertilizer N. Urea tablets result in low emission of N_2O and granular urea results in high levels of the gas. Efficient use of fertilizer use leads to lower emissions of N_2O. On the other hand, according to Stevens *et al*. (1999), use of fertilizer N in abundance can increase crop damage due to pest attack and disease, lengthen crop duration and cause lodging.

Abolition of fertilizer subsidies by the government does not necessarily lead to farmers using less fertilizer because they have become accustomed to using high dosages. As a result, the operating costs of farmer tend to remain high. In this context, technology relating to optimum fertilizer use efficiency, especially of fertilizer N, is required. Site-specific nutrient management aims at fertilization as per crop requirements and increased absorption of N by the crop.

The objective of our study was to increase the efficiency of fertilization of nitrogen, increase productivity and farmers' income in Tigabinanga subdistrict at a location characterized by Inceptisols.

Materials and Method

Our research was carried out during March-June 2007 at a dryland location 600-700 m above sea level. Nutrient and soil analysis was done at the BPTP Laboratory in North Sumatra. We used a factorial completely randomized block design with two treatment factors (variety and fertilization) with three replications. The experimental varieties were P 12 (V1), BISI 9 (V2), NK 99 (V3) and DK 3 (V4). The four

Table 1. Fertilization dosage for site-specific nutrient management of maize in Tigabinanga subdistrict, Indonesia.

Location	Fertilizer	N0(kg ha^{-1})	N1(kg ha^{-1})	N2(kg ha^{-1})	N3(kg ha^{-1})
Sp. Gunung	$NP_2O_5K_2O$	07290	1607290	2077290	2507290
Simolap	$NP_2O_5K_2O$	07290	1607290	2077290	2507290
Kutabangun	$NP_2O_5K_2O$	07290	1607290	2077290	2507290

Table 2. Soil analysis results at three locations in Tigabinanga subdistrict, Indonesia.

Location	Texture Pasir %	Organic Debu %	C/N Clay	Olsen-P C P_2O_5 (ppm)	Bray 1-P N			
Sp.Gunung (0-20)	48	13	39	0.92	0.06	15	188	65
Sp. Gunung (20-40)	50	12	38	0.78	0.06	13	128	174
Simolap (0-20)	36	29	35	0.85	0.07	12	64	56
Simolap (20-40)	36	29	35	0.85	0.07	12	64	56
K.bangun (0-20)	36	23	41	0.89	0.07	13	119	146
K.bangun (20-40)	36	29	35	0.83	0.07	14	70	60
Soil texture	Clay loam to clay							
Organic C and N		Low	Wide					
P available				High				

Location	pH H2O	KCl	Acidity Al	H	Exchangeable Bases Ca	Mg	K	Na	SUM	CEC	Base saturation %
			------------------------------- cmol+/kg -------------------								
Sp.gunung (0-20)	5.4	4.5	0.00	0.19	3.39	1.22	0.78	0.17	5.56	8.31	8.31
Sp.gunung (20-40)	5.3	4.3	0.00	0.11	3.44	1.06	0.79	0.10	5.39	8.10	8.10
Simolap (0-20)	5.2	4.3	0.13	0.13	3.48	0.74	0.52	0.16	4.86	7.70	7.70
Simolap (20-40)	5.2	4.3	0.13	0.13	3.28	0.89	0.52	0.17	4.86	7.71	7.71
Kutabangun (0-20)	4.8	4.0	0.50	0.25	1.41	0.81	0.51	0.17	2.90	6.93	6.93
Kutabangun (20-40)	5.3	4.3	0.07	0.13	3.11	2.45	0.62	0.14	6.32	8.83	8.83
pH	Acid-neutral										
Acidity			Low								
Exchange Bases					Low-high		Med-high	Low			
CED										High	
Base saturation											Low-high

nitrogen treatments were: N0 = without nitrogen; N1= 160 kg ha^{-1} N; N2= 207 kg ha^{-1} N; N3 = 250 kg ha^{-1} N. There were 48 (4×4×3) trial units.

Fertilization (Table 1) was done at 7 HST with 30% of the dosage of urea, SP36 entirely and 50% KCl; 35% of the urea was applied at 25 HST; and 35% urea and 50% KCl at 50 HST for treatment N1. But for treatments N2 and N3 fertilization was done two times: 50% at 25 HST and 50% at 50 HST. Thinning was done at 7 days to leave 1 seed per hole.

Weed control was done manually but pest and disease control operations were done as per recommended practices. Crop husbandry was done from planting to harvest. Harvest done by selecting 6 samples from plots of 4 × 3 m to compute productivity and agronomic efficiency of N.

Observations were recorded on:

- Productivity (t ha^{-1})
- Agronomic efficiency of N = (Production with NPK - Production without N)/fertilizer amount of N)
- Profitability ratio = Net profit which/Cost which required by every treatment

Treatment influence was determined by analysis of variance (tested F) at 5% and 1% levels and continuation test DMRT.

Results

Productivity (t ha^{-1}) was highest (10.21 t ha^{-1}) in treatment N1 (Table 3) and lowest in the without-N treatment (N0) with an average of 5.95 t ha^{-1}. Statistical data analysis indicated that treatment N1 was significantly different from treatments N0, N2 and N3.

Fertilization as per SSNM (N1) had a real effect on crop productivity (10.21 t ha$^{-1)}$, and proved to be better than farmers' fertilization practices by 11.5% compared with the FFP2 treatment (9.03 t ha^{-1}). This supports the findings of Sanchez (1993) that nitrogen is important as a chlorophyll compiler with an important role in photosynthesis and growth of bar and leaves.

Agronomic efficiency of N (Table 4) was highest in the SSNM treatment (N1) with 26.59 kg grain per kg N and lowest for the FFP 1 treatment (N2) with 11.96 kg grain per kg N. Data analysis indicated that the SSNM treatment was significantly different from treatments FFP 1 (N2) and FFP 2 (N3).

Results presented in Table 4 show that agronomic efficiency of N (AEN) for the SSNM treatment included in low category (25 kg per kg). Agronomic efficiency N was highest for the SSNM treatment at 26.59 kg grain per kg N and lowest for FFP 1 at 11.96 kg grain per kg N with increasing of efficiency agronomy 53.6% mean in increasing of 26 result kg require N counted 1 kilogram. This supports the findings of Witt (2000) expressing agronomy efficiency excelsior a in location hence progressively a few the application of given by fertilizations is crop for yielding optimal production. For treatments FFP 1 and FFP 2 AEN each = 11.96 and 12.32 kg grain per kg N (low), this thing is as according to the application of fertilization and amount of fertilizers given by farmers unmatched to productions are yielded. SSNM treatment kg grain per kg N (low), this

Table 3. Maize productivity (t ha^{-1}) under site-specific nutrient management (SSNM) in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P 12)	V2(BISI 9)	V3(NK 99)	V4 (DK 3)	Average
N0 (control)	6.20	6.00	5.60	6.00	5.95d[1]
N1 (SSNM)	10.30	10.10	10.02	10.40	10.21a
N2 (FFP[2] 1)	8.50	8.45	8.10	8.20	8.31c
N3 (FFP 2)	9.16	9.00	8.78	9.18	9.03b
Average	8.54a	8.39a	8.13a	8.45a	

[1.] Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
[2.] FFP = Farmers' fertilizer practice.

Table 4. Agronomic efficiency of N (kg grain per kg N) under site-specific nutrient management (SSNM) for maize in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P12)	V2 (BISI 9)	V3 (NK 99)	V4 (DK 3)	Average
N0 (control)	0	0	0	0	0
N1 (SSNM)	25.63	25.63	27.63	27.50	26.59a[1]
N2 (FFP[2] 1)	12.08	13.04	12.08	10.63	11.96b
N3 (FFP 2)	11.84	12.00	12.72	12.72	12.32b
Average	16.51a	16.89a	17.47a	16.95a	

[1.] Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
[2.] FFP = Farmers' fertilizer practice.

Table 5. Recommendations for N fertilization of maize crop on the basis of agronomic efficiency of N and increase in yield if fertilized by N compared with without-N treatment.

Agronomic efficiency (kg grain increase per kg N)	Low (25 kg/kg)	Medium (29 kg/kg)	High (33 kg/kg)
Yield response (t ha^{-1})	Fertilizer N rate (kg ha^{-1})		
1	40	35	30
2	80	70	60
3	120	105	90
4	160	140	120
5	200	175	150
6	240	210	180
7	Ä%	245	210
8	Ä%	Ä%	240

Ä%= select a lower yield response
Sumber: Witt (2008).

Table 6. Benefit:cost (B/C) ratio under site-specific nutrient management of maize compared to other treatments in Tigabinanga subdistrict, Indonesia.

Treatment	V1 (P12)	V2(BISI 9)	V3(NK 99)	V4(DK 3)	Average
N0 (control)	1.26	1.22	1.09	1.22	1.20d[1]
N1 (SSNM)	1.77	1.74	1.73	1.78	1.76a
N2 (FFP[2] 1)	1.40	1.39	1.32	1.35	1.37c
N3 (FFP 2)	1.53	1.50	1.46	1.53	1.51b
RATAAN	1.49a	1.46a	1.40a	1.47a	

[1.] Numbers followed by the same letter in the same column indicate nonsignificant differences at 5% level of the distance test Duncan.
[2.] FFP = Farmers' fertilizer practice.

thing is according to the application of fertilization and amount of fertilizers given by farmers unmatched to productions yang are yielded. In the SSNM treatment fertilization was done in three applications (I = 30%; II = 35%; and III = 35%) using a dibber and providing cover. In the farmers' practices, fertilization was done in two applications (I = 50%; II= 50%) as side dressing without cover. As per research results (Garcia 2007), fertilization with the same dosage but different time and manner of application may result in a difference of 1 t ha^{-1} in yield. According to the findings of Witt (2000), the maize plant requires N during three phases of growth: early growth, flower formation and grain filling. If in this phase assessed BWD hence vegetative growth and generative of crop will be pursued so that produce yield low production.

Computatio of the benefit:cost (B/C) ratio (Table 6) indicated that the highest advantage was afforded by at the SSNM treatment with an average B/C ratio of 1.76. The lowest B/C ratio (1.20) was for the without-N treatment (N0). The SSNM treatment was statistically different from the other three treatments.

The benefit by using SSNM was equal to 25%.

Conclusion

From these research results, the recommended fertilization under site-specific nutrient management for maize in Tigabinanga subdistrict is: urea = 347.8 kg ha^{-1}; SP36 = 200 kg ha^{-1} and KCl = 150 kg ha^{-1}. Under SSNM treatment we obtained a yield increase of 11.5%, increased farmers' income by 25% and agronomy efficiency of N equal to 53.6%. Fertilization of N should be done by a dibber and then covered.

Acknowledgement

We thank Dr. Christian Witt and the Assessment Institutes for Agricultural Technology North Sumatra (AIAT) for their inputs to this research.

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Strategy of Maize Farming System Development for Subsistence/Semicommercial Farmers

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Abstract. Subsistence/semi-commercial farmers tend to be slow to adopt new technologies of maize cultivation even when they make economic sense and are compatible with their sociocultural values. Generally, that is because of the high cost of inputs (such as seed of superior varieties, fertilizer and pesticides). Also, their orientation is toward food security rather than commercial farming. Moreover, input shops at the subdistrict/village level are scarce. As a consequence, new technologies do not reach these farmers, who fall back upon existing technologies after the awareness building stage. This research involving three farmer groups and maize planted area of 15 ha was conducted in South East Timor Tengah district in East Nusa Tenggara province of Indonesia during 2007-2008 with the aim of finding an alternative strategy for maize farming by subsistence/semicommercial farmers. The research indicated that problems of technology adoption can be overcome by guiding farmers/farmer groups on the uses of new technologies over 1-2 planting seasons and building institutional support structures such as technology clinics at the village level. Extension workers and farmer groups (GAPOKTAN) can manage these technology clinics. The clinic's functions would be to disseminate information on new technologies and undertake the responsibility of supplying inputs to farmers/farmer groups. However, to ensure continuity, farmers need loan assistance. Farmers/farmer groups borrow inputs from the clinics and repay the loan in kind in the form of maize grain after harvest. The clinic also processes the grain and sells it in the early rainy season. It builds a maize seed industry in the village level and buys inputs (fertilizer and pesticides) from shops at the district/province level. This strategy could guarantee sustainability of maize farming systems and has the potential to develop maize farming in other village.

Key words: Maize, adopt, subsistence/semicommercial farmers, technology clinic

Introduction

Seventy per cent of the people of East Nusa Tenggara (ENT) in Indonesia live in rural areas and maize is their staple food (BPS NTT 2004). Maize productivity has been poor (1.5-2.0 t ha^{-1}) and subsistence farm households suffer from food shortages. However, by improving technology adoption, productivity can be increased to 2.3-4.8 t ha^{-1} (Bobihoe *et al.* 1999), or even 3.4-6.7 t ha^{-1} (Subandi 1999).

External interventions to increase productivity have been attempted, but technology adoption by farmers has been very slow. That is mainly because maize cultivation in this province is oriented toward food security and farmers tend to be satisfied with their maize productivity (Yusuf *et al.* 2001). The Indonesian government has taken up many projects such as PELITA III (Indonesian development plan phase III) but social change (farmers' attitude and adoption of new technologies) has been limited.

Extension of new technology to farmers has been attempted but adoption by farmers has been slow (Lidjang 1995). These external interventions relating to seed, fertilizer and pesticide technologies did increase farmers' agricultural productivity but there have often been relapses after the initial stages and farmers continued to produce maize for self-consumption purposes.

Farmers in dryland areas of ENT province are of the subsistence and semicommercial type (Subandi *et al.* 1997). They find it difficult to relate to maize farming as an agribusiness because of their orientation toward food security (Sumarno and Bamualim 1999). So technology information dissemination and innovation adoption efforts have to be adapted to these conditions. It needs an institution building dimension to achieve sustainable technology introduction.

Maize farmers in this province use very simple technologies. Use of production inputs such as fertilizer and pesticide is very limited. Land fertility has been gradually decreasing because of nutrient depletion (Murdolelono and Beding 2006).

Generally, poverty of subsistence/semicommercial farmers is an outcome of poor natural resources, uncertainty of climate, low levels of cultivation technology and limited capital. Dissemination of information regarding cultivation technology and capital access may address the problems of maize cultivation in this province. This could be achieved by strengthening farmer groups.

The purpose of our research was to find a maize agribusiness model for subsistence/semicommercial farmers in ENT.

Materials and Method

Field design and measurements

The study was conducted with the involvement of three farmer groups in Tobu village in Mollo Utara subdistrict of South East Timor Tengah district during 2007-2008. The innovations sought to be promoted were new maize cultivation techniques and building of farmers' institutions. The improved maize cultivation techniques included new varieties and improved plant spacing, fertilization and seed production techniques. The institutional initiatives included strengthening of farmers' group capital, management of input supplies and seed production (Table 1).

The technology clinics were managed by extension workers and farmer groups (GAPOKTAN). The clinics' functions were to disseminate information on technology and inputs to farmers. They The clinics bought the inputs (fertilizer and pesticide) from shops at the subdistrict or district level and supplied them to farmers for which the latter paid in grain after harvest. Seed was sourced from seed production unit operated by farmer groups.

Table 1. Improved cultivation techniques and farmers' institutional initiatives.

Innovation	Existing technology	Technology improvement
A. Maize cultivation		
· Varieties	Local OPVs	White Srikandi OPVs
· Plant spacing	Not uniform; 80-125 × 80-125 cm; 4-5 seeds per hole	100 × 40 cm; 2-3 seeds per hole
· Fertilizer	Not used	N 90 kg ha^{-1} + K_2O_5 36 kg ha^{-1}
· Seed production	Selection from farmers' own maize production	Maize seed production procedure
B. Improving farmers' institutions		
· Strengthening farmers' group capital	None	Farmers get out maize product equal than inputs price
· Management of input supply	None	Supply of inputs by technology clinics and loaned to farmers againt postharvest repayment in grain
· Management of seed production	None	Farmers produce quality seed

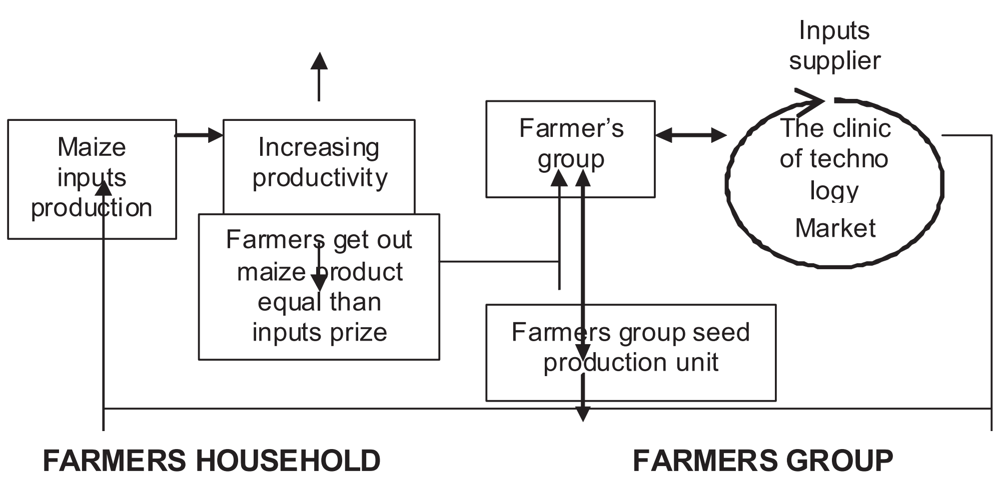

Figure 1. Strategy for improving farmers' institutions.

Table 2. Data collection and data analysis method.

Innovation	Data collection	Analysis technique
Maize cultivation		
Maize production	In the same year: 9 samples of existing maize cultivation and 9 samples of improved maize cultivation	T-test
	Previous year: measurement of maize production storage before introduction of innovation (exiting technology) and after introduction on the same land	Wilcoxon signed test
Farmers' response	Farmers' response to technology introduction	Descriptive
Institutional innovation		
Strengthening of farmers' group capital	Farmers' response to revolving system	Descriptive
Management of input supply	Farmers' response to input supply system	Descriptive
Management of seed production	Farmers' response to maize seed supply system	Descriptive Observation

Data analysis

Production data were collected by measurement and other data were collected by observation and Rapid Rural Appraisal (Table 2)

Results and Discussion

Maize cultivation

The results showed that plant height and tree diameter of local OPVs and Srikandi OPVs were not significantly different under the t-test of equality of means at 5%, but were significant for amount of plant and productivity. Amount of plant under existing technology was 13.25 holes per $10m^2$ and under introduced technology 17.56 holes per $10m^2$. Introduced technology increased maize productivity from 1.753 kg ha^{-1} to 3.438 kg ha^{-1} (Table 3).

The duration of Srikandi OPVs was 105 days and that of local varieties 120 days. The shorter duration offers an advantage to Timorese farmers because the rainfall period in this area is only 3-4 months, so farmers can plant other commodities after maize is harvested. Another advantage of Srikandi OPVs is their high content of lysine (0.410%) and tryptophan (0.087%) (Azrai 2004). The problem of poor nutrient levels in this area needs to be tackled.

Technology intervention increased farmers' maize storage by 173.2% per household. Average maize storage before intervention (previous year) was 80.2 kg per household and after intervention 219.1 kg per household (Table 4). This was net production after farmers repaid the cost of inputs to the technology clinic. The good results were a surprise to farmers and increased their motivation to adopt improved maize cultivation.

Table 3. Maize plant height, diameter, amount of plant per $10m^2$ and productivity under existing and introduced technologies.

Variable	Existing	Introduction	Notation
Plant height (cm)	149.62	149.78	ns[1]
Tree diameter (cm)	2.42	2.41	ns
Amount of plant (holes per $10m^2$)	13.25	17.56	**
Productivity (kg ha^{-1})	1.753	3.438	**

[1.] ns: not significant under t-test for equality of means at 5%.
** very significant under t-test for equality of means at 1%.

Table 4. Farmers' maize production before and after intervention.

Item	Number of sample	Farmers' production (kg per household**)
Before intervention	10	80.2
After intervention	10	219.1

** Very significant under 2-tailed Wilcoxon signed ranks test.

Farmer human resources in this area were very low. Their number of years of education ranged from 0 to 12 years and averaged 4.2 years. It influences acceptance of technology information. So to increase maize cultivation technology adoption, farmers need guidance over 2-3 times planting seasons.

Seed production

Maize seed production for this study was conducted at the farmer group level on 0.15 ha with white Srikandi OPV seed foundation. Seed production seed was 315 kg (productivity 2.100 kg seed ha^{-1}). These seed were sold at affordable rates to farmers in the farmer group and others.

Before this seed production initiative, farmers used to rely on their own seed of local OPVs. Generally, they selected the bigger grain for use as seed. Since the local OPVs had low production potential, introduction of new OPVs of maize was necessary.

To ensure that new varieties are adopted, it is necessary that the seed industry is located in proximity of farmers. Unfortunately there was no such seed industry in the study area. Maize seed had to be brought in from the provincial capital, which was about 150 km away. This only adds to the farmers' cost. Seed production at the farmers' level is a solution to this problem, but proper seed production practices ought to be followed.

The main problem affecting seed production in this area is availability of water for irrigation. Seed production must be done during the dry season when water availability is poor. So seed production must be done near rivers by the watering technique, which requires much labor.

Institutional innovation

Generally, subsistence/semicommercial farmers live far from the city under conditions of poor road infrastructure. This restricts farmers' mobility and access to communication. In such a situation, government and church organizations both can help disseminate agricultural information in this area. For instance, agricultural technology information can be disseminated at the traditional weekly market on Saturday where a large number of farmers gather. It is also imperative that the agricultural clinics be located near these traditional markets.

Generally, poverty of subsistence/semicommercial farmers is caused by (a) low level of natural resources and poor agricultural cultivation technologies resulting in low productivity; (b) climatic uncertainty, mainly during the rainy season; (c) limited capital of farmers and limited access to credit; and (d) poor knowledge of improved agricultural technologies and nonavailability of information. The lack of capital leaves farmers in a poor bargaining position forcing them to sell their commodities before maturity.

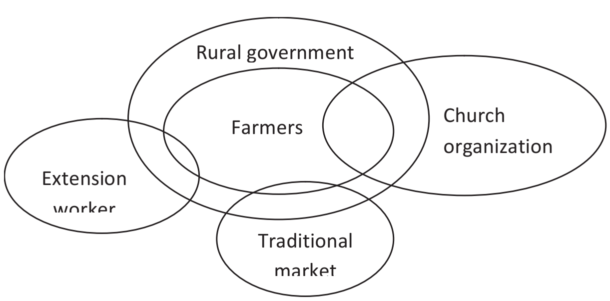

Figure 2. Venn diagram showing the farmers' relationship with different institutionals in the rural areas.

Strengthening farmer groups of subsistence/ semicommercial farmers is difficult. They break easily and are difficult to develop. Generally, farmer groups are built by extension workers for the purpose of a new government project and not upon the farmers' own initiative. To impart independence to the farmer group, we need to strengthen the group capital by building a seed production unit with the help of the farmers' own labor.

Farmer's maize production can be increased by adopting new technologies. However, they need capital to purchase the needed improved inputs (new varieties, fertilizer, pesticides and others). Loan assistance under a semigrand system was offered to farmers who participated in this research. This concept was chosen because stagnation of credit is very common in the grand system of loan assistance. Generally, if farmers default on instalment payments, the government finds it difficult to collect the debt. In the semigrand system, loan assistance is given to a farmer group. It is used to buy production inputs. The risk of stagnation of credit in this system is very limited because farmers tend to be deterred from defaults by the possibility of social sanctions.

Strengthening of farmer's group capital can be done in two ways. The first strategy is to increase maize production for consumption and the second is to increase maize production for a combination of consumption and home industry.

In the first strategy, suppliers give inputs such as seed, fertilizer and pesticides on a semigrand assistance basis to the farmer group. Members of the group access these inputs on the condition of repaying them in the form of grain after harvest. The farmer group sells the grain and with the proceeds buys inputs for next season. This model guarantees supply of inputs.

In the second strategy, the service provider supplies production inputs to the farmer group on a semigrand assistance basis. In addition, he gives assistance to the group to process the maize production. This has the opportunity of engaging the services of the farmer group members in the processing in return for payment for their labor.

Management of input supplies

The technology clinic was built at the village level because there were no input supply shops in the village. The nearest shop was located in the district capital about 50 km away and another was located in the provincial capital about 200 km away. This added to the high cost of cultivation for subsistence farmers.

The combination of high cost of inputs, limited agricultural knowledge and low capital is the reason why adoption of agricultural technology by these farmers tends to be very slow. The clinic was built to solve this problem.

The sale agricultural inputs at the technology clinic was not a profit-oriented activity, but to invite farmers to visit the clinic, interact with extension workers and read any agricultural information available at the clinic. It was a policy of the clinic that the farmers must come of their own volition to the clinic. Extension workers and clinic workers only supplied the information sought by the farmers.

The clinic management was drawn from the deputy heads of the farmer groups. The management had three divisions. The first division administered the supply of inputs, mediating between the farmers and the suppliers. The second looked after marketing by connecting farmers and commodity buyers. The third division looked after rural development planning.

Conclusions

Extension workers and farmer groups (GAPOKTAN) manage the technology clinics at the village level. Farmers/ farmer groups borrow inputs from the clinic and repay it in grain after harvest. Maize grain is processed at the clinic and sold during the early rainy season. The clinic built a maize seed industry unit at the village level and bought inputs (fertilizer and pesticides) from agricultural shops in the district/provincial capital. This strategy can guarantee sustainability of maize farming systems and has the potential for development in other villages.

Acknowledgement

The authors express their sincere appreciation for all the support provided to them.

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Maize Technology Transfer to the Poor: Lessons Learned from Community-Based Approaches of Technology Transfer to the Hill and Mountain Farmers of Nepal

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Abstract. Maize (Zea mays L.) contributes about 25% of the total food requirement of Nepal. It covers 80% (mountain region 10% and hill region 70%) of the total cultivated land in Nepal and plays a vital role in the livelihoods of hill and mountain dwellers. Nepal has a long history of maize research. Nineteen maize varieties including one hybrid have been released for cultivation so far. But production improvement is still insufficient. Technology transfer is undertaken through different community-based approaches – mainly District Seed Sufficiency Program (DISSPRO), Community-Based Seed Production (CBSP) and participatory varietal selection (PVS). Community mobilization by forming groups and cooperatives and technology transfer through demonstrations (production and results), training programs, mini-kits and visits are keys to increasing technology adoption by farmers and increasing food production. The District Seed Sufficiency Program (DISSPRO) was started in 1998 with the basic principle of encouraging and strengthening local seed production and marketing to meet local and district level seed demand. A few years later the Crop Development Directorate (CDD) through the Hill Maize Research Program (HMRP) joined hands with CIMMYT and several nongovernmental agencies to execute CBSP and PVS in maize particularly in the hill and mountain districts. Theoretically, DISSPRO, CBSP and PVS are similar at least from the community participation perspective; there are, however, important improvements in CBSP and PVS over DISSPRO. Both are more holistic, inclusive and localized. We argue that integration of the community approach of DISSPRO, the technology transfer approach of PVS and the inclusive approach of CBSP will be effective in achieving technology transfer to the poor in the future.

Key words: Maize, seed, technology, DISSPRO, PVS, CBSP

Objective of This Paper

The objective of this paper is to share the experiences of the Government of Nepal's Department of Agriculture/ Crop Development Directorate (DOA/CDD) in maize technology transfer to poor farmers and highlight the possible ways of improving the technology transfer approaches involving multiple actors: public (research and extension), civil society and private and farmer groups/ cooperatives.

Maize in Nepal

Maize is the major traditional cereal crop in the rainfed ecosystem of the hills and mountains of Nepal which cover 80% (mountain regions 10% and hill regions 70%) of the total cultivated land (MOAC 2007) in the country. It contributes about 25% of the total food requirement and plays a vital role in the livelihoods of the people living in these regions. It is cultivated for food, feed and fodder purposes on sloping *bari* land (rainfed uplands). It is grown under rainfed conditions during the summer (April-August) as a single crop or relayed with millet later in the season. In the *terai*, inner *terai,* valleys and low-lying river basin areas, maize is also grown in the winter and spring with irrigation. The *terai*/foothills (lowlands), midhills and high hill maize growing environments are depicted in the map in Figure 1. Although the map accurately represents the elevation bands, it does not indicate whether the area is cropped or if maize is grown there. More than two-thirds of the maize produced in the midhills and high hills is used for direct human consumption at the farm level, and the ratio of human consumption to total production is higher in the less accessible areas. In the *terai*, less than 50% of the maize is used for human consumption and a significant proportion goes to the market (Paudyal *et al.* 2001). Maize contributes about 6.88% of the total agricultural GDP of the nation (MOAC 2008). In 2006/2007, maize was grown on about 870 401 ha which represents 26.3% of the total area planted to cereals in Nepal. In the same period, 1 819 925 t of maize was produced, representing about 25% of Nepal's total cereal production. Out of the total cereal production in the mountains and hills, maize contributes only about 43.5%. In recent years, maize has come up as a new and promising cash crop in the *terai* region due to the growing demand from the feed industry. Maize demand has been growing by 5% annually throughout the last decade.

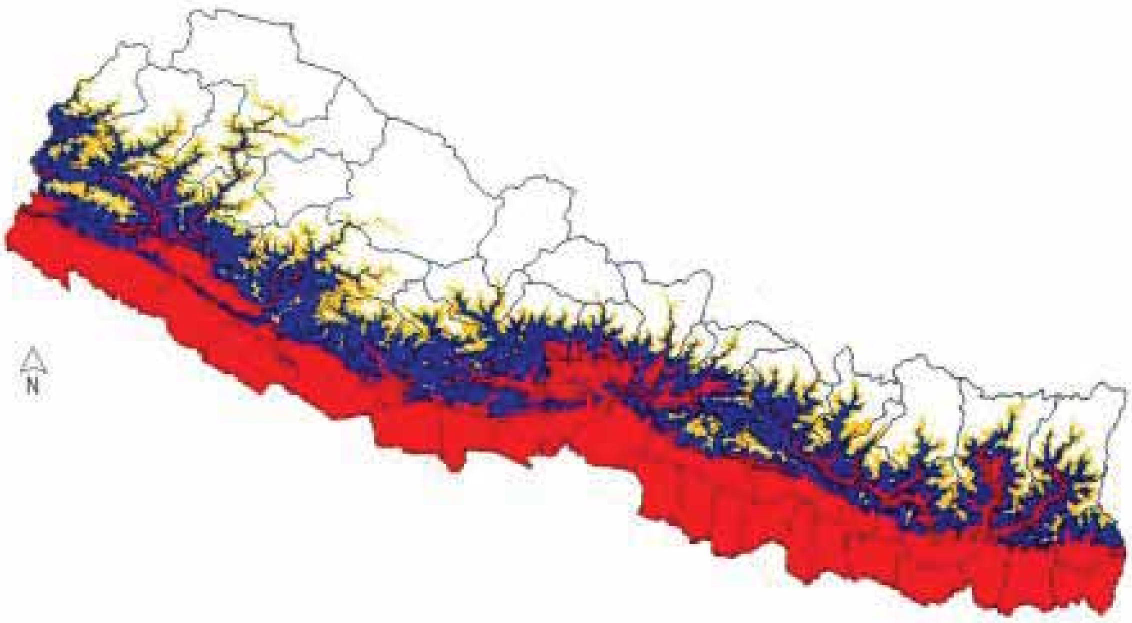
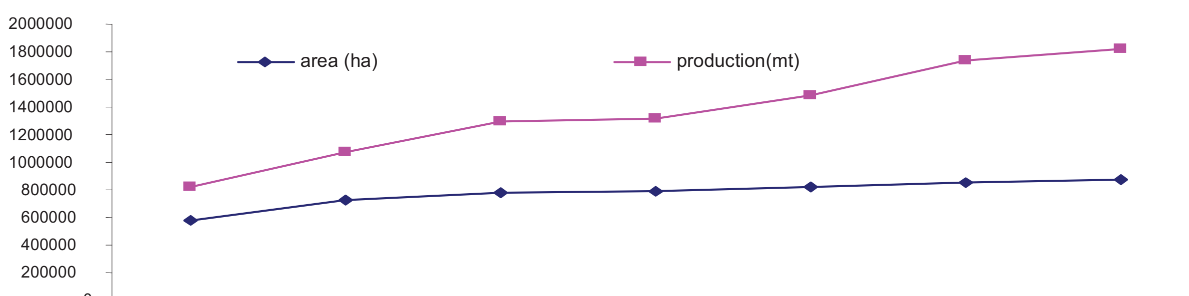


Figure 1. Distribution of lowland (red), mid-hill (blue) and high hill (yellow) maize growing environments in Nepal. Source: Paudyal *et al.* (2001).



	1984/85	1988/89	1992/93	1996/97	2000/01	2005/06	2006/07
area (ha)	578720	721870	775220	793720	824525	850947	870401
production(mt)	819850	1071610	1290500	1316840	1484112	1734417	1819925

Figure 2. Maize production trends in Nepal. Source: (MOAC 2007).

Area, Production and Productivity Trends

Although data (Fig. 2) show that there has been a constant increase in area, production and productivity of maize in Nepal, there has been very little yield improvement compared to nationwide yields some 20 years ago. This is probably due to the expansion of maize cultivation into less suitable terrain, declining soil fertility and sluggish adoption of improved management practices. Compared to 1984/85, the percentage increase in area, production and productivity of maize in 2006 was 50.4%, 121.9% and 47.5%, respectively in 2006 (MOAC 2007). Though most of the maize area (80%) lies in the hills and mountains, productivity there (hills 1.99 t ha^{-1} and mountains 1.73 t ha^{-1}) is rather less than the national average (2.09 t ha^{-1}) (Table 1 and 2).

Table 1. Area, production and productivity in the hill regions of Nepal, 2006/07.

Region	Area (ha)	Production (t)	Productivity (t ha^{-1})
Eastern Hills	141 779	271 603	1.916
Central Hills	138 098	292 542	2.118
Western Hills	208 695	473 301	2.268
Midwestern Hills	104 174	198 662	1.907
Far-western Hills	21 028	37 003	1.760
Total	613 774	1 273 111	1.993

Source: MOAC 2007.

Table 2. Area, production and productivity in the mountain regions of Nepal, 2006/07.

Region	Area (ha)	Production (t)	Productivity (t ha^{-1})
Eastern Mountains	39 065	69 448	1.778
Central Mountains	28 740	62 397	2.171
Western Mountains	691	1145	1.657
Midwestern Mountains	9252	13 690	1.480
Far-western Mountains	10 540	16 929	1.606
Total	88 288	163 609	1.738

Source: MOAC 2007.

Maize Research and Extension

Varietal research and extension activities are vital for technology generation and transfer. Sustained efforts have been made in Nepal to develop high-yielding varieties. Altogether 19 maize varieties including one hybrid have been released since 1960 (Bhatta and Bajracharya 2008). Efforts are underway to develop hybrids and quality protein maize (QPM) varieties to further improve productivity and nutrition. In extension activities taken up by the Government of Nepal (GON), technology transfer is underway through different approaches like DISSPRO, CBSP and PVS. Despite four decades of endeavor toward maize production improvement, progress has been insignificant. Although 19 maize varieties have been released so far, the annual seed replacement rate (SRR) is only 5.8% and average productivity 2.09 t ha^{-1}. Maize seed supply from the public sector is less than 1% and adoption of improved maize varieties is low. Our past experience shows that the policies, programs and approaches of followed in these research and development efforts were erroneous.

What Are Our Existing Approaches?

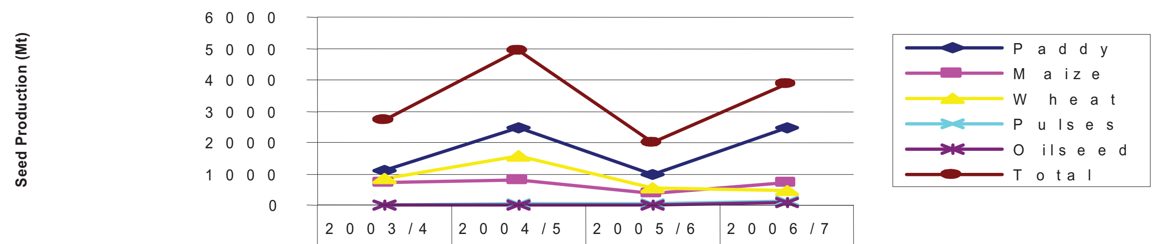
Technology transfer has been undertaken through different community-based approaches, mainly DISSPRO, CBSP and PVS. Community mobilization through formation of groups and cooperatives and technology transfer through demonstrations (production and results), training, mini-kits and visits are keys to increasing technology adoption by farmers and increasing food production. DISSPRO is a major public sector seed production program at the community/farmer level with the policy strategy of forming and strengthening of farmers groups to produce quality seed of the desired verities and in the required quantity. It has the basic principle of encouraging and strengthening local seed production and marketing to meet local and district level seed demand. This is becoming a very effective approach. DISSPRO was started in 1998. A few years later CDD through the HMRP joined hands with CIMMYT and several nongovernmental agencies to execute community-based seed production (CBSP) and participatory varietal selection (PVS) in maize, particularly in the hill and mountain districts.

District Seed Sufficiency Program (DISSPRO)

DISSPRO was envisaged in the Ninth Five Year Plan (1998/99 to 2001/03). The basic underlying principle of DISSPRO is to encourage and strengthen local seed production and marketing to meet local and district level seed demand and develop small-scale seed entrepreneurship and facilitate development of a larger seed market in the future; create awareness about quality seed among farmers; disseminate and multiply newly released varieties. Training, 25% subsidy on source seed, provision of a seed fund and a seed revolving fund of Nepali rupees 5000 and 60000 respectively for each group and provision of 200.00 Nepali rupees per ha for insect pest control are the major support programs. Formation and strengthening of farmer groups to produce quality seed of the desired varieties in the required quantity is a major policy strategy of DISSPRO. To assure supply of source seed as per demand, seed balance seed is prepared each year.

Implementation status of DISSPRO

Since the initiation of DISSPRO many districts have adopted the program by utilizing available funds and also by getting minor support (technical and financial) from the Crop Development Directorate. Provision of seed fund of Nepali rupees 5000 for subsistence groups and Nepali rupees 60000 for commercially oriented groups were major attractions of this program. In 2007, a total of 63 districts



	2003/4	2004/5	2005/6	2006/7
Paddy	1126.6	2453.3	967.95	2483.1
Maize	731.25	823.71	402.45	740.5
Wheat	840.31	1592.5	547.15	452.2
Pulses	5.2	39.3	52.25	138.21
Oilseed	1.1	16.2	19.3	67
Total	2704	4925	1989	3881

Years

Figure 3. Seed production trends under DISSPRO, 2003-2007. Source: CDD 2007; 2008).

(out of 75) implemented the DISSPRO program. In the same year, 3881 t of quality seeds of cereals, pulses and oilseed crops were produced under the program (Fig. 3).

Lessons learned from DISSPRO

- Supply-driven (eg, identification and seed multiplication of farmer-preferred varieties)
- There is a scarcity of source seed of major cereals, especially rice, oilseed and pulses.
- The existing seed production technology dissemination system is not adequate.
- The seed quality control mechanism should be made easy and effective. The present mechanism is not sufficient and not effective.
- Participation of stakeholders is encouraging in the districts and should be increased.
- Community-based infrastructure development should be supported through government as well as private sectors/NGO efforts.
- The seed marketing and market information system is poor and needs strengthening.
- Farmer groups and farmer cooperatives must be strengthened with different support programs and capacity building activities through training in seed business skill development, entrepreneurship development and managerial skill development.
- Agro-vets and seed traders should be involved for sustainable seed marketing channel development.
- Rural youth, especially educated and unemployed youth, should be trained and involved in seed production and marketing activities to run it in a sustainable manner.

Participatory varietal selection and community-based seed production in maize

In 1999, HMRP/CIMMYT for the first time worked with the DOA/CDD to improve food security through increased maize production in the four hill and mountain districts of Nepal. In the second phase of this initiative, the number of districts was increased to 11. After successful completion of the first and second phases, the third phase of this project (2008-2010) has been implementing technology transfer to poor and socioeconomically disadvantaged communities in the hills and mountains (NMRP 2008). Participatory Technology Development (PTD) using the mother/baby trial concept and the community-based seed production program are the major technical interventions. In 2007, 105 t of maize seed was produced in the 11 districts. For the year 2008, this is expected to be at least 210 t from 25 districts.

Lesson learned from CBSP

- A cheap and successful intervention to increase food production and make it sustainable.

- Identification of location-specific maize varieties through PVS and production and supply of quality seed of farmer-preferred varieties through CBSP are promising approaches.
- Participation of multiple stakeholders in maize technology research and extension activities create an enabling environment to realize the interests of each actor in a coordinated way.
- PVS/CBSP have increased access of the poor, women and disadvantaged farmers to available technology and inputs.
- However, focus on marketing and development of community structures for seed storage and marketing is insufficient.
- Involvement of enterprising community groups in seed production, processing, storage and marketing is still weak in a majority of cases.
- Increase in maize yield by 17% was realized in the DOA/CDD project areas mainly due to use of quality seed of the improved varieties and minor changes in technology.
- Through PVS (mother-baby trial approach) farmer-preferred varieties were selected and included in the CBSP which facilitated the availability of quality seed to farmers. Rampur Composite, Manakamana 1, Manakamana 3, Deuti, Shitala varieties were released under the national variety registration and release system.

Conclusion

The DISSPRO is designed to transfer seed production technologies and produce and market quality seed at the community level. The main benefit of this approach is community participation and empowerment. It is cost-efficient. PVS on the other hand has proved to be an important approach and tool to generate and promote locally suitable technologies where farmers are involved in the whole process. CBSP has gained strength since CBSP groups are very inclusive and the support package is more complete and holistic. Considering these strengths of various approaches, it is recommended to integrate community participation, participatory technology development and community empowerment to transfer technology to the poor in future. DOA is giving priority to promote approaches of PVS and CBSP in the national extension system.

Acknowledgement

The authors are thankful to Dr. G. Ortiz Ferrara, team leader HMRP/CIMMYT; Dr. T.P. Tiwari, Agronomist, CIMMYT and are also grateful to the Director General, Department of Agriculture and the Swiss Agency for Development and Cooperation/Nepal. Similarly, we also acknowledge the contribution of Dr D.B. Gurung, Dr. D.P. Serchan and B.B. Hamal (DADO Syangja).

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Development of Parboiled Polished Corn [*Jagung Sosoh Pratanak JSP*)] as Practical Raw Material for Many Local Corn-Based Foods

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Abstract. There are many indigenous corn-based foods consumed as a carbohydrate source such as bassang in South Sulawesi and binte biluhuta in Gorontalo and kambewe in South East Sulawesi. However, the duration of processing and the method of serving are less practical. Therefore, the foods have disappeared from the common menu. Research has been carried out to solve this problem and the result was Jagung Sosoh Pratanak (JSP) or parboiled polished corn which is a practical raw material for making many corn-based foods such as bassang. JSP is produced through a series of procedures such as polishing, soaking, cooking and drying. Usually the process for making bassang takes a long time, ie, 18-24 hours, but by using JSP, the time could be reduced to 15-30 minutes. Research results relating to JSP production showed that the yield was 64.98%, rehydration time 15 minutes, water content 11.46%, fat 0.36%, protein 8.59%, carbohydrate 79.63%, ash 0.24% and crude fiber 1.1%. JSP packed in cartons, polypropylene and polyethylene could be stored for 498 days, 771 days and 899 days, or 16.6 months, 25.7 months and 30 months, respectively. Besides bassang, JSP can be used as a raw material for corn soup, corn rice and corn porridge with various serving options. By producing JSP, people's interest in corn-based foods can be revived, which would meet the objectives of food diversification programs.

Key words: Parboiled polished corn, carbohydrate source, bassang, diversification program

Introduction

Corn is a major source of carbohydrate food apart from rice. It is relatively cheap. Many kinds of corn-based traditional foods are widely known in Indonesia, such as *bassang* in South Sulawesi, *bintebiluhuta* in Gorontalo, *kambewe* in South East Sulawesi and grontol in Jawa. However, some of these traditional foods are losing place in people's menus because their preparation is perceived as impractical and their taste and appearance as nonappealing. These foods are considered as food for marginalized communities.

Bassang, which for generations was a widely consumed food in South Sulawesi, is made of corn combined with coconut milk and salt or sugar. It is served for breakfast or as a between-meal-time food by people in South Sulawesi. Its preparation takes 18-24 hours, including 8-20 hours for soaking and 2-3 hours for cooking. Survey results have shown that a lot of people in South Sulawesi still prefer bassang. It is commonly consumed in traditional markets (50%), at people's residences (20%), houses (20%) and schools (10%). *Bassang* is consumed by merchants (20%), *becak* drivers (16.7%), office staff (16.7%), housewives (16.7%), drivers (10%), laborers (6.7%) (Tawali *et al*. 2003). These results indicated that consumption is limited and confined mainly to marginalized communities. Against this background, we investigated how to make *bassang* more attractive to consume and convenient to prepare in order to revive people's interest in corn-based foods.

Materials and Methods

Raw materials and chemicals

Corn for this study was bought from the Takalar regency. All the chemicals used in this research were bought from chemical distributors in Makassar.

Method

This research was carried out in three steps. The first was production of Jagung Sosoh Pratanak (JSP); the second step involved determination of the physical and chemical profile of JSP and its yield; and the last step was prediction of the shelf life of JSP.

Measurement parameters

The parameters measured in this research were the JSP yield, water content, fat, protein, carbohydrate, ash and crude fiber (AOAC 1980) and shelf life prediction using the sorption isotherm method (Labuza 1982)

Data analysis

Research data were processed by descriptive and quantitative methods.

Results and Discussion

Production of parboiled polished corn (JSP)

Use of whole maize corn as a raw material of food is not widely known because of its use specifically as a traditional food in some regions. Processing of parboiled polished corn for this research generally consisted of the following steps: polishing, soaking, cooking (parboiling) and drying. Polishing was carried out to lose the bran and pericarp of corn using a polishing machine. Cleaned corn is dripped and then watered well to ensure the pericarp comes off. Research showed that in order to have polished corn with a high percentage of whole corn, the corn should be processed three times with the polish machine. Besides, maize corn was watered at a ratio of 1:1. This leaves the outside of corn wet and the inside in a hard and dry condition.

Soaking was done to let the product adsorb water rapidly and uniformly (Wimberly 1983). In order to make the corn tissue open and apart, amylase enzyme and $CaCl_2$ were added to the soaking water. As a consequence, some of the starch chains are broken and rendered loose so that the starch can swell optimally. The final result showed that there was a significant difference between rehydration time and soaking with and without the enzyme. Soaking was done for 24 hours. Cooking was aimed at gelatinizing the starch (Miah *et al*. 2002). Cooking of corn previously soaked in the enzyme solution was done for 2 hours. During cooking, starch gelatinization occurs, making the corn texture soft.

The soft corn was then dried until the water content is down to less than 13%. To reach this level of water content, drying is done for 7-8 hours at 50-70^{o}C using an electric drying machine. Figure 1 illustrates the method used to produce JSP. By using JSP, *bassang* can be produced in just 15-30 minutes depending on the consumer's preference regarding corn texture.

Product and nutrition profile of JSP

The rehydration time of JSP was 15 minutes. It is defined as the time needed by JSP to absorb water and become soft when recooked. The time was calculated since water was boiled until *bassang* became soft and ready to eat. Soaking in the enzyme solution is aimed at breaking the starch molecules and loosen the carbohydrate chain, thereby opening the pores. As a consequence, water rehydration was accelerated after the corn is dried.

During the processing of JSP, there was a loss of the corn matrix particularly during polishing and parboiling. The JSP yield based on the standard operational procedure was 64.98%. Yield from the polishing process was 75.86% and from the parboiling process 85.66%.

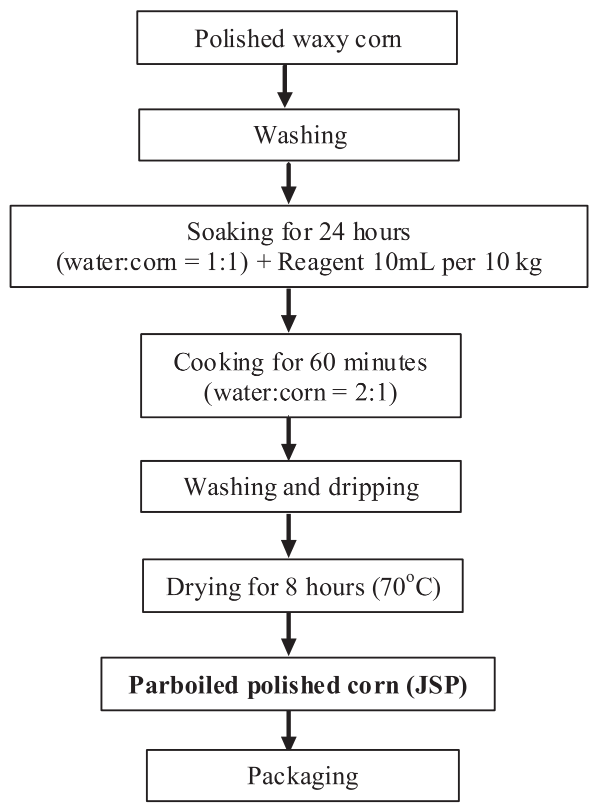

Figure 1. Processing of parboiled polished corn.

Table 1. Nutrition profile of JSP.

Component (%)	Content
Water content	11.46 ± 0.4
Fat	0.36 ± 0.01
Protein	8.59 ± 0.01
Carbohydrate	79.63 ± 0.33
Ash	0.24 ± 0.06
Crude fiber	1.10 ± 0.10

Table 2. Permeability, thickness and water vapor transmission rate (WVTR) of packaging material and the shelf life of JSP.

Material	Thickness (mm)	WVTR ($g/m^2/hr$)	Permeability ($g/mm.m^2/mmHg/hr$)	Shelf life of JSP (months)
Carton	410.00	125.088	0.305	16.6
Plastic PE	30.70	5.195	0.169	30
Plastic PP	37.00	7.303	0.197	25.7

The steps used to process from dry corn into JSP caused physical change as a consequence of polishing, cooking and drying. The complete analytical results of the JSP component are displayed in Table 1. The fat and ash content of JSP were lower because the germ (where the fat is concentrated) and pericarp are removed.

Prediction of the shelf life

Prediction of the shelf life of JSP was carried for packaging in cartons, polyethylene (PE) and polypropylene (PP). The shelf life of JSP is determined by the sorption isotherm method for each packaging material. The permeability, thickness and water vapor transmission rate (WVTR) of the packaging material are displayed in Table 2. The results showed that JSP can be stored for 16.6 months in carton packaging, 25.7 months in PP plastic and 30 months in PE plastic. The best packaging material to pack JSP in is PE plastic with a permeability value 0.169 $g/mm.m^2/mmHg/hr$. PE plastic had the lowest permeability compared to other materials. Syarief *et al*. (1989) reported that PE plastic protects the product from moisture.

Conclusions

1. The yield of JSP from standard operational procedure was 64.98%.
2. The rehydration time of JSP was 15 min with the following nutrition profile: water content 11.46%, fat 0.36%, protein 8.59%, carbohydrate 79.63%, ash 0.24% and crude fiber 1.1%.
3. The shelf life of JSP packaged in carton material, PP plastic and PE plastic was 16.6, 25.7 and 30 months, respectively.

Acknowledgement

We are thankful for the funding provided by the Indonesian Ministry of Research and Technology through Riset Unggulan Strategis Nasional-Diversifikasi Pangan Pokok (RUSNAS-DPP; National Strategic Research-Staple Food Diversification) for this research.

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Increasing Farm Income through Maize-Based Strip Intercropping Practices during the Winter Season in Nepal

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Abstract. A study was undertaken during two consecutive winter seasons (2006 and 2007) at the research farm of the National Maize Research Program (NMRP) in Rampur, Chitwan (228 masl), Nepal to assess the effect of maize varieties, row distances for strips on different intercrops and maize yield. Promising results were tested in farmers' fields in the second year of the study. Among the different intercrops evaluated, maize + potato was found to be the most productive with the highest maize grain equivalent yield (MGEY) of 9836 kg ha^{-1} followed by maize + kidney bean (7423 kg ha^{-1}). However, maize + kidney bean in lieu of sole maize gave a higher marginal rate of return (MRR) of 1.32 compared to maize + potato (1.02) when partial budgeting was performed. The Land Equivalent Ratio (LER) obtained with maize + kidney bean (1.37) was also significantly higher compared to maize + potato (1.16). Rampur Composite gave significantly higher MGEY (7937 kg ha^{-1}) compared to early-maturing Arun-2 (7328 kg ha^{-1}). The MGEY obtained with strip intercropping with 1.8 m space was significantly higher (8190 kg ha^{-1}) even with lower maize yield (3703 kg ha^{-1}) than strip intercropping with 1.5 m space (7076 kg ha^{-1}). This maize + kidney bean practice was also found beneficial in farmers' fields (B:C ratio 1.6, MRR 1.48) compared to maize + potato (B:C 1.05, MRR 0.79) and maize + lentil (B:C 1.38, MRR 0.75). The two years' experiment results indicated that growing maize + kidney bean with strip intercropping was found more productive and economic in comparison to sole maize as well as other tested intercrops in Chitwan conditions.

Key words: Strip intercropping, maize, kidney bean, LER, equivalent yield

Introduction

Maize (*Zea mays* L.) is the second most important cereal grain in Nepal and a priority staple food crop in the hill regions of the country. It is commercially grown in the *terai* (plains) area. Maize is also a major constituent of poultry feed for which demand is increasing. Similarly potato (*Solanum tuberosum* L.) is one of the main vegetable crops grown in the winter in the *terai* and mid-hill areas and in the summer in the high hill regions as well. Kidney bean/common bean (*Phaseolus vulgaris* L.) is an important legume crop grown for green vegetable and dry seed purposes in a wide range of agroclimatic conditions ranging from the *terai* (300 m amsl) to the high hills (2500 m amsl). It is a summer rainfed crop often grown in association with maize in the hills although sole cropping has been practised in recent years in the *terai*. Lentil (*Lens culinaris* M.) is equally an important winter legume crop grown as a sole or mixed intercrop with mustard in the *terai* and inner *terai* of Nepal.

Intercropping is a way to increase the diversity of farming systems. It not only produces more than two kinds of commodities at a time in the same field but also increases the amount of humus in the soil due to the decaying of crop remains. It averts the risk of crop failure and increases land-use efficiency. Maize is one of the best crops for intercropping due to its physiological nature. Crops like legumes, cereals, vegetables and spices are different intercropping options. Therefore, the potentiality of intercropping different crops with maize in the *terai* and inner *terai* has been studied with the objective of reducing poverty by improving farm production and farmers' income.

Maize being a major crop in the hilly areas, most studies on maize-based intercropping have concentrated on the hilly areas of Nepal. Mixed cropping is a traditional practice in Nepal, which may be one of the major countries where multiple cropping is practised mainly for subsistence (Bhattarai 1992). Wolfswinkel reported that each crop in an intercropping system should have adequate space to maximize cooperation and minimize competition between them. The benefits from intercropping are higher when two crops with different rooting systems, different patterns of water and nutrient demand and different above-ground habits are planted together. Water, nutrients and sunlight are thus used more efficiently, and strip intercropping may result in benefits greater than from a sole crop. Winter maize yields are better from intercropping than summer maize. Shiblee *et al.* (2000) found the highest marginal rate of return (MRR) of 598% from maize + chilli or potato or cucumber intercropping.

Materials and Method

On-station experiment

Our research included an experiment carried out during two consecutive winter seasons in 2006 and 2007 at NMRP Rampur, Chitwan (228 masl). Three different factors – two maize varieties, two row distances for the strip and three different intercrops – were evaluated in three replications. The net harvested plot was 24 m^2. The experiment was carried out in a sandy loam soil which was acidic (pH 5.5), low in organic matter (1.7%) and total nitrogen (0.10%), medium in available phosphorus (277 ka ha^{-1}) and potassium (134 ha^{-1}). Two maize varieties, Rampur Composite (full season) and Arun-2 (early-maturing) were planted on Sep 21 in 2006 and Oct 6 in 2007 and all the intercrops (potato, kidney bean and lentil) were planted 20-30 days after maize sowing. Maize was planted in paired rows (60 × 180 cm and 75 × 150 cm) resulting in 1.8 m and 1.5 m strips for intercrop planting. Intrarow spacing for maize was 40 cm with double plants per hill in the paired row (60 × 180 cm) design and a single plant and 25 cm in the other arrangement. Maize was fertilized with 120:60:40 kg ha^{-1} $N:P_2O_5:K_2O$ and common bean, potato and lentil were grown with 80:50:30, 140:100:60 and 18:46:30 kg ha^{-1} respectively. The crops were irrigated once during the maize silking stage. But two irrigations were given when no rainfall occurred in 2007. The potato crop was infected with late blight disease and four sprays of Mancozeb @ 2.5 g L^{-1} of water were applied at 10-day intervals in the later stages in both seasons. Maize (early), maize (full-season), potato, kidney bean and lentil were harvested 146, 158, 95, 130, and 142 days after planting, respectively.

On-farm experiment

Four treatments were tested in farmers' fields (considered as a replication) in both upland and lowland conditions during winter 2007. Three different intercrops (potato, kidney bean and lentil) and one sole maize crop were evaluated. The planting space available for intercrops was 1.8 m in strip and potato was planted with 60 × 25 spacing, kidney bean with 45 × 10 cm, and lentil in 25 cm × continuous. The net harvested size of each experimental unit was 48 m^2. The following activities were performed in different farmers' fields.

Activities	Farmers/sites				
	1	2	3	4	5
Planting date (maize)	Sep 16	Sep 20	Oct 26	Nov 5	Nov 8
Planting date (intercrop)	Oct 18	Oct 18	Nov 15	Dec 5	Nov 30
Irrigation	Dec 2	-	Dec 31	Dec 6	Dec 6
Previous crop	Maize	Sesame	Rice	Rice	Rice

Results and Discussion

Maize

The combined analysis of variation and yield data are presented in Table 1 and Table 2. The good performance recorded in 2006 (Table 2) might have been due to better rainfall and early planting (Sep 21) compared to 2007. Full-season maize variety Rampur Composite produced significantly higher maize grain yield (4369 kg ha^{-1}) compared to early-maturing Arun-2 (3321 kg ha^{-1}). Maize plant population was significantly higher (41 000 ha^{-1}) with 1.8 m strip space and planting in 60 × 40 cm (two plants per hill) compared to 1.5 m strip space and planting in 75 × 25 cm (35 500 ha^{-1}). However, strip space 1.5 m yielded significantly more maize grain yield (3987 kg ha^{-1}) compared to 1.8 m strip space which might be due to the planting of two plants per hill. Maize yield did not vary significantly due to different intercrops.

Intercrops

The results for intercrop yield were the reverse of results for maize yield which was due to the shading effect for a longer duration caused by the full-season variety which was lower (2438 kg ha^{-1}) compared to the early-maturing maize variety (2757 kg ha^{-1}). Strip space 1.8 m yielded significantly more intercrop yield (3138 kg ha^{-1}) compared to the 1.5 m strip space (2057 kg ha^{-1}). The highest maze grain yield treatment yielded a lower intercrop yield and vice versa for the lower maize grain yield treatment. This indicates strong competition between maize and the intercrops. Potato was yielded the highest (6316 kg ha^{-1}) followed by kidney bean (1001 kg ha^{-1}) and lentil (476 kg ha^{-1}). The performance of potato could have been much higher but for the late blight infection in both study years. This disease reduced yield and increased the cost of cultivation.

Table 1. Combined analysis of variance for maize variety, strip space and intercrops in intercropping trials during the winter seasons of 2006 and 2007.

Source of variation	Mean square			
	Maize grain yield	Intercrop yield	MGEY	LER
Year (Y)	889 284.9*	13 674 532.3**	46 134 131.5**	0.049
Maize variety (A)	19 804 413.5**	1 827 999.5*	6 670 874.5*	0.048
Strip space (B)	1 448 686.6**	21 069 137.0**	22 336 085.1**	0.200
Intercrops (C)	44 824.8	250 509 932.8**	106 444 679.9**	0.270*
AB	114 186.9	1 154 288.2	2 744 870.6	0.047
AC	5 270.7	1 093 732.5	1 750 091.9	0.106
BC	32 293.8	14 898 008.5**	15 908 157.6**	0.005
ABC	2 035.8	636 254.3	166 185.0	0.028
YABC	115 222.5	847 147.8	849 203.1	0.042
Error	154 446.7	378 280.6	984 572.9	0.071

* and ** indicate significance at p = 0.05 and 0.01 respectively.

Table 2. Mean yield (kg ha^{-1}) and land equivalent ratio (LER) of maize variety, strip space and intercrops evaluated over two years in intercropping trials (W).

Treatment	Maize grain yield (kg ha^{-1})	Intercrop yield (kg ha^{-1})	Maize GEY (kg ha^{-1})	LER
Y. Year				
2006	3956	3033	8434	1.30
2007	3734	2162	6833	1.24
A. Maize variety				
Rampur Composite	4369	2438	7937	1.24
Arun-2	3321	2757	7329	1.30
B. Strip space				
1.5 m	3987	2057	7076	1.22
1.8 m	3703	3138	8190	1.32
C. Intercrop				
Potato	3864	6316	9836	1.16
Kidney bean	3876	1001	7423	1.37
Lentil	3796	476	5640	1.27
CV %	10.2	23.7	13.0	20.9
LSD (p = 0.05)				
Y, A, B	132.4	207.1	334.3	ns
C	ns	253.6	409.2	0.11

Analysis of crop mixture

Due to variation in the yield potential of different intercrops, their market price and profitability should be considered prior to making inferences. Therefore, we calculated the maize grain equivalent yield, and performed an economic analysis. Given the good performance of maize as well as the intercrops, MGEY and LER were better in the first year of the experiment. Rampur Composite performed better in terms of MGEY than Arun-2 but the results for LER were the opposite of that. Strip space of 1.8 m resulted in significantly higher MGEY (8190 kg ha^{-1}) and LER (1.32) compared to 1.5 m strip space. The potato intercrop yielded a higher MGEY but lower LER (1.16) compared to kidney bean while lentil was found poor in producing maize and intercrop yield.

Economic analysis

The highest biological yield, and consequently total income, were obtained with the full-season maize variety, 1.8 m strip space and potato intercrop. However, kidney bean produced the highest marginal rate of return (revenue gain:additional cost ratio), of 1.32 than potato (<1.0) when partial budgeting was performed based on high-yielding sole maize among two varieties. The reason behind this result is the remarkably variable cost of cultivation of intercrops. The wide strip space and full-season variety

Table 3. Partial budgeting-based economic analysis of maize-based intercropping study in the winter season for treatments having >1.0 MRR (revenue gain: additional cost ratio) at Rampur, Chitwan, Nepal, 2006-2007.

Treatment	Revenue(NRs '000)		Cultivation cost(NRs '000)		Revenue gain: add. cost
	Total	Gain	Variable	Additional	
1. Rampur Composite; strip 1.5 m + Kidney bean	109.94	27.89	34	23	1.21
2. Rampur Composite; strip 1.8 m + kidney bean	115.12	33.07	36	25	1.32
3. Arun-2; Strip 1.8 m + potato	157.57	75.52	85	74	1.02
4. Rampur Composite (sole)	82.05	=	11		

Table 4. Performance of maize-based intercropping practice (W) in farmers' fields (5) of Chitwan, Nepal and its economic analysis (partial budgeting), 2007.

Treatment	Maize GY[1] (t ha^{-1})		Intercrop yield (t ha^{-1})		MEGY[2] (t ha^{-1})	Revenue (NRs '000)		Cost (NRs '000)		MRR[3]	B:C ratio[4]
	Mean	Range	Mean	Range		Total	Gain	Vary	Ad[5]		
Maize + potato	3.174	2.8-3.5	5.270	4.2-6.0	7.915	111	58.4	85	74	0.79	1.05
Maize + Kidney bean	3.155	2.6-3.5	1.005	0.7-1.5	6.385	89.4	36.9	36	25	1.48	1.60
Maize + lentil	3.084	2.5-3.3	0.412	0.4-0.5	4.558	63.8	11.3	26	15	0.75	1.38
Maize (sole)	3.748	2.4-5.1	0		3.749	52.5		11			1.69
CV%	21.8				15.8						
LSD (p=0.05)	ns				1.228						

[1] GY = Grain yield.
[2] MEGY = Maize equivalent grain yield
[3] MRR = marginal rate of return (revenue gain:additional cost)
[4] B:C = Benefit:cost ratio
[5] Ad = Additional

treatments gave a higher MRR than the narrow strip space and early-maturing variety treatments respectively (Table 3). For calculating partial budgeting, the prevailing price of produce for maize, potato, kidney bean and lentil was considered (NRs 10, 10, 35, 40 in 2006 and 14, 12, 45, 50 in 2007 respectively) at the time of harvesting.

On-farm results

Though sole maize yielded the highest grain yield (3748 kg ha^{-1}) it was nonsignificant with other intercrop treatments. Naturally intercropping of potato produced the highest economic tuber yield than kidney bean and lentil and consequently high MGEY. But intercropping of kidney bean gave the highest economic return, ie, MRR and benefit:cost ratio (1.48 and 1.60 respectively) compared to intercropping of potato (0.79 and 1.05) and lentil (0.75 and 1.38) in farmers' fields (Table 4). The trials were conducted in both lowland and upland conditions with different dates of planting (Sep 16 to Nov 8). However, the results obtained were comparable for all planting dates and conditions.

Conclusion

Rampur Composite yielded higher MGEY (7937 kg ha^{-1}) and maize yield (4369 kg ha^{-1}) without additional cost for maize variety. Strip space 1.8 m was more profitable with higher intercrops, MGEY, higher LER and MRR than 1.5 m space. Among the tested intercrops, potato yielded higher tuber yield as well as MGEY but the potato intercrop was found less beneficial due to the higher cost of cultivation. Intercropping of kidney bean gave the second highest MGEY with higher LER (1.37) and MRR (1.32) compared to potato and lentil. Maize + kidney bean intercropping also gave the highest MRR (1.48) and B:C ratio (1.60) in farmers' fields. Therefore, growing maize + kidney bean in 1.8 m strip space was found more productive, economic and promising compared to other tested intercrops and sole maize in Chitwan conditions.

Acknowledgement

The authors would like to thank NMRP management for their kind support in conducting our research activities.

We are also thankful to NMRP's Mr. R.K. Shrestha for his sincere support in trial management and data collection. The financial support of HMRP/CIMMYT and nomination from NARC to participate in the 10th ARMW is also duly acknowledged.

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Demand for Technology Innovation and Transfer to Maize Farmers in South Sulawesi, Indonesia

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Abstract. This paper illustrates the demand for technology innovation and transfer to maize farmers in South Sulawesi Province. Data for this study was gathered through Participatory Rural Appraisals (PRA) conducted in several subdistricts of this province and also from secondary data sources. The pattern of maize varieties used in this province has been changing. In 1995 local/white varieties accounted for 40.0% of the usage, open-pollinated superior varieties 48.6% and hybrid maize varieties 14.4%. Over the next 10 years, the use of local varieties decreased to 16.2% and that of open-pollinated superior varieties to 23.4% including Quality Protein Maize (QPM) varieties. The share of hybrid maize varieties rose to 55.6% in 2007 although in 2006 it was only 29.6%. Innovation technologies have been used by some farmers. Farmer groups are using open-pollinated superior varieties and hybrid varieties. The other technology innovations adopted have been use of zero and minimum tillage, site-specific nutrient recommendations, improvement in postharvest handling technologies like threshers, drying and processing equipment. These technologies were recommended by the Indonesian Government through the Agricultural Technology Committee. Initiatives to transfer technology innovations to farmers have included demonstrations, case studies and communication development. The strategy and methods to disseminate technology innovations have included (1) direct communication to the clients; (2), learning through media like leaflets, folders, and booklets; and (3) a combination of both methods.

Key words: Maize farmers, technology innovation, transfer methods

Introduction

Maize is a strategic commodity in Indonesia because of its multiple uses as food, feed and raw material for industry. As a food commodity, maize is second in importance to rice. Suryana (2006) reports that the contribution of maize to Indonesia's economy has doubled in three years, from 2004 to 2006, growing from Rp.9.4 trillion to Rp.19.2 trillion. However, maize production is still not sufficient to meet domestic demand, and imports are needed. The Ministry of Agriculture planned to achieve maize self-sufficiency in 2007, and then to expand production for export. The targets were not achieved. Farmers hope to increase production by adopting new technologies.

Farmers of South Sulawesi used to cultivate local white varieties of maize in the upland areas for food purposes. Later they used to sow open-pollinated superior varieties in the dryland in the rainy season. Under the government-supported maize development program named BIMAS Palawija (Mass Guidance for Second Crop Intensification), farmers have been planting not only white corn and open-pollinated superior varieties, but also hybrid maize varieties in the drylands in the rainy season, and in irrigated lands after the rice crop. The Mass Guidance or *BIMAS* approach delivered both technology innovations and production resources like fertilizers and insecticides.

Innovation is defined as a new idea, practice or object that is successfully introduced into economic or social processes (Hartwich *et al.* 2007). In agriculture, innovations can include new knowledge or technologies related to primary production, processing and commercialization all of which can positively affect productivity, competitiveness and livelihoods of farmer and others. Both maize price and good seed assistance from the government can motivate farmers to adopt technological innovations and increase their production. The government's objective of increasing maize production by 2.24% per year can be attained by increasing productivity and expanding the planting area.

This assessment paper is aimed at strengthening activities to increase maize production by adoption of innovation in South Sulawesi, Indonesia.

Methodology

Data for this paper was collected through Participatory Rural Appraisals (PRAs) in several subregencies of South Sulawesi, especially the regencies of Bulukumba, Takalar, Bantaeng and Bone. Secondary data was obtained from related institutions and assessment data from AIAT of South Sulawesi.

Results and Discussion

Maize planting area, production and trends

Development of maize in South Sulawesi has been related to the other staple foods of the people of this area, especially rice. When rice used to be sown during only one season (rainy season) every year until the 1960s, farmers used to plant while local varieties of maize in the upland dry areas and in rainfed lowlands area after rice. The pattern of food consumption of rice for six months and maize or rice mixed with maize in the other six months was reflected in the management of maize.

Initiatives have been undertaken in recent decades to increase maize production and productivity in South Sulawesi. There have been changes in the composition of maize varieties used. In 1995, 40.0% of the maize varieties used were local/white varieties, 48.6% open-pollinated superior varieties and 14.4% hybrid maize varieties. In the one decade since then, the composition has changed: the share of local varieties has decreased to 16.21% and that of open-pollinated superior varieties to 23.42% including Quality Protein Maize (QPM) varieties such as Srikandi Kuning-1, while the share of hybrid maize varieties rose to 55.56% in 2007 although in 2006 it was only 29.6% (Table 1). The sharp increase in the total area of maize hybrids was mainly due to seed assistance from the government. Local white varieties used to be the staple food of people in some districts, but with the increase in rice production in South Sulawesi, the number of people using maize as the main food has decreased.

Adoption of modern varieties, including hybrid varieties and inputs, have helped increase yields. Production has grown 8.86% from 639 414 t to 696 084 t, and productivity 5.08% from 3.210 t ha-1 to 3.373 t ha-1.

Table 1. Distribution of maize varieties, 2006 and 2007.

Maize varieties	Year of production			
	2006		2007	
	Planted area (ha)	%	Planted area (ha)	%
Local (white, glutinous)	32 590.48	15.1	34 751.05	16.2
Superior open-pollinated including QPM	86 979.80	40.3	50 198.64	23.4
Hybrid				
F_2 or F_3 hybrid	63 885.98 32 374.65	29.6 15.0	119 129.88 10 321.67	55.6 4.8
Total	215 831	100	214401.21	100

Source: UPTD BPSB Tanaman Pangan dan Hortikultura Propinsi Sulawesi Selatan 2007.

Demand for technology innovations

Several agricultural technologies have been introduced to farmers and farmer groups in this region. Some of them have been successfully adopted such as open-pollinated superior varieties and hybrid varieties and zero and minimum tillage. Site-specific nutrient management has also been recommended but not successfully adopted by farmers yet. Improvement in postharvest handling technologies like using threshers, drying and processing has been recommended. The government's recomendations for the maize planting seasons of 2008 and 2008/2009 are summarized in Table 2.

The other innovations include growing certified seed, of both open-pollinated superior varieties and hybrid maize varieties, by farmer groups as part of the Seed Grower Base Community System and local enterprise. Farmers have adopted hybrid maize seed as a production technology innovation which could increase their production and income. Production of certified seed of open-pollinated superior varieties including Quality Protein Maize (QPM) variety Srikandi Kuning-1, and hybrid maize varieties will be developed as part of the community-based development initiative.

Adoption of technology is dependent on farmers' motivation. In the case of adoption of new crop varieties, farmers not only consider the capacity of the crop to boost productivity, but also other characteristics such as resistance to drought, flooding, pests and disease. A lack of assets such as land, education or equipment (eg, pumps), can also limit adoption. So more attention needs to be paid to technologies that require fewer assets and less expensive inputs.

Transfer of technology innovations

Innovators are agents, farmers, processors or other private sector entities who introduce and adopt innovations. Researchers and extension personnel are inventors, knowledge communicators who assist the innovator in the conduct of the innovation, which in any case may also occur without their contribution. Training farmers and bringing to them training materials that are understandable to those with low literacy can help them adopt a new technology. Cultural characteristics influence adoption in many different ways, such as preferences for certain tastes and textures.

Table 2. Summarized recomendations for development of maize in South Sulawesi Province of Indonesia for planting seasons 2008 and 2008/2009.

No.	Subject	Wet season	Dry season
1.	Land Resources		
	Upland areas	Upland rice + maize	-
		Maize	Soybean
	Irrigated lowland areas	Rice	Dry season 1 : Rice
			Dry season 2: Maize zero tillage
	Rainfed lowland areas	Rice	Maize zero tillage
	Rainfed lowland areas with pump irrigation	Rice	Maize zero tillage (dry season 1)
2.	Technological innovations		
2.1	Seed and varieties	Certified seed (commercial seed) Varieties recommended: Open-pollinated superior varieties like Lamuru, Sukmaraga, Bisma, Gumarang, Srikandi Kuning-1 (QPM) Hybrid varieties: BISI, Pioneer, Bima 1, Bima 2, Bima 3	
2.2.	Fertilizer amount	250-300 kg urea ha^{-1} 100-150 kg SP 36 ha^{-1} 50-100 kg KCL ha^{-1}	
	Fertilizer application	Application of urea 3 times: 1. At 7-10 DAS; 2. 28-20 DAS; 3. 40-45 DAS Third application based on LCC measure	
2.3.	Weed control	Manual weeding two times: 14 DAS and 30 DAS. Or using herbicides	
2.4.	Pest control	Seed treatment with fungicide Metalaxyl for downy mildew; Using resistant varieties and proper planting times; Maize stem borer controlled by insecticide if more than one group of larvae or eggs hatched per 30 plants. Cutworm or armyworm controlled if more than two larval groups from eggs hatched per 36 plants. Applied Carbofuran 3 G 10 kg ha^{-1} through tip of maize.	
2.5.	Postproduction handling	Threshing and cleaning with machine, and drying manually or with dryer.	

[1.]DAS = Days after sowing.
Source: Dinas Pertanian Tanaman Pangan dan Hotikultura Propinsi Sulawesi Selatan (2008).

Dissemination pathways, how people learn about or obtain a technology play a fundamental role in determining who will adopt a new technology. Aidar *et al.* (2002) reported that of the media used to communicate effectively, 88% learn from leaflets and only 50% from booklets. Both media could grow up of maize production by 43.3%, time efficiency by 35% and cost efficiency by about 21%. A broad range of dissemination methods were used to promote maize in South Sulawesi Province for three decades. However, there was no one "best" method of dissemination for all regions or groups of farmers within one region. So, dissemination is specific to location, ethnic group, knowledge level, attitudes and practices of farmers or farmer groups.

According to Sumarno (1997), some of the technologies developed by research were not adopted by farmers because: (1) there was a barrier of communication between the source of innovation and the farmer; (2) many innovations are developed away from the farmers' fields without involving the participation of farmers; (3) there are problems relating to socioeconomic conditions and environmental resources; and (4) there are problems internal to farmers.

Show case of technology innovation is a dissemination method successfully used in maize because: (1) it establishes a direct relation with the client (farmers and their families); (2) it is conducted on farmers' lands with the participation of farmers, (3) it uses multimedia, visual, verbal and printed material; (4) it can be documented and used again by other people efficiently and effectively combining with other instructional media (Aidar *et al.* 2002). A case study on zero tillage for maize in Takalar Regency (with 50 farmer respondents) showed that 50% of farmers found it easy to obtain herbicide, all of them said zero tillage in preparing land for maize was useful, and 80% respondents stated that production costs were lower with zero tillage than complete land preparation.

Each method is affected by local histories and social dynamics. Therefore, we must know about local cultural and power relationships to understand how people interact and learn. The Government has been involved in projects for the development of maize, especially agribusiness activity, playing a significant role in dissemination of information and providing infrastructure support. The private sector has been involved too in dissemination technology, but companies have been more concerned about the needs of larger, commercial or successful farmers and much less about fulfilling the needs of poor farmers.

One approach used to encourage seed enterprises is to supply inputs for maize production and buy the production at the end of the season, a system that is called *"bayar setelah panen/yarnen"*. Decision makers need to take this perceptions into account when considering using the private sector to develop and disseminate technologies. The other development approach is using farmer groups. The groups are intended to make dissemination more efficient by reaching a number of farmers at once, building capacity by encouraging trained groups to train others and empowering farmers through collective action.

Conclusions

Technology innovations are needed especially in varietal engineering and use of natural resources ie, minimum tillage, zero tillage, fertilization, pest, disease and weed control and others. Seed production by farmers must be supported for both open-pollinated and hybrid varieties, including under the community-base development approach. Site-specific nutrient management for maize can conserve and develop land resources, save costs and obtain optimum production. Farmers will adopt a simple new innovations if it doesn't require expensive inputs and investment.

Transfer or diffusion of innovations for increasing production is also need of vital importance. Continuing communication of innovations through multimedia, visual, verbal and printed material is needed. For innovations that need inputs and materials, they must be easy to obtain locally, at low cost and lead to higher production. The marketing approach called "yarnen" (pay after harvest) can encourage farmers and farmer groups to work toward future improvement.

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Community-Based Maize Seed Production as a Means of Food Security in the Western Hills of Nepal

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Abstract. Maize is critical to livelihoods and food security in the mid-hills of Nepal but area, production and yields during 1990-2006 were not encouraging. Improved seed contributes about 25% of the production increment, which ought to be a focal point of interventions. The public seed supply during last ten years has been plagued by problems (145 t in 1996 and 2.11 t in 2005). Against that context, participatory maize varietal selection (PVS) was carried out to determine farmers' preferred varieties of maize for two years in a 5Ĩ 3 sq m plot. Based on the results of the PVS, highly preferred varieties Man-3 and Sitala were selected for seed production. A community-based seed production (CBSP) program was carried out in Palpa district of Nepal with the aim of establishing a maize seed production and marketing system as an enterprise for women farmers. Women farmer groups were involved in dissemination of improved maize production technologies after receiving hands-on training. The area used for this program ranged from 4.20 ha in 2003 to 12.45 ha in 2007 and income from US$ 689 in 2003 to US$ 3239 in 2007. The net benefit per ha from maize grain and maize seed was US$ 212 and US$ 525 respectively. Additional household income from CBSP ranged from US$ 54 to US$ 77. In essence, maize seed production can be an opportunity for improving household incomes and the food security status of women farmers.

Key words: Food security, participatory varietal selection, community-based maize seed production, preferred variety

Introduction

Agriculture is the largest sector of Nepal's economy and has been the main basis of food security and rural employment. Agriculture contributes about 38.9% of the national GDP and employs about 66% of the economically active population. Maize is second in importance to rice among cereal crops, and is the main food for 55% of the population. However, trends in maize area, production and yield druing 1990-2006 were not encouraging. Seed is the first link in the food chain and seed-based technologies offer the earliest and cheapest options for increasing crop productivity in the farmers' fields (Joshi 2001). Improved seed contributes about 25% of the production increment. This therefore suggests that seed should be the focal point of interventions for increasing maize productivity and production.

Justification

Improved maize varieties contribute substantially to productivity. Demand for maize is expected to grow by 6-8% per year over the next two decades in Nepal. However, average productivity stands at only 2.038 t ha^{-1}. Moreover, the yield difference between research stations and farmers' fields is high (6.33 t ha^{-1} at research stations as opposed to an national average of 2.038 t ha^{-1} in farmers' fields) Net income per unit of maize is too low. The seed replacement rate is very low in the hill regions of the country. Public sector supply of maize seed is only 0.062%, and therefore seed availability of farmer-preferred varieties remains the biggest bottleneck to increasing production. The maize seed supply system in rural Nepal is very poor in terms of quality, quantity, availability and affordability. What is needed is viable profit-oriented seed entrepreneurship at the local level, managed by farmer groups. Community-level seed production seems to be the only solution to Nepal's maize seed problems.

Objectives

- To increase the accessibility of improved maize seed at the local level.
- To establish maize seed enterprise as an element contributing to rural women's livelihoods.

General description of the project site

Palpa district lies in the mid-hills belt of the western development region of Nepal. The oldest Siddhartha highway passes through this district and is the main north-south outlet for local produce. Most of the land here is rainfed steepland (*bari*). The approximate elevation of the research site ranged from 900 m to 1200 m above the mean sea level. The aspect of the land mostly is south-faced. Bramins, Chhetris, Janajatis and Dalits are the major social groups inhabiting this area. Almost 100% of the participant farmers in this project own less than 2 ha of land. The total annual rainfall in this district is 1903 mm, 90% of it distributed from mid-May to mid-August.

Materials and Method

Seed producer group formation: The Shivashakti Seed Producer Group was constituted comprising 19 women farmers in Pokharathok in Palpa district. Farmers from poor and disadvantaged sections participated in the group, of which 18% were from socially underprivileged sections.

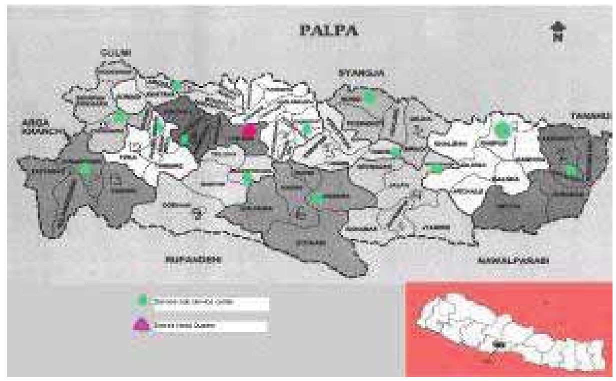
The group members were given an orientation on the conduct of PVS and CBSP, their roles and responsibilities in trial management and technical aspects of maize production and marketing.

Farmer-preferred varieties selected through PVS (mother trial) for two years.

PVS is a process of active and functional involvement of farmers in planning, implementation, monitoring and evaluation of maize varieties and their promotion.

Baby trials were conducted over a 250 sq m area independently by the participants.

Baby trials select a single new variety from among the mother trial set which is given to farmers to grow in his/her



own way and compare it with the variety they had been using.

Recently released and pipeline varieties of maize (Manakamana-3, Hill Pool White, Hill Pool Yellow, Deuti (ZM 601), Population 45, Shitala (Population-44), Quality Protein Maize (S99TLWQ-HG-AB) and farmers' variety) were included in the PVS.

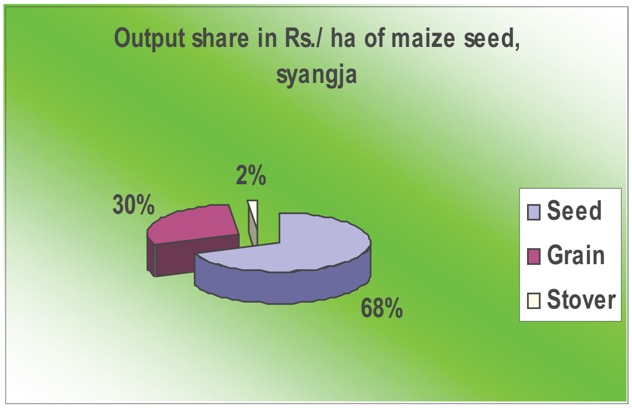
Periodic visits to the research sites were made by maize experts and seed technologists to monitor the standing crop and advise the farmers accordingly.

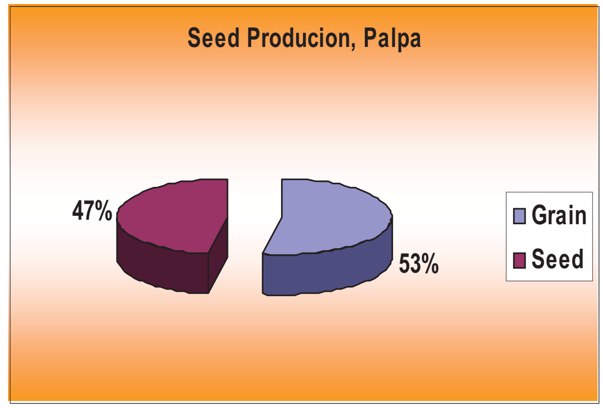
Focus group discussions were carried out to evaluate the qualitative and quantitative performance of each maize variety.

Based on the farmers' preference, Manakamana-3 and Shitala were selected for seed production.

Training on maize seed production technology was imparted to the farmers three times in a season:

- Before sowing
- Flowering time
- Postharvest and marketing





The women farmer group conducted monthly meetings and interacted regularly on maize production-related technology and savings and credit schemes.

A marketing subcommittee was formed to undertake seed marketing activities through a group marketing approach.

The marketing subcommittee had the mandate of selling the maize seed, and needy/poor members of the group received money immediately or sometimes in advance.

Price differential at the beginning and end of the sale process, at the end total sale amount will be calculated distributed in equal rate to all the members.

The group collects a percentage of the income for the group fund with which to take up group activity.

Some male members were selected as advisers to facilitate the seed marketing process.

Results

A community-based maize seed production program was conducted in 12.45 ha of land, producing 39.23 t in 2007. The area and production data are given in Table 1. Of the total participants, 18% were from the socially underprivileged (Dalit) groups. The women farmer group was actively involved in seed production and marketing. Manakamana-3 and Shitala varieties were used for seed production. Breeder seed of Manakamana-3 and Shitala were also sown by the farmer group. The farmers showed a very positive response to the program, and were actively involved in every activity. About 47% of their produce was taken as seed. The benefit:cost ratio was computed including all the possible cost components incurred in the seed production process. The net benefit from seed production was calculated to be NRs. 34 147.50 (525.34 US \$) ha^{-1} whereas the net benefit from maize grain production

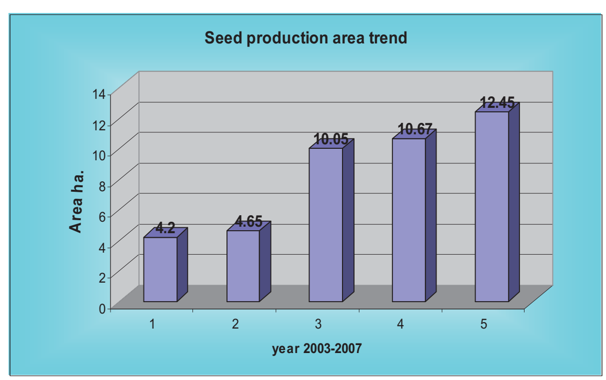
Table 1. Area (ha) and seed and grain production (kg) under the community-based seed production program.

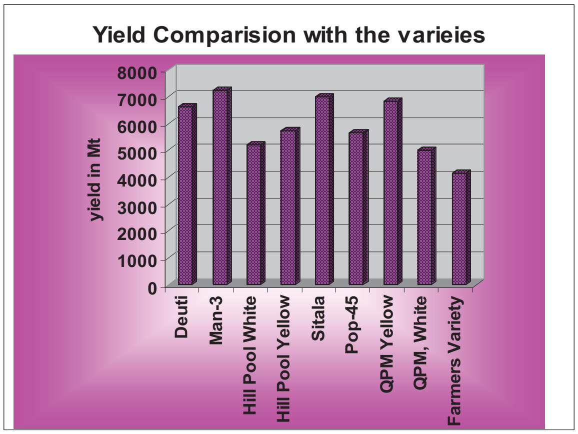
Year	Area	Seed production	Grain	Total
2003	4.20	3,963	6,175	10,138
2004	4.65	5,234	5,314	10,548
2005	10.05	8,476	8,563	17,039
2006	10.67	9,473	15,118	24,591
2007	12.45	15,832	23,420	39,252

Table 2. Income of the seed production program.

Year	Seed (kg)	Seed sold (%)	Cash earned by group (NR[1]s)
2003	3963	60	42800
2004	5234	63	62651
2005	8476	70	112731
2006	9473	85	185197
2007	15832	80	253632

[1.] 1 US\$ = NRs. 65 at harvesting time in 2007.





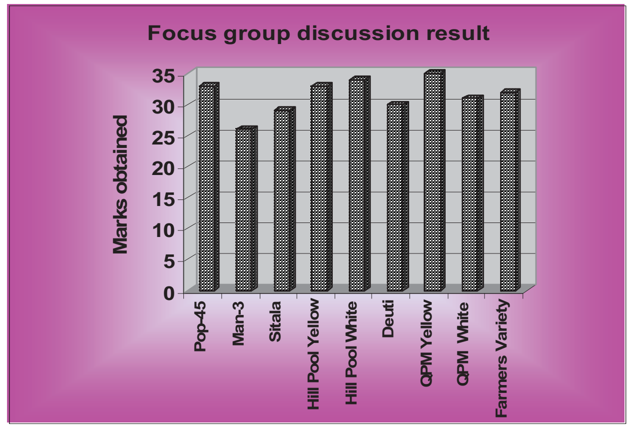


Figure 1. Cumulative qualitative and quantitative comparison of different maize varieties.

Focus group discussion on participatory varietal selection for maize, 2007. Ranking: 1 = best; 6 = worst.

SN	Specific character	Variety								
		Pop-45	Man-3	Sitala	Hill Pool Yellow	Hill Pool White	Deuti	QPM Yellow Kernel	QPM White Kernel	Farmers' variety
1	Germination	1	1	1	1	1	1	1	1	1
2	Thick stem and nonlodging	2	1	2	2	1	1	1	2	5
3	Height	2	2	1	2	2	2	1	1	5
4	Drought resistant	1	2	2	2	2	1	1	1	3
5	Good in less fertile soil	2	2	1	1	2	2	2	2	3
6	Stay green after riping	3	1	3	3	3	3	3	2	5
7	More stover	1	1	2	2	2	2	2	2	2
8	Shade tolarant	2	2	2	2	2	2	2	2	3
9	Less pest incedence	2	1	2	2	2	1	3	2	2
10	Early maturity	4	3	2	4	4	2	1	2	3
11	Large cob size	3	1	1	1	3	1	2	2	4
12	Tight grain	1	1	2	2	1	2	1	1	4
13	Grain color	1	1	1	2	1	1	2	2	2
14	High productivity	2	1	1	1	3	1	2	3	5
15	Grain filled more	1	1	1	1	1	1	1	1	3
16	Husk cover	3	2	3	1	2	3	5	2	1
17	Cob rot tolerant	1	1	1	2	1	1	3	1	2
18	Good for roasted use	1	2	1	2	1	3	2	2	1
	Total marks	33	26	29	33	34	30	35	31	32
	Rank	VI	I	II	V	VII	III	VIII	IV	V

was NRs.13 787.5 (212 US$). The monetary share of maize seed was 69%.

Dissemination of farmer-preferred seed was done through the Informal Research and Development (IRD) approach, in which 500 gm of seed is given to individual farmers of a new area to evaluate the adaptability and potentiality of the new cultivar. The seed produced by the women farmer group was thus distributed in nine mid-hill districts of the country.

Impacts of the program

- Members of farmer groups are capable of selecting their preferred variety.
- Production of maize seed is increasing.
- Farmers used to regard maize farming as a cash fetching commodity.

- Farmer group institutionalized and vested ownership of its property such as land, house, seed bin, weighing machine, sewing machine, etc.
- Women farmers empowered to develop entrepreneurship through seed production program.
- Group cohesiveness has increased due to group dynamics.
- The bargaining power of farmers has increased; in 2007 they sold the maize seed from NRs. 21 (0.32$) to NRs. 35 (0.54$) per kg.
- The maize seed producer group of Palpa was able to win DISSPRO (District Seed Self-sufficiency Program) support of NRs. 60 000 (US$ 923) from the Crop Development Directorate of the Department of Agriculture.
- Farmers growing Deuti variety as a baby trial received the national level first prize for its recorded yield of 7.5 t ha^{-1} on the occasion of World Food Day 2007.
- CBSP accepted as a vehicle of improved maize seed availability at the local level in affordable price.
- CBSP approach of seed production is recognized and integrated in the government extension system.
- Yield contribution by improved maize varieties:
    - o Local variety: 2.1 t ha^{-1}
    - o Improved variety: 3.5 t ha^{-1}
- Contribution to food security:
    - o Before: 7 months from own produce.
    - o After: 8 months from own produce.

Farmer Group empowerment due to CBSP

- Regular group meetings
- Started conducting savings and credit programs
- Conducted adult education program
- Conducted environmental protection program.
- 125 metal seed bin with a capacity of 18750 kg
- Have weighing balance, sprayer, corn sheller, electric sack sewing machine, land and meeting cum training hall
- Approach road to seed production area
- Started to communicate with regional seed lab, National Maize Research Program and adopted truthful labeling

- Started to market their seed by themselves through group marketing
- Technically able to produce foundation seed
- New farmers are highly interested in joining the CBSP

Conclusions

Community-based maize seed production is a means to disseminate improved seed in remote areas. The CBSP can solve the problem of supply of affordable seed, timely availability, quantity and quality of seed.

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Utilization of Maize Stover Use as Cattle Feed in Bangladesh

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Abstract. The study was conducted, both on-station and on-farm, to determine the use of maize stover as cattle feed. In the on-station study, maize stover was collected and chopped, using a forage chopper, into pieces 2-3 cm long and silage was prepared. After 3 months, a feeding trial was conducted with 12 growing Pabna bulls. We found that the DM intake (kg day^{-1}) of BHM-5 and BHM-3 stovers did not significantly ($P<0.05$) differ between the two treatment groups. The values of DM intake were 3.83±0.10 and 3.85±0.1 for BHM-5 and BHM-3 respectively. The DM intake percentage live weight (LW) did not vary either. The DM intake g per kgW$^{0.75}$ also did not significantly ($P<0.05$) differ between the treatment groups. The values for estimated intake by the growing bulls (MJ kg^{-1}) were 26.81±0.7 and 27.05±0.75 for BHM-5 and BHM-3 respectively, and the difference was not significant ($P<0.05$). Similarly, the estimated metabolizable protein (MP) intake did not differ significantly ($P<0.05$) between the treatments. The values for MP intake (g day^{-1}) by growing bulls were 257.37±6.76 and 259.76±7.19 for BHM-5 and BHM-3 maize stovers respectively. Here, it was observed that digestibility of DM, OM and CP significantly ($P<0.05$) differed between the two varieties of maize stover but the ADF digestibility was not significant ($P<0.05$) between the varieties. The values for weight gain were 1.20±0.15 and 1.00±0.06 for the BHM-5 and BHM-3 treatment groups, respectively.

Key words: Maize stover, forage chopper, digestibility, growing bulls

Introduction

Smallholder dairies are an important economic activity for the landless and smallholder farmers in Bangladesh. They help to reduce the daily per capita milk intake deficiency (only 34 mL as opposed to the 250 mL requirement) and to limit the use of powdered milk. The average annual growth rate of milk is about 4.21%. Smallholder dairies are a preferred option for small-scale farm households to increase their income and to accumulate assets. However, the productivity of livestock holdings remains low mainly because of the scarcity of quality fodder. This situation is most serious during the lean season from March to May and in areas recurrently affected by drought and salinity.

Green fodder plays a very significant role in increasing the productivity of farm animals. In Bangladesh year-round availability of grass for large ruminants is about 1 kg per head per day (Tareque and Saadullah 1988), most of which comes from agricultural weeds and/or roadside grasses. This amount is far below the maximum requirement of about 6 kg per adult ruminant per day on a straw-based diet (Chowdhury and Khan 1997). It has been reported that the overall feed deficit in terms of dry matter, digestible nutrient and crude protein in Bangladesh are 45%, 50% and 80% respectively (BARC 1983). Naturally grown grasses available in fallow land, playgrounds and the wayside are the main sources of such forage for the rural people of Bangladesh. Feed scarcity, which is pronounced in the lean season from March to May, is a major factor limiting milk production. A countrywide survey on smallholder dairy farms revealed that shortage of feed, disease control and marketing facilities are the major constraints raised by 48%, 39% and 32% of farmers, respectively (Huque *et al.* 2002). Therefore, scarcity of feed and fodder is the biggest problem for livestock and poultry development in the country. To sustain the production systems of livestock round the year, there is a need for technological intervention in the existing production systems of cattle and buffalo. The challenge for researchers working with smallholder farmers is to identify fodder and feed technologies that complement the current cropping practices and feeding systems to increase the availability of feed and fodder without incurring unacceptable changes in inputs and risks. Keeping these considerations in view, the Bangladesh Livestock Research Institute (BLRI) has already developed some technologies and packages suitable for the livestock and poultry sectors. It has been reported that the Internal Rate of Return (IRR) of BLRI-developed technologies was 56-315% whereas the IRR of crop technologies was only 15-59% (Caliveto 2001). Hence, a farmer can earn 56-315 taka by investing only 1 taka in field livestock and poultry, whereas in the crop sector a farmer can earn only 15-59 taka by investing the same amount of money. Further, the labor coefficient of the livestock subsector is 81.3 person years per million taka of gross output (Planning Commission 1988), which is about five times higher than that of the average for all sectors. The potential of smallholder livestock production systems needs to be exploited by giving priority to four critical areas: feed supply, animal health and diseases, genetic stock and marketing network. These issues are intertwined and require a sequenced approach. Improving feed supply needs first priority since it will lead to increased output by the existing stock of animals. That will have a direct impact on the production and income of small producers. It will also contribute to better health and higher disease resistance. Therefore, cost-effective intervention of feed and fodder technology needs to be disseminated among farmers. Dissemination of technologies should be strengthened through extension services so that farmers will become more aware of the benefits and take steps to adopt technologies without any risk and uncertainty.

Due to the growth of the poultry industry, there has been an increasing demand for maize because it is a major feed ingredient. Maize is a unique crop that has the highest capacity to convert solar energy into carbohydrates. At present the demand for maize is 70 000 t but the potential demand is 270 000 t. Realizing the importance of maize because of its diversified potentialities, the Government of Bangladesh had placed emphasis on its cultivation since 1990. During 1995-96, 25 000 acres of land were under maize cultivation. The potential area for growing maize is very high in Bangladesh. The crop can be grown in the *rabi* or postrainy seasons (86%) as well as the *kharif* or rainy (14%) seasons. The total potential area is about 2.8 million ha. However, the area potential is not evenly distributed in all divisions of the country: 54% of it lies in Rajshahi, 25% in Dhaka, 14% in Chittagong, 6% in Khulna and 1% in Barisal divisions. Maize cultivation has increased in Rajshahi and Dhaka divisions but not as much in Chittagong and Khulna divisions.

Maize is a multipurpose crop producing both grain and stover. After harvesting the grain/cobs, a huge amount of stover is produced. Some of this remains in the field and some is used as fuel. Farmers usually spend large amounts of money to remove the stovers from the field. Land area under maize cultivation did not increase in the last 1976 but land area under maize cultivation has slightly increased from 19th to 2000. Though land under maize cultivation did not increase too much, production has almost doubled as compared to the 17th. This is possibly due to the cultivation of hybrid maize. Hybrid maize produces 6-10 t and 4-5 t of grain during the *rabi* and *kharif* seasons respectively. The ratio of extraction of maize grain and stover is 1:1.3-2.0. Therefore, from one ha of land on an average it may be possible to produce about 25 t of stover. The main advantage of hybrid varieties is that after the harvest of cobs the plant remains green. As a result the stover is suitable for cattle feed. Production of stover of hybrid maize has increased from 200 000 metric t to 400 000 metric t from 1995 to 2000. Hence, on the basis of location-specific availability of production of maize, the Bangladesh Livestock Research Institute has developed a processing, preservation and utilization technology of maize stover as a source of cattle feed which will help in minimizing scarcity of feed in the country.

In order to increase the adoption of technology by farmers, a project was designed with the following objectives.

- Demonstrate maize stover technology to farmers
- Improve the handling and transportation techniques of maize stover
- Assess the impact of technology on milk production and income
- Identify the problems at the farmer level
- Compare the nutritive values of maize stover of different varieties.

Materials and Methods

On-station trial

At the on-station level, to determine the intake and digestibility of maize stover, an intake and digestibility trial was conducted for a period of 35 days with 12 Pabna growing bulls of similar age. The animals were divided into two groups that had been previously grazed as a group on natural pasture and stall-fed napier grass. All animals were dewormed with broad-spectrum anthelmentic at the start of the experiment. They were then randomly allocated to two feeding treatment groups (six animals each), taking into account age and initial live weight differences. At the end of the experiment, a five-day collection was done to determine the intake and digestibility of different nutrients in maize stover.

Preparation of maize stover silage

Maize stover from the on-station research farm was collected and chopped, using a forage chopper, into pieces up to 2-3 cm long. The stover was ensiled in silo pits, 3 feet wide and 3 feet deep, with 8 feet with in the middle and 10 feet at the top. Weighed batches of chopped stover were placed in the pit and sprayed with molasses plus water solution (preparation: 3 kg molasses + 3 kg water per 100 kg chopped stover) by using a garden watering can. The stover was mixed and trampled by two to three people to compress and compact it. More batches of stover were then added and treated until the pit was filled. Finally, the pit was covered with polythene sheets and a thick (about 45 cm) layer of soil. The experimental animals were fed maize stover silage prepared with molasses.

On-farm demonstration

On-farm demonstrations were conducted in five maize-growing areas – Nilphamari, Jinaidha, Pabna, Natore and Lalmonirhat – in different parts of Bangladesh with the help of DLS and BRAC. In addition to these locations, one demonstration of maize stover silage was carried out in Dhamrai with maize stover processing and utilization techniques. Ten farmers (ie, a total of 50) were selected for the on-farm demonstrations. On-farm feeding trials with preserved maize stover silage will be conducted during the lean period when no green fodder is available to farmers.

Results and Discussion

The chemical composition of maize stover used in this experiment is shown in Table 1. The dry matter DM, OM, CP, ADF and ash of two varieties of maize stover (BHM-5 and BHM-3) were 29.38%; 31.15%, 89.22%; 90.79%, 7.52%; 7.97%, 42.72%; 38.64% and 10.78%; 9.21%, respectively. The chemical compositions of the two varieties were almost similar with little variation in DM, ADF and ash contents. There was a nonsignificant ($P<0.05$) difference in CP.

The DM intake, growth and digestibility of maize stover by the growing bulls are presented in Table 2. The DM intake (kg day^{-1}) of BHM-5 and BHM-3 did not significantly ($P<0.05$) differ between the two treatment groups. The values of DM intake were 3.83 ± 0.10 and 3.85 ± 0.1 for BHM-5 and BHM-3, respectively. The DM intake per cent live weight (LW) did not vary due to the treatment groups. The values for estimated energy intake (MJ kg^{-1}) were 26.81 ± 0.7 and 27.05 ± 0.75, for T_1 and T_2 respectively, and the difference was nonsignificant ($P>0.05$). Similarly, the estimated metabolizable protein (MP) intake did not differ significantly ($P>0.05$) between the treatments. The values for MP intake (g day^{-1}) by the growing bulls were 257.37 ± 6.76 and 259.76 ± 7.19 fed BHM-5 and BHM-3 maize stover, respectively. It was observed that digestibility of DM, OM and CP did not differ significantly ($P>0.05$) due to the varieties of maize stover. Further, it indicated that though the growing bulls were fed sole maize stover they gained weight. The values for weight gain were 1.20 ± 0.15 and 1.00 ± 0.06 for the BHM-5 and BHM-3 treatment groups, respectively.

Table 1. Chemical composition (% DM basis) of maize stover silage.

Nutrients(%)	BHM-5	BHM-3
DM	29.38	31.15
OM	89.22	90.79
CP	7.52	7.97
ADF	42.72	38.64
Ash	10.78	9.21

On-farm utilization of maize stover

Maize leaves are one of the most important sources of livestock feed in Bangladesh. About 52% of farmers use maize leaves as feed for dairy cows and 48% used them for fattening animals. Maize leaves are usually harvested from the plant at 70 days and feeding the top of the plant (tassel) takes place at 105 days after sowing. Of the farmers who participated in the demonstraton, about 45% preserved maize stover as silage and rest used it fresh. Farmers usually supplied 3-5 kg of maize stover for their cattle. But all the respondents were interested in receiving training on utilization of maize stover.

Table 2. Effect of feeding maize stover silage on nutrient intake, growth and digestibility by growing bulls.

Parameter	BHM-5	BHM-3	Significance
DM intake (kg d^{-1})	3.83±0.10	3.85±0.1	NS
DM intake (% LW)	1.66±0.03	1.68±0.02	NS
Dry matter intake (g/kg $W^{0.75}$/d)	64±0.001	65±0.009	NS
ME intake (MJ d^{-1})	26.81± 0.7	27.05±0.75	NS
ME intake (kJ/kg $W^{0.75}$/d)	440±7.0	450±6.0	NS
MP intake (g d^{-1})	257.37±6.76	259.76±7.19	NS
MP intake (g/kg $W^{0.75}$/d)	4.32±0.06	4.35±0.06	NS
DM digestibility	63.38±1.98	60.00±2.14	NS
OM digestibility	61.68±1.89	57.57±2.14	NS
ADF digestibility	55.46±1.71	58.26±2.11	NS
CP digestibility	68.19±1.83	64.50±1.88	NS
Growth			
Initial live weight (kg)	227.50±30.10	230.67±31.70	NS
Final live weight (kg)	248.70±28.94	249.00±32.24	NS
Daily live weight gain (kg)	1.20±0.15	1.00±0.06	NS

±: Standard error of mean; *Significant at 5% level.
BHM= BARI Hybrid Maize.

Table 3. Existing feeding practices of maize leaf and maize stover silage under farmers' conditions.

Category	Time
Leaf feeding	70 days after DAS
Feeding top (tassel)	108 days after DAS
Type of animals	
Dairy cows	10
Fattening	10
Amount of supply (kg d^{-1})	7.0
Maize stover silage	
Dairy cattle (kg d^{-1})	5.3
Fattening cattle (kg d^{-1})	3.0

Table 4.

Type of uses	No. of respondents	Percentage
Fuel	4	20
Normal burning	-	-
Fuel + cattle feed	13	65
Only cattle feed	3	15

Uses of maize stover

The results (Table 4) show that about 19% of the farmers used maize stover as fuel, 14% as cattle feed and 67% used it both as both fuel and cattle feed. Maize stover contributes significantly as ruminant feed particularly during the dry season.

Problems of maize stover utilization

- Most of the farmers are not aware of maize stover as a suitable cattle feed.
- Lack training about processing and utilization of maize stover as ruminant feed.
- Hand chopping sometimes is time consuming and entails higher labor costs for processing of maize stover. Chopping machine are not available to the farmers.

Conclusions and Recommendations

Every year a huge quantity of maize stover remains unutilized or wasted due to lack of awareness about the benefits of its utilization as cattle feed. The following improvements can be made:

1. Improving handling and transportation of maize stover by reducing bulkiness by chopping to minimize wastage.
2. Studying optimum ration formulations by using maize stover on a least-cost basis.
3. Where technology allows it, grinding of maize cobs provides an opportunity of utilizing a feed resource that is otherwise wasted.
4. Measures should be taken to develop awareness among farmers about the benefits of utilization maize stover as cattle feed.
5. Farmers should be trained on maize stover utilization technology.

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The use of quality protein maize (QPM) in the ration of native chickens raised semi-intensively

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Abstract. The severity of avian influenza endemic in Lampung province, Indonesia forced the implementation of a tight bio-security standard in the chicken farming system with the consequence of an obligatory to provide feed exclusively in the confined native chicken semi-intensive farming. An experiment was conducted to evaluate "Yellow Srikandi" quality protein maize (QPM) as a feed ingredient for native chickens. Either "P-12" common hybrid maize (CM) or QPM was used as the sole source of protein and amino acids of a 16% crude protein and 2698 kcal/kg ME ration, and the rations were balanced for vitamins and minerals by the addition of a vitamin-mineral premix. Two hundred and forty pullets, 6 weeks of age, were allotted randomly into 24 cages, and fed either one of the two treatment rations for 10 weeks. At the termination of the trial, 3 birds per cage were killed for carcass analysis. Body weight at 16 weeks of the QPM fed birds was significantly higher ($P<0.05$) compared to those of birds fed the CM (1234.01 vs. 1148.57 g). Feed consumption of the two was similar, averaging 3822.5 g/bird/10 weeks, but the feed conversion of the QPM birds was significantly better ($P<0.05$) than those of the CM (3.09 vs. 3.39). Carcass percentage and carcass components did not show significant differences between the two groups of birds fed either the QPM or CM ration. It is concluded that QPM is more superior to common hybrid maize on its nutritive value that could improve performance of native chickens.

Key words: Quality Protein Maize, Nutritive Value, Native Chicken, Performance.

Introduction

Native or local chicken, that commonly raised extensively by small farmers, play important roles in the family economy in Indonesia. During the last five years, the severity of avian influenza endemic in Lampung province forced the implementation of a tight bio-security standard in the chicken farming system with the consequence of an obligatory to provide feed exclusively in the confined native chicken semi-intensive farming (Disnak-Keswan Lampung, 2008).

Normal or common hybrid maize contributes up to a third or more of the crude protein content of chicken rations (Qi et al., 2004). On the other hand, maize is low in protein in addition to its general deficiency in essential amino acids, particularly lysine and tryptophan. Thus, the feeding of common hybrid maize necessitates the use of expensive protein ingredients, including fish meal and soybean meal.

Development of maize varieties with high quality protein (QPM) in Indonesia has been started in 2002 and for the first time, two QPM's, "White Srikandi-1" and "Yellow Srikandi-1" were released in 2004 (ICERI, 2006). According to Swastika et al. (2004); the "Yellow Srikandi" QPM has higher protein (8.49 vs. 10.38 %), lysine (0.28 vs. 0.48 %) and tryptophan (0.06 vs. 0.09 %) contents than the "Lamuru" common hybrid maize. It has also been demonstrated that the use of "White Srikandi" QPM up to 15 % and "Yellow Srikandi" QPM up to 20 % to substitute a commercial ration for broilers have no detrimental effects on the growth performance of the birds, therefore the QPM substitution for commercial ration for broilers scheme could be recommended to farmers (Arsana and Wiguna, 2005).

Furthermore, reported nutritional evaluation studies of QPM have proved the superiority of QPM over common hybrid maize in the feeding of broilers and laying hens but none has been done for native chickens (Osei et al., 1998; Osei et al., 1999; Bai, 2002; Zhai, 2002). Therefore, an experiment was conducted to evaluate the "Yellow Srikandi" QPM as a feed ingredient for native chickens.

Materials and Methods

Two hundred and forty pullets, 6 weeks of age, were selected from the local chicken population. Birds were then allotted randomly into 24 slated floor covered by rice hull cages, fenced using bamboo and sized 200 x 100 x 100 cm (length x width x height), to simulate field conditions under semi-intensive farming. The density of birds in a cage was 10 pullets/cage and one unit of cage was used as an experimental replication.

Birds were fed ad libitum either one of the two treatment rations for 10 weeks. Drinking water was also provided freely. Either "P-12" common hybrid maize (CM) or QPM was used as the sole source of protein and amino acids of a 16 % crude protein and 2698 kcal/kg ME ration, and the rations were balanced for vitamins and minerals by the addition of a vitamin-mineral premix. Composition of the experimental rations is shown in Table 1.

Observations were made for body weight, by weighing the birds every week; daily feed consumption by subtracting the amount of leftover feed to the amount of feed given; and the feed conversion ratio (FCR) by calculating the ratio between the amount of feed consumed and the weight gain during the experiment.

At the termination of the trial 3 birds per cage were killed, after being fasted without feed for 24 hours, for carcass analysis. The analysis covers carcass weight, carcass percentage, percentage of eviscerated yield and percentage of eviscerated yield with giblet and abdominal fat percentage.

Data of the birds growth performances and carcass characteristics were analyzed using t-test (Steel and Torrie, 1980) to compare means between the two treatments.

Table 1. Composition of the experimental rations

Ingredients	Treatment	
	CM Ration	QPM Ration
Common hybrid Maize (CM)	39.80	-
QPM	-	39.80
Groundnut cake	19.20	19.20
Wheat offal	27.90	27.90
Maize offal	9.60	9.60
Bone meal	3.00	3.00
Common salt	0.30	0.30
Vitamin-mineral premix*	0.20	0.20
Total	100.00	100.00
Calculated analysis		
ME (Kcal/kg)	2696	2700
Crude protein (%)	15.86	16.18
Crude fiber (%)	9.42	9.56
Calcium (%)	1.24	1.19
Available Phosphorus (%)	0.73	0.75
Lysine (%)	0.58	0.79
Methionine (%)	0.36	0.32

*Vitamin-mineral premix provides per kg of ration: Vitamin A, 13.340 IU; D3, 2680 IU; Vitamin E, 10 IU; Vitamin K, 2.68 IU; Calcium pantothenate, 10.68 mg, Vitamin B12, 0.022 mg; Folic acid, 0.668 mg; Choline chloride, 400 mg; Chlortetracycline, 26-28 mg; Manganese, 133.34 mg; Iron, 66.68 mg; Zinc, 53.34 mg; Copper, 3.2 mg; Iodine, 1.86 mg; Cobalt, 0.268 mg, Selenium, 0.108 mg.

Table 2. Performance of native chicken fed the experimental rations

Variable	Treatment	
	CM Ration	QPM Ration
Number of birds	120	120
Experimental unit	12	12
Initial weight (g)	864.94 ± 55.15	935.91 ± 57.16
Final weight (g) *	1148.57 ± 26.55	1234.01 ± 24.96
Feed intake (g)	3751.4 ± 152.78	3893.6 ± 133.87
Weight gain (g)	283.63 ± 55.26	298.10 ± 57.14
Feed conversion ratio (FCR) *	3.09 ± 0.19	3.39 ± 0.21

*Significantly different ($P<0.05$).

Results and Discussion

The growth performance of experimental chickens is shown in Tables 2. Body weight at 16 weeks of the QPM fed birds was significantly higher ($P<0.05$) compared to those of birds fed the CM (1234 vs. 1149 g). Furthermore, although the feed consumption of the two was similar, averaging 3822.5 g/bird/10 weeks, the feed conversion of the QPM birds was significantly better ($P<0.05$) than those of the CM (3.09 vs. 3.39).

These results indicate that QPM has a superior quality to CM. A possible explanation of this result is due to the lysine content of QPM in the formulated 16 % protein ration that is higher than those of CM and may increase the digestibility of critical essential amino acids. Similar results were obtained by Bai (2002) and Osei et al. (1998) in broiler; and Osei et al. (1999) and Zhai (2002) in layer pullets

Carcass characteristics of the two groups of birds fed either the QPM or CM ration were similar (Table 3). There was no significant difference on carcass percentage, percentage of eviscerated yield, percentage of eviscerated yield with giblet, and abdominal fat percentage between CM and QPM ration treatments.

Table 3. Carcass characteristics of native chicken fed the experimental rations

Variable	Treatment	
	CM Ration	QPM Ration
Number of samples	36	36
Experimental unit	12	12
Carcass weight (g)	887.84 ± 23.97	950.06 ± 24.71
Carcass percentage (%)	77.30	76.99
Percentage of eviscerated yield, %	68.03 ± 5.64	69.41 ± 5.47
Percentage of eviscerated yield with giblet, %	79.60 ± 7.14	80.13 ± 7.98
Abdominal fat, %	2.03 ± 4.02	2.05 ± 3.89

Results of a study reported by Arsana and Wiguna (2005) showed that the use of "Yellow Srikandi" QPM up to 20 % and the "White Srikandi" QPM up to 15 % to substitute a commercial ration for broilers have no detrimental effects on the carcass characteristics of the birds. Similar results were also reported by Bai (2002). At a given digestible lysine content, using QPM tended to increase weight gain with no statistical significance. Therefore, using QPM to replace CM in the broiler diet may have economical benefits due to improved weight gain and FCR and decreasing of dietary lysine supplementation.

Conclusions

It is concluded that QPM is more superior to common hybrid maize on its nutritive value that could improve performance of native chickens. It is more economical to use rations incorporating QPM due largely to the adequacy of lysine content to improve growth.

Acknowledgements

The author wish to acknowledge and thank the Ometraco Company and the PINTAR – Poultry Industry Asscociation, Lampung for their cooperation.

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ISBN: 978-979-1159-41-8