13th Asian Maize Conference
and Expert Consultation on
Maize for Food, Feed, Nutrition and Environmental Security

Ludhiana, India
October 8-10, 2018

EXTENDED SUMMARIES

ORGANIZERS

भाकृअनुप ICAR
CIMMYT International Maize and Wheat Improvement Center
भाकृअनुसं IIMR
Punjab Agricultural University
CGIAR Research Program on Maize
BISA Borlaug Institute for South Asia

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Editors:
BM Prasanna, Aparna Das and Kelah K. Kaimenyi

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Maize in Asia – Status, Challenges and Opportunities

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Introduction

Maize is cultivated on more than 180 million hectares (M ha) globally, contributing ~50% (1,170 million metric tons or MMT) to the global grain production. About 60-70 per cent of the cultivated area under maize is in the developing world, with a predominant proportion in the low- and lower-middle income countries. The crop provides over 20% of total calories in human diets in 21 countries, and over 30% in 12 countries that are home to a total of more than 310 million people (Shiferaw et al. 2011).

Asian countries are making rapid strides in maize production and productivity. In most of Asia, especially in South and South East (SE) Asia, maize is predominantly grown under rainfed conditions by the smallholder farmers. Despite several constraints, including overdependence on rainfall, frequent climatic extremes, including drought, heat and/or waterlogging, yield losses due to pre- and postharvest pathogens and insect-pests, weeds, and lack of access to quality seed in some areas, several of the Asian countries have registered impressive growth rates in terms of maize area, production and productivity in the last 4-5 years.

China now cultivates much more maize than the USA. Compared to 2013 when China's maize area was around 33.5 M ha, in 2016 the harvested maize area in China touched almost 39 M ha (FAOSTAT, 2018). In 2017, Chinese farmers harvested about 35.84 M ha of maize, down by 2.5% from 2016. China is estimated to produce nearly 250 MMT of maize, almost 25% of the global maize production. Maize yields have registered impressive increases in China, reaching 5.95 t/ha in 2016 (Table 1).

South Asia has approximately 13-14 M ha under maize cultivation, while SE Asia grows maize on 9-10 M ha. SE Asia, however, fares slightly better in terms of average maize yield (~4.2 t/ha), compared to that of South Asia (`3.8 t/ha). India is the second most important maize growing country in Asia, with an estimated maize area of ~11 M ha in 2017. India's maize production rose from 11.15 MMT in 2002-03 to 22.5 MMT in 2012-13, and to 26.26 MMT in 2016 (Table 1). Maize grain production increased from about 7 million tons in 1980/81 to 11.5 MMT in 2002/03, to ~22 million tons in 2010/11, and to 26.26 MMT in 2016 (Table 1). This impressive growth has been largely driven by the increasing demand for maize grain as feed for the rapidly expanding poultry industry, coupled with adoption of maize in non-traditional areas, the strong role of the private sector in the maize seed industry, and the development and delivery of higher-yielding, single-cross hybrids. Indonesia is the third major maize growing country in Asia, with nearly 20 MMT of maize in 2016, way up from 9 MMT in 2013.

Animal feed is the largest end use segment for maize in Asia with ~70% of total volumes used by feed industry. Demand for maize will be further fueled by population growth and increasing inclination towards higher protein consumption in the form of meat and eggs (Shiferaw et al., 2011). Apart from feed and industrial applications, food processing industry is another crucial end use segment, as maize is being used for making food additives and sweeteners. With processed food industry slated to grow at 10%+ rate in the next five years in most countries of the region, maize demand is expected to further escalate.

Table 1. Maize area, production and yield in some major maize-producing countries in Asia (2016 data; Source: FAOSTAT 2018).

Sub-region / Country	**Area harvested (M ha)**	**Production (tons)**	**Yield (t/ha)**
East Asia			
China	38.98	231.84	5.95
South Asia			
India	10.20	26.26	2.57
Pakistan	1.33	6.13	4.60
Nepal	0.89	2.23	2.50
Bangladesh	0.33	2.45	7.30
Afghanistan	0.15	0.31	2.05
SE Asia			
Indonesia	3.79	20.37	5.37
Philippines	2.48	7.22	2.91
Viet Nam	1.15	5.24	4.55
Thailand	1.14	4.81	4.23
Myanmar	0.49	1.83	3.75
Lao PDR	0.26	1.55	6.00
West Asia			
I.R. Iran	0.13	0.90	6.89
Turkey	0.68	6.40	9.42
USA (only for comparison)	35.11	384.78	10.96

Note: Only those countries with at least 100,000 hectares of harvested area under maize are included in the table.

Adaptation to the Changing Climates

Abiotic stresses, especially drought, heat, waterlogging, acidity, combination of drought and heat, have a huge effect on rainfed maize yields in Asia. In South and South East Asia, more than 80 percent of the maize-growing area is rainfed and prone to various climatic extremes/variabilities. While we tend to focus mostly on abiotic stresses in the context of changing climates, it is equally important to consider the changing spectrum of pathogens and insect-pests. In the future, pest species are likely to differ in their responses to global warming, with changes in their relative impacts both geographically and among various crops. Deutsch et al. (2018) highlighted that global yield losses of three of the most important cereal staples – rice, maize and wheat – are projected to increase by 10 to 25% per degree of global mean surface warming. Crop losses will be most acute in areas where increase in temperatures may lead to increases in both population growth and metabolic rates of insects.

In this context, development and deployment of improved maize varieties with tolerance to abiotic stresses (drought, heat, waterlogging, salinity, and combination of drought and heat stress), nitrogen use efficiency, disease and insect-pest resistance, and improved nutritional quality, are crucial for building resilience and adaptive capacity of the farming communities in the tropics to the changing climates (Shiferaw et al. 2014). Through the USAID-funded Heat Tolerant Maize for Asia (HTMA) project, a large heat-stress phenotyping network, comprising 23 sites in the four Asian countries, has been established. Several drought tolerant and heat-tolerant CIMMYT-derived elite maize varieties have been released during 2016-2018 by public and private sector partners in South Asia, and several more are in pipeline. Tesfaye et al. (2017, 2018) highlighted the potential benefits of incorporating drought, heat and combined drought and heat tolerance into maize varieties in the climate-vulnerable tropical environments. The magnitude of the simulated benefits from drought tolerance, heat tolerance and combined drought and heat tolerance and potential acceptability of the varieties by farmers could vary across sites and climate scenarios, indicating the need for proper targeting of varieties where they fit best and benefit most.

Climate-smart Sustainable Intensification Practices

While enhanced adoption of climate-resilient maize varieties is undoubtedly important, one must not ignore the need for complementary uptake of other climate-smart, sustainable intensification practices in Asia. Drought tolerant maize can only tolerate (not resist) spells of drought, especially during the most sensitive flowering stage, but such varieties cannot effectively tolerate prolonged drought during the vegetative and grain-filling stages. Therefore, building climate resilience in Asia requires a multi-disciplinary and multi-institutional strategy. This includes more extensive awareness creation and adoption of climate-smart agronomic management practices, strengthening of local capacities, and focusing on sustainability.

Precision-conservation agriculture, scale-appropriate mechanization and integrated nutrient management can help support sustainable intensification of maize-based cropping systems, helping to improve efficient use of resources (soil, labor, water and nutrients). Scale-appropriate mechanization can also have significant social benefits like increased income, employment, food security, and less drudgery. Adoption of agricultural mechanization in Africa, Asia, and Latin America has reaped many benefits. For example, farmers in many parts of Africa and Asia are saving up to 45 days of labor with direct-seed machinery in conservation agriculture systems, compared to conventional methods. In addition to the above, intensive and deliberate efforts need to be made to provide farmers with usable climate risk information and skills to build their comprehensive adaptive capacity.

Increasing Genetic Gains in the Stress-prone Tropics

Increasing genetic gain in grain yield in stress-prone environments of the tropics could be a challenge, but certainly possible with a clear product development and deployment strategy (Cairns and Prasanna, 2018), The “breeders’ equation” provides the focus around which new technologies can contribute to increased genetic gain.

Doubled haploid (DH) technology

One of the simplest ways to increase genetic gain is to reduce the breeding cycle time - if selection intensity, accuracy and variability remain constant, halving cycle time will double the genetic gain (Xu et al. 2017). Breeding cycle times are typically 10 years or more in the tropics, compared to less than five in temperate regions (Challinor et al. 2016). Faster product cycle times are not only important for adaptation to the changing climates, but also for countering emerging pests and diseases. Doubled haploid (DH) technology has now been optimized and deployed in sub-Saharan Africa, reducing the time taken to develop parental lines (Prasanna et al. 2012). CIMMYT’s work on DH-based maize breeding has greatly expanded in the past few years. Through dedicated maize DH facilities in Kenya and Mexico, CIMMYT Global Maize Program produces annually over 100,000 DH lines (up from less than 5000 in 2011) and selects the best out of these lines in breeding pipelines. Recognizing the scope to further improve the first-generation tropicalized haploid inducers for various traits, CIMMYT team recently developed superior second-generation haploid inducers for tropics using marker-assisted breeding (Chaikam et al. 2018). These inducer lines (called CIM2GTAILs) have high haploid induction rates (~10-13%), better agronomic performance in terms of plant vigor, synchrony with tropical populations, better standability, resistance to tropical foliar diseases and resistance to ear rots compared to first-generation TAILs in trials at different locations in Mexico and Kenya. Inducer hybrids developed using these CIM2GTAILs exhibit greater heterosis for plant vigor and pollen production while maintaining similar haploid induction rates as the parents and are well suited for open pollinations in isolation nurseries.

Maize breeding programs of most of the national agricultural research systems (NARS) and small- and medium-enterprise (SME) seed companies in South and SE Asia are yet to tap the benefits of DH technology. This issue was highlighted and discussed during the 12th Asian Maize Conference in October 2014, but four years hence, the situation largely remains the same. This issue needs to be addressed soonest.

High-throughput field-based phenotyping

The development of low-cost, high-throughput phenotyping tools has the potential to play an important role in reducing breeding costs, thus allowing resources to be allocated to generation and management of larger populations, enabling an increase in selection intensity within a fixed budget (Araus et al. 2018). Recently, there have been many advances in the development of high-throughput phenotyping tools for traits extensively used within maize breeding programs. Zaman-Allah et al. (2018), in this volume, highlighted the potential opportunities in this regard, including the power of proximal and remote sensing tools to reliably phenotype important plant traits (Makanza et al. 2018a), and for using image analysis to quantify maize yield components (Makanza et al. 2018b).

Genomics-assisted breeding

For effectively meeting the challenge of developing improved cultivars with combinations of relevant adaptive traits, including biotic and abiotic stress tolerance, and nutritional quality, it is imperative that breeding programs routinely use molecular tools in product development. With the rapid reduction in genotyping costs, new genomic selection technologies have become available that allow the maize breeding cycle to be greatly reduced, facilitating inclusion of information on genetic effects for multiple stresses in selection decisions. Nair et al. (2018), further in this volume, discussed in detail the progress and prospects of genomics-assisted maize breeding in the tropics, including trait-marker discovery and marker deployment and genomic prediction, and other enabling technologies that complement genomic tools.

It is noteworthy some of the Asian countries, especially China and India, have also made significant progress in developed biofortified maize varieties using molecular marker-assisted breeding. For example, at the ICAR-Indian Agricultural Research Institute, marker-assisted introgression of *opaque2* have recently led to the commercial release of three QPM hybrids viz., ‘Pusa HM4 Improved’, ‘Pusa HM8 Improved’ and ‘Pusa HM9 Improved’ (Hossain et al. 2018a). These hybrids possessed 3.49% and 0.84% lysine and tryptophan in protein, respectively. Also, pyramiding of *opaque2* and *opaque16* showed an increase of 64% lysine and 86% tryptophan over o2-based hybrids. ‘Pusa Vivek QPM9 Improved’, India’s first provitamin-A rich maize hybrid was developed through introgression of *crtRB1*. This hybrid showed 8.15 µg/g of provitamin-A compared to 1-2 µg/g in normal maize (Muthuswamy et al. 2014; Hossain et al. 2018b).

Strengthening Asia’s Maize Seed Systems

Targeted deployment of improved climate-resilient varieties by GIS-based prediction of areas of climate vulnerability, emphasis on quality assurance/quality control (QA/QC) throughout the seed value chain (Gowda et al. 2017), improving varietal turnover (with newer and better genetics), recommendations on appropriate agronomic management practices for realizing the genetic potential of improved varieties, especially in stress-prone environments, and creating better linkages of the smallholder maize farmers to output markets (for providing greater incomes to the farmers) are all absolutely critical for strengthening maize value chains in Asia.

For new climate-resilient maize varieties to contribute towards smallholders' adaptation to climate variability in Asia, it is important to further strengthen the seed systems. Delivering low-cost improved maize seed to smallholder farmers with limited purchasing capacity and market access requires stronger public-private partnerships, and enhanced support to the committed local seed companies, especially in terms of information on access to new products, adequate and reliable supplies of early-generation (breeder and foundation) seed, and training on hybrid seed production, quality assurance/quality control (QA/QC), seed business management, market segmentation and territory planning.

While an array of climate-resilient maize varieties have been released in the recent years, especially through the CIMMYT-Asia product pipeline, a lot still remains to be done in South and SE Asia in terms of market-oriented adoption of these varieties, replacing the old/obsolete climate-vulnerable varieties that are presently grown by the resource-poor farmers in the stress-prone rainfed areas. This requires intensive awareness creation, on-farm demonstrations and extension efforts, coupled with appropriate government policies and institutional innovations for enhancing affordability and timely access of quality seed. Appropriate government policies and adoption of progressive seed laws and regulations are critical for improving smallholder farmers' access to improved climate-resilient seed, and for overcoming key bottlenecks affecting the seed value chains, particularly in the areas of policy, credit availability, seed production, germplasm and marketing (Cairns and Prasanna, 2018).

Tackling the Emerging Threat of Fall Armyworm

In the 12th Asian Maize Conference in 2014, I have highlighted the outbreak and rapid spread of maize lethal necrosis (MLN), caused by a combination of two viruses – Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV) – in eastern Africa, affecting the food security and income of smallholder maize farmers in the region. A new and complex challenge called Fall Armyworm (*Spodoptera frugiperda*; FAW), a highly aggressive and invasive insect-pest with devastating effect, has been officially reported in the beginning of 2016 in Nigeria, and since then, rapidly spread across the African continent. Presently, more than 40 countries in Africa have officially reported the incidence of FAW. In July 2018, the southern state of Karnataka in India was the first to officially report the incidence of FAW.

FAW has a strong appetite for maize; therefore, the implications of the incidence of this pest in maize-growing countries in Africa and India is indeed a major concern. CIMMYT is at the forefront in the fight against FAW in Africa, in collaboration with several national, regional and global partners, focusing on an integrated pest management (IPM) strategy (Prasanna et al., 2018). There is an urgent need to generate widespread awareness and to empower the farming communities with knowledge of the pest, along with suitable technologies/management practices for its sustainable management. We continue to learn from the experiences of countries like the US and Brazil where the pest has been successfully managed for several decades. At the same time, in Africa, we are getting to understand various ecological factors that influence the pest ecology in the new environment in Africa and developing technologies that are useful for its management as relevant to the African agro-ecologies and cropping systems landscapes. The same needs to be done in India, and possibly elsewhere in Asia, as the pest has high capacity to migrate.

The pest migrates very fast (almost 100km per night, and nearly 500 km before laying eggs), and thus, can invade new areas quickly. It can complete its life cycle within 1-2 months (depending on weather conditions), with each female moth capable of laying on average 1500 eggs). It is one of the most destructive crop pests, with a wide spectrum of host range (including maize, rice, sorghum, sugarcane, soybean, vegetables etc.). Based on a recent study (Early et al. 2018), the strongest climatic limits on FAW's year-round distribution are the coldest annual temperature and the amount of rain in the wet season. Much of sub-Saharan Africa can host year-round FAW populations. South and Southeast Asia and Australia have climate that would permit fall armyworm to invade. Current trade and transportation routes reveal Australia, China, India, Indonesia, Malaysia, Philippines, and Thailand face high threat of FAW invasions originating from Africa.

Effective monitoring, surveillance and early warning systems, coupled with capacity to quickly respond to any new insect-pest threat through IPM, are vital for safeguarding the crops and to protect the income and livelihoods of the smallholders that dominate the Asian agrarian landscape. Based on the experiences so far, it is indeed clear that there is no single solution for sustainable management of FAW, and we need to have an evidence-based, inclusive and well-balanced IPM strategy. An effective IPM strategy for control of FAW will employ host plant resistance, biological control, cultural control, and environmentally safer synthetic and biopesticides to protect the crops from economic injury while minimizing negative impacts on people, animals, and the environment. Many organizations, including both public and private sector, have been intensively working on identifying/validating/developing technologies/management practices that can help manage the pest in Africa, as well as creating awareness among the stakeholders on monitoring, surveillance and IPM-based FAW control in Africa and Asia.

Sources of native genetic resistance (partial, polygenic resistance) to FAW have been developed through intensive work at CIMMYT-Mexico during 1970s to 1990s (Mihm et al. 1997), and through research work conducted by USDA-ARS, University of Florida, and Embrapa-Brazil. Some of these sources of insect-pest resistance were specifically tested against FAW, while others were tested for resistance to other insect-pests but have potential to confer resistance to FAW. While identifying materials with native resistance to FAW, it is important to consider not only foliar rating but also ear/kernel ratings, as FAW can also cause significant ear/kernel damage, especially when the larvae gain entry into the developing ears. CIMMYT team in Africa is now intensively screening maize germplasm for native genetic resistance to FAW under artificial infestation (under net-houses) in Kiboko, Kenya, and some promising inbred lines have been identified.

CIMMYT and IITA, under the CGIAR Research Program MAIZE established a FAW R4D International Consortium in which more than 35 international / regional organizations are now part of, for a collective and synergistic R4D action. The Consortium aims to bring together diverse institutions in public and private sectors to explore ways to synergistically work on short-, medium- and long-term solutions to tackle the challenge of FAW in Africa, and in other parts of the world where the pest is prevalent.

Conclusions

Intensive multi-institutional efforts are required to identify and utilize climate-resilient tropical/subtropical maize germplasm in product development pipelines. There is an increasing body of evidence confirming the benefits of climate-resilient maize varieties to increase yields, reduce yield variability and, ultimately, increase food security. To increase genetic gains through maize breeding in the stress-prone tropics, and for enhancing the the pace, precision and efficiency of breeding progress, judicious and effective integration of modern tools/strategies, especially high-density genotyping, high throughput and precision phenotyping, DH technology, molecular marker-assisted and genomic selection-based breeding, and knowledge-led decision-support systems, is vital.

Genetics and breeding alone cannot solve the complex challenge of enhancing maize productivity at the smallholder farm level, especially in the face of depleting/degrading natural resources and changing climates. There is a distinct need for effective complementation of improved maize cultivars with suitable precision-conservation agriculture practices, integrated nutrient management, scale-appropriate mechanization, as well as institutional and policy innovations for strengthening maize value chains.

Emerging seed enterprises in Asia need to be strengthened to become more market-oriented and dynamic, and for providing smallholders with greater access to affordable climate-resilient improved seed. Understanding the smallholder farmers' constraints for adoption of modern maize varieties, enhancing affordability and access to quality seed, and improving linkages of resource-poor farming communities to the input and output markets should be accorded top priority.

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Why CIMMYT Genotyped its Maize Germplasm Bank Collection

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Introduction

CIMMYT genotyped its 28,000 maize germplasm bank accessions using the DarTSeq genotyping platform, at a cost of about US$25 per sample. CIMMYT spent a lot of money to obtain this information, which is or will become a global public good. Scientists and funders are justified to ask what value is being derived from this investment. This presentation will describe the uses of genotypic data to guide the choice of materials for use in pre-breeding projects and to initiate innovative 'big data' research to discover useful genetic diversity in CIMMYT's germplasm bank collection.

Seeds of Discovery (SeeD) Project

The Seeds of Discovery (SeeD) project was developed with the ambitious goal of sequencing the CIMMYT bank to enhance the effective use of maize (and wheat) genetic diversity. The project was succinctly described in a recent publication, so the reader is directed to Pixley et al. (2018) for an overview. In brief, the SeeD project includes maize and wheat genotyping, phenotyping, pre-breeding and capacity development activities. The SeeD project has developed a platform consisting of 1) high-density genotypic data and extensive phenotypic data characterizing maize and wheat germplasm bank accessions, 2) software tools to enable bioinformatics analyses of these and relevant germplasm bank data, and 3) maize and wheat lines incorporating novel diversity for priority traits from exotic germplasm into breeder-preferred, elite genetic backgrounds (Pixley et al., 2018).

The genotypic data for maize bank accessions has been used for various purposes. The first example was to identify likely candidate accessions where we might find resistance to viruses causing maize lethal necrosis (MLN). After making a preliminary selection using geographical location data, genomic data were used to study genetic similarity among candidates and reduce the number of accessions to a manageable number for subsequent phenotypic evaluation. Pre-breeding work is ongoing for MLN resistance, as well as for tar spot, drought (Figure 1) and heat tolerance, and for nutritional quality conferred by anthocyanins (blue-pigmented maize).

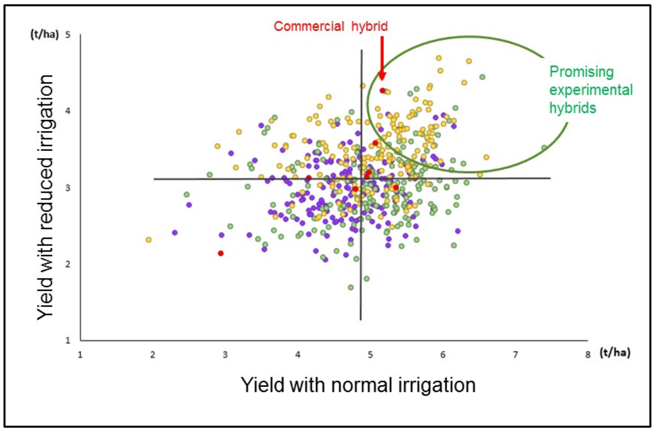

Figure 1. Graphical presentation of testcross trial results for hybrids of pre-breeding experimental lines crossed with three elite tester lines and evaluated at drought stressed (Y-axis) and well-irrigated (X-axis) locations. Pre-breeding lines producing hybrids within the upper-right quadrant of this graph are the most promising, because they performed well under drought and unstressed conditions.

The second and third examples of using genomic data are for discovery of useful genes and discovery of useful accessions in the germplasm bank. Environmental genome-wide association studies (EnvGWAS) associate precise geographical, climate and soil data for the collection sites, with genomic data for each collection (Figure 2) (Romero et al., 2017). Genomic regions that are associated with good or poor performance are identified in environments of interest, for instance, acid soils or high temperatures during grain fill. This method has been expanded to perform genomic predictions using thousands of molecular markers - as is now commonly done in genomic selection - to predict which bank accessions are likely to perform well in target product environments (TPE).

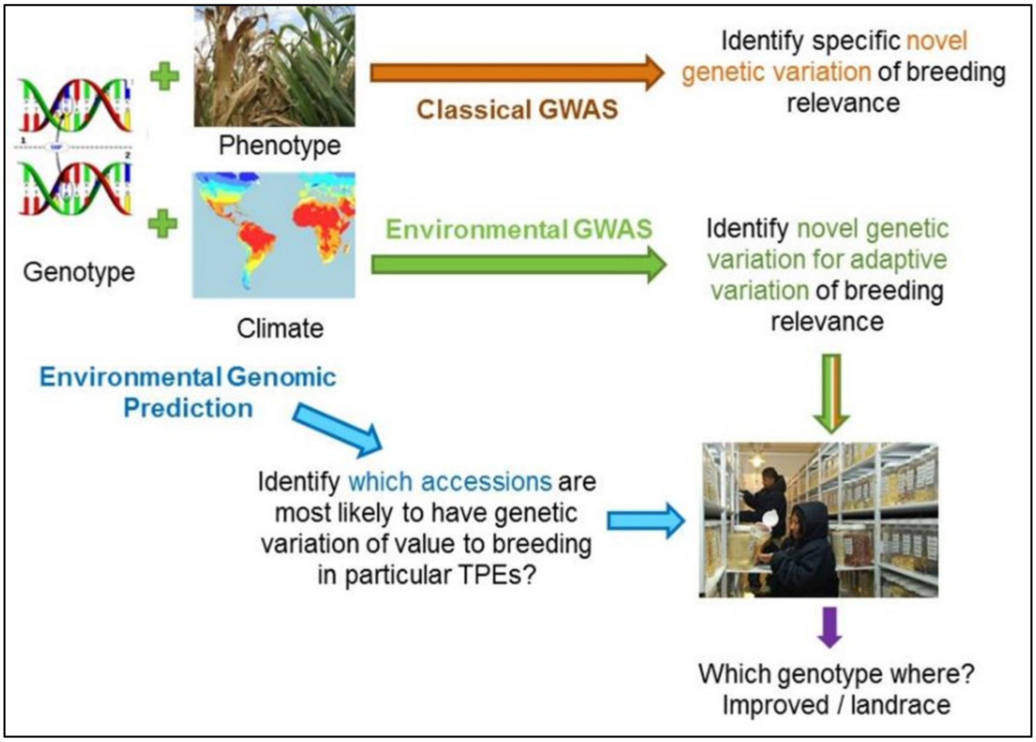

Figure 2. Schematic illustrating the difference between classical genome-wide association study (GWAS) and environmental GWAS (EnvGWAS). EnvGWAS and environmental genomic prediction are exciting, novel methods elucidated and under validation by the SeeD project. These depend on use of 'big data' from genotypes and geographic information systems (data on climate, soils, geographical location, etc.) to identify genomic regions (QTL and eventually genes) associated with good or bad performance in an environment of interest (e.g. drought, heat, acid soils, cold during grain fill, etc.). TPE = target product environment.

Several journal publications summarize the maize-specific work of the SeeD team (Brandenburg et al., 2017; Chen et al., 2016; Faux et al., 2016; Figueroa et al., 2013a and 2013b; Gorjanc et al., 2016; Hellin et al., 2014; Hickey et al., 2014a and 2014b; Pixley et al., 2018; Romero et al., 2017; Shaw et al., 2017; Swarts et al., 2014). Finally, CIMMYT has a quantitative geneticist with expertise in machine learning to further explore what guidance these genomic data can provide towards the effective use of the 28,000 maize accessions in CIMMYT's germplasm bank.

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High-throughput Field-based Phenotyping in Maize

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Background

Good phenotyping is one of the most critical piece of a successful breeding program. Methods for the measurement of most breeder-preferred traits have largely remained unchanged over the past few decades and are manual, laborious and time consuming; with some being prone to human error or lacking repeatability. The use of data collection methods that allow reliable assessment of crop traits at reasonable cost and faster than the methods currently in use, can significantly improve resource use efficiency and contribute to increased genetic gain through improved selection efficiency (Araus et al. 2018). The selection cost reduction will allow resources to be allocated to the generation and management of larger populations, enabling an increase in selection intensity within a fixed budget (Araus et al. 2018). Sensor technology coupled with progress in image processing offer radically new perspectives for field-based HTP and are anticipated to enable a better integration of phenotyping approaches into breeding programs by helping to (i) extract more value from every research plot and (ii) improve phenotypic data quality. Few examples of current developments on low-cost field phenotyping tools targeting breeder-preferred traits in CIMMYT maize breeding program are presented.

Plant and ear height measurements using hand-held laser distance meter

Plant and ear height have routinely been measured in the field using a telescopic stick with decimeter marks or a ruler and the data captured manually; making the process prone to errors and time and resources consuming. In the case of maize, the process is even more difficult because some genotypes/varieties can be as tall as 2m or more. Various sensors are currently available for measuring plant height but are not all applicable for measuring ear height. Those include LiDAR (Light Detection and Ranging), ultrasonic sensors, and RGB camera (Crommelinck and Höfle, 2016, Friedli et al. 2016, Hämmerle and Höfle, 2016). Assessment of plant height from images is relatively complex and the level of accuracy of the data still needs improvement before implementation in crop breeding. This can be done using Real Time Kinematic (RTK) GPS (Xiong et al. 2017) but the associated cost is still very high. Recently at CIMMYT, the use of sensors like the laser distance meter (Hand-held Leica Disto D110, Leica Geosystems AG, Heerbrugg, Switzerland) have provided new perspective for plant/ear height data collection. The sensor which can be used directly or mounted on a phenopole, connects to mobile phones or tablets through Bluetooth, providing a simple, very low-cost estimation of plant and ear height (<200 USD per sensor). Estimated plant and ear height were highly correlated with measurements collected using a ruler. Only one person is required to take measurements with a laser distance sensor compared to two people when a ruler is used, thus reducing the cost of data collection by 50%. The data are also captured automatically in an excel sheet, which significantly reduces the time required for measuring plant or ear height.

Aerial sensing for early vigor and canopy senescence assessment

Crop early vigor and canopy senescence are often assessed based on visual scores that are qualitative, and often subjective. Imaging methods can provide a standardized, rapid, cost effective and more objective way of collecting these data. The common methods include the use of canopy reflectance (for example Normalized Difference Vegetation Index (NDVI)) to monitor crop cover or leaf senescence but the cost of sensors is often high or at least higher than that of an RGB camera. Recently at CIMMYT, RGB images taken with consumer-grade digital cameras onboard low-cost unmanned aerial vehicle (UAV) were used to derive a senescence index based on the ratio of senesced canopy to total canopy cover under low nitrogen conditions. The senescence index was highly correlated with grain yield compared to visual measurements of canopy senescence, while broad-sense heritability was equal to or higher than visual measurements (Makanza et al., 2018a). The time required for phenotyping using a UAV was reduced by 95% relative to visual measurements. With advances in image analysis methods, the rapid cost reduction of sensors, and effective image processing software, there is still potential for wider applications of field-based phenotyping by UAVs.

	A
1	0.8992
2	1.4016
3	1.0407

Figure 1. Plant and ear height data collection using the hand-held Leica Disto D110 (Leica Geosystems AG, Heerbrugg, Switzerland) at the CIMMYT-Harare research station in Zimbabwe.

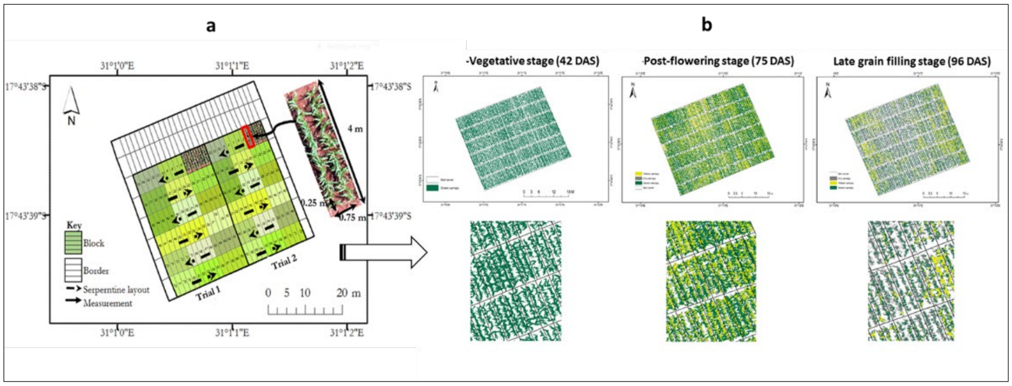

Figure 2. (a) Pre-processed details of a portion of a maize field with plot details and (b) time sequence processed aerial images of maize hybrids at three different developmental stages grown at the CIMMYT-Harare research station in Zimbabwe.

Digital ear phenotyping

Recent open-source image analysis protocols have been developed to measure maize yield components using both a line scanner and conveyor belt and flatbed scanner (Liang et al. 2016; Miller et al. 2017). These methodologies are generally slow (only a couple of ears per photo i.e 1-5) and not easy to use in the field. Besides, they do not provide a comprehensive data (all ear and kernel traits) set from a single image of unthreshed ears. Ear digital imaging (EDI) is a simple, low-cost, high-throughput and robust method for extracting yield components (ear and kernel attributes) from harvested maize ears developed at CIMMYT (Makanza et al., 2018b). The method provides estimates of ear and kernel attributes i.e., ear number and size, kernel number and size as well as kernel weight from photos of ears harvested from field trial plots. The image processing method uses a script that runs in a batch mode on ImageJ; an open source software. Kernel weight was estimated using the total kernel number and the average kernel size. Estimated yield components (including kernel weight and number) were significantly correlated with manual measurements of yield components (r >0.80). Current investment in combine harvesters at key breeding locations may supersede this technology; however, it will continue to provide an important quality control feedback in on-farm trials where yields are generally measured by non-researchers. Furthermore, the cost and maintenance of harvest equipment does not make them accessible for small breeding programs, especially within national programs.

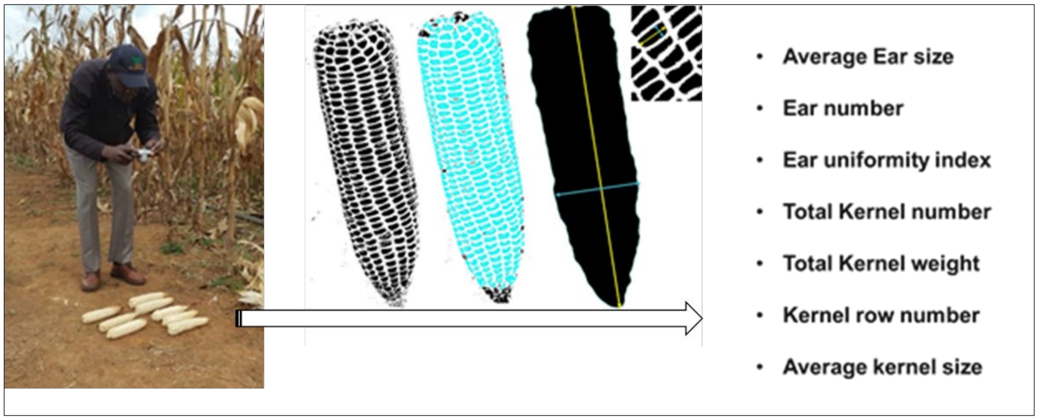

Figure 3. Photo acquisition, simplified processing steps and ear and kernel attributes than can be generated using the ear digital imaging (EDI) method.

Future prospects

Selection cost reduction is an essential component of the breeding efficiency improvement process. The development and increased availability of robust sensing-based crop phenotyping methods to plant breeders will significantly assist in improving the efficiency of the breeding process. Aerial sensing is anticipated to play a major role because it enables the generation of data at the high resolutions needed for accurate crop parameter estimations, and allows in-season dynamic assessment of the crop due to the ability to fly missions at high temporal frequencies.

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Genomic and Enabling Technologies for Enhancing Genetic Gain in Tropical Maize

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Introduction

By 2050, world annual demand for maize, rice and wheat is expected to reach some 3.3 billion tons, or 800 million tons more than 2014's record combined harvest, and this will have to happen from similar or perhaps much lower land resources (FAO, 2016). At the same time, changing climates, environmental degradation, and devastating pathogens and insect pests are known threats to crop production and productivity, especially in the tropics. Crop yields are a result of the interplay of improved genetics and agronomy (Duvick, 2005). For consistent increase of yields against all the above-mentioned threats facing agriculture, improved genetics need to play an important initial role, while the potential needs to be effectively translated at the farm-level through improved crop management.

Genetic gain in simple terms refers to the gain in population mean achieved through each breeding cycle. Conventional maize breeding, although successful, is a relatively slow and resource-intensive process. The increasing demands for high-yielding, multiple stress tolerant and nutritionally enriched maize varieties warrant accelerated breeding that makes use of modern tools and technologies, including doubled haploids, molecular markers, high-throughput and reliable phenotyping, off-season nurseries and decision support tools.

Maize genome: challenges and opportunities

The maize genome is approximately 2500 Mb in size, comparable to the size of the human genome. Maize inbred lines have an average nucleotide diversity of around 1% in the genic regions (Tenaillon et al., 2001; Wright et al., 2005), similar to the divergence between humans and chimpanzees (Mikkelsen et al., 2005). Read-depth variants, representing moderate sized deletions, insertions and duplications add to this complexity of maize (Chia et al., 2012), along with the structural changes leading to gene non-collinearity among inbred lines caused by activities of transposable elements like Helitrons (Fu and Dooner, 2002). Whereas the genetic diversity shown by this amazing crop has helped drive its domestication and adaptation to diverse agro-ecologies worldwide, and helped in improvement through breeding and selection, the size and complexity of the genome poses a challenge in terms of discovery and deployment of genomic tools for improving some key traits relevant for smallholders, and continuously increasing genetic gains.

The genetic architecture of maize is more dispersed, as compared to self-pollinated crops like rice; majority of the agronomically important traits in a cross-pollinated crop like maize appear to be controlled by many small-effect genes (Morrell et al., 2012; Wallace et al., 2014). The most important consequence of mating system is on the levels of heterozygosity and the amount of effective recombination which induces fundamental differences in trait architecture and patterns of linkage disequilibrium or LD (Morrell et al., 2012), and thus, breeding strategies. This could also apply to genomics-assisted breeding strategies, as such strategies differ among various crop species, based on their genome complexities and trait architecture. Although highly diverse maize populations show rapid decline of LD, as in humans, it is possible to define populations with strong LD (Rafalski and Morgante, 2004), like a closed breeding pool, that is more amenable to genomics-assisted breeding.

Genomics and other omics: bottlenecks anymore?

From the time when DNA-based markers like RFLPs were developed and used in the 1980s (Tanksley et al., 1989) to the present time about three decades later, genomic technologies have been constantly evolving. The most substantial improvements happened by the advent of single nucleotide polymorphisms (SNPs) and the whole genome sequencing of crop plants. The whole genome of maize, specifically of the popular temperate inbred line B73, was sequenced in 2012 (Schnable et al., 2012). Through the years, genomes of 1218 maize lines have been sequenced and enormous amounts of variation of more than 83 million variants documented (HapMap Version 3, www.panzea.org; Bukowski et al., 2018). Maize SNP chips like Illumina® MaizeSNP50 BeadChip (Ganal et al., 2011) have been available for a long time; however, their utility in analysis of the tropical maize germplasm was found to be limited due to ascertainment bias (caused by use of the temperate germplasm for developing the chip). There have been SNP chips with larger SNP number like the Axiom 600K (Unterseer et al., 2014) and with inclusion of SNPs related to specific traits, such as the Maize 55K Axiom (Xu et al., 2017). These are yet to find their place in routine trait analysis and breeding applications. Apart from fixed arrays, there have been NGS platforms like Genotyping by Sequencing (GBS) (Elshire et al., 2011), DarT-Seq (www.diversityarray.com) and rAmpSeq (Buckler et al., 2016) that provide SNP calls anywhere between 2K–1000K variants in maize.

Apart from genomics, there have been a lot of new omics that emerged and evolved, with varying degrees of application and utility in maize breeding, including; transcriptomics, proteomics, metabolomics, epigenomics etc. How best such "omics" can be used in maize breeding, especially for improving genetic gains for key traits, depends on various factors, including cost and capacity to convert the enormous amount of data into selection decisions during the product development pipelines. Though there are a number of genotyping platforms available at present (and likely to further evolve in the future), there is a gap in terms of access for breeding programs in many developing countries to low-cost/ affordable, high-throughput genotyping platforms. Recent developments in terms of establishment of HTPG (http://cegsb.icrisat.org/high-throughput-genotyping-project-htpg/) and Excellence in Breeding (EiB) platform (http://excellenceinbreeding.org/) offer opportunities to have low-density trait-based marker analyses in crops, as well as medium-density genotyping for applications of forward breeding and genomic prediction in crop plants.

Genetic gains in maize breeding

Breeding being a cyclical process of crossing, evaluating, selecting and crossing again, the efficiency (both in terms of time and cost) with which breeding programs can make considerable shift in population mean from one cycle to the other determines the genetic gains. Regardless of the trait of interest, or the breeding methods employed, genetic gain represented by "ΔG" serves as a simple universal expression for expected genetic improvement (Falconer and Mackay, 1996). The genetic gain equation that represents the factors leading to genetic gain (rather than a formula to calculate the same) came to be known as the "breeders' equation". The genetic gain equation can be represented as $\Delta G = i.r.\sigma A/t$, where 'i' represents selection intensity, 'r' represents selection accuracy, 'σA' represents genetic variance and 't' represents cycling time (Araus et al., 2018). These components are discussed in greater detail later in the article.

Under optimal conditions, genetic gain for maize grain yield was estimated at 94.7 kg ha^{-1} yr^{-1} in China over a period of 30 years (Ci et al., 2011), 132 kg ha^{-1} yr^{-1} in Argentina over a period of 32 years (Luque et al., 2006), 80 kg ha^{-1} yr^{-1} in Canada over a period of 100 years (Bruulsema et al., 2000), and 65 to 75 kg ha^{-1} yr^{-1} in the United States over a period of 70 years (Duvick, 2005). Badu-Apraku et al. (2013, 2016) estimated genetic gain by era studies for a period of 23 years in OPVs in West and Central Africa and estimated a genetic gain of 40 kg ha^{-1} yr^{-1} under optimal conditions and 13.5 kg ha^{-1} yr^{-1} under random stress. Over a period of 10 years, an era study conducted by CIMMYT team in eastern and southern Africa (ESA) estimated the genetic gains from the CIMMYT hybrid maize breeding program at 109.4, 32.5, 22.7, 20.9, and 141.3 kg ha^{-1} yr^{-1} under optimal, managed drought, random drought, low-N, and maize streak virus (MSV) infection, respectively (Masuka et al., 2017). There are not many reports of evaluating genetic gains for key traits made by the maize breeding programs in the tropics. It is imperative to constantly monitor the gains in the breeding programs and take appropriate measures to maintain the gains in the face of emerging threats and constraints. In this article, we have discussed how genomic tools and other enabling technologies can potentially impact different components affecting genetic gain.

Genomic technologies for maize breeding

Trait-marker discovery and marker deployment

Most of the agronomically and economically important traits in maize are quantitatively inherited (Wallace et al., 2014), with several small effect genetic loci with epistatic and environmental interaction. When two genetically diverse parents are crossed producing a progeny with maximum genetic variation for a particular trait, linkage mapping can be done to elucidate linkage/association between specific markers and the genetic loci controlling the trait; this is called QTL mapping. Considering its major limitation, that only allelic diversity that segregates between the parents of the particular population can be assayed, genome-wide association study (GWAS) in a panel of breeding-relevant diverse lines came into vogue. After the first GWAS reported in maize a decade ago (Belo et al., 2008), there have been numerous publications on GWAS in maize for traits ranging from nutritional quality to abiotic and biotic stress tolerance, and grain yield (Xao et al., 2017). The plethora of articles on GWAS, especially after the whole genome sequencing in maize, created an unfounded perception that GWAS is the method of choice for genetic mapping, against QTL mapping. But, it must be emphasized that QTL mapping and GWAS are quite complementary, and, when effectively combined, can overcome each other’s limitations (Korte and Farlow, 2013). Based on this understanding, huge resources for joint linkage and association mapping have been created in temperate maize (Yu et al., 2008; Dell’Acqua et al., 2015), but such populations based on tropical maize germplasm, which could be genotyped at high density and shared across the tropical maize-based breeding programs, is not developed yet. Several association mapping panels have been assembled in CIMMYT, like the DTMA (Drought Tolerant Maize for Africa) panel, IMAS (Improved Maize for African Soils) panel, CAAM (CIMMYT Asia Association Mapping) panel and HTMA (Heat Tolerant Maize for Asia) panel, which have been used in GWAS of many traits relevant to the tropics (Suwarno et al., 2015; Zaidi et al., 2016; Nair et al., 2015; Gowda et al., 2018; Rashid et al., 2017; Vemuri et al., 2018; Cao et al., 2017). Similarly, hundreds of articles have been published on QTL mapping for various traits relevant to tropical maize, but there are few studies where both these approaches are judiciously employed to discover and validate trait-associated markers.

The routine deployment of trait-based markers in the forward breeding pipeline requires high-throughput low-density marker system at an economical cost with a fast turn-around time. The cost efficiency will be decided based on the accuracy of the markers being used in the breeding pool and the relative cost and time advantage with respect to existing phenotyping methods. High throughput platform for genotyping (HTPG) is an initiative funded by the BMGF to broker access to low-cost and fast turn-around genotyping facilities to CGIAR institutions, and NARS and SME seed company partners. This initiative envisions that lowering the genotyping cost will enable CGIAR and other NARS/ SME breeders to utilize marker-based selection in forward breeding and to take advantage of low-cost genotyping in breeding applications. As part of this initiative, a set of 10 SNPs could be genotyped in a turn-around time of two weeks at a cost of 1.5 USD (http://cegsb.icrisat.org/high-throughput-genotyping-project-htpg/).

Trait-based markers are developed and deployed in tropical maize for various quality traits like *opaque 2*, *opaque 16* and provitamin-A in various breeding programs in the tropics (Babu et al., 2005; Gupta et al., 2009; Muthusamy et al., 2014; Zunjare et al., 2018; Sarika et al., 2018). Two of them are causal gene-based markers and are associated with high effect size. Similarly, other trait-based markers based on causal genes are being used in marker-assisted breeding for waxy and sweet corn (Yang et al., 2013; Faqiang et al., 2015). There are many efforts to discover, validate and deploy trait-based markers for resistance to major diseases in the tropics. *Msv1*, a major effect locus contributing to MSV resistance has been fine-mapped (Nair et al., 2015) and is being deployed widely in a forward breeding pipeline in ESA. Maize lethal necrosis (MLN) is another devastating disease where genomic tools were efficiently discovered and are being deployed in the CIMMYT maize breeding programs in Africa (Gowda et al., 2018). Markers for *qhir1*, a major QTL for haploid induction in maize, have been successfully used by CIMMYT Maize Program in developing second-generation tropicalized haploid inducers (Chaikam et al., In press). In CIMMYT-Asia Maize Program, association mapping for resistance to turcicum leaf blight (TLB) was carried out in three association mapping panels, and SNPs/haplotypes have been identified for TLB resistance. Five biparental populations have been used for validating 14 low-moderate effect SNPs/haplotypes for TLB resistance. This is in accordance to the understanding of the genetic architecture of most of the resistance traits in maize (Wallace et al., 2014; Yang et al., 2017). Two of the validated haplotypes were already in mapped genomic regions for TLB race-specific major genes, *ht1* and *htn1* (www.maizegdb.org; Hurni et al., 2015). These were analyzed on a set of 124 breeding lines from the CIMMYT-Asia breeding pool, and a set of 10 (two SNPs and four haplotypes) have been identified, that individually led to reduction in disease severity among the breeding lines to the tune of 10.6-20.1%. Combination of any of the two SNPs reduced the disease severity in breeding lines by 20-30%. The favorable allele frequency of these SNPs ranged from 0.43-0.82 among the set of breeding lines (Nair et al., unpublished). These SNPs/ haplotypes are being pilot tested in the CIMMYT breeding populations to deploy them in forward breeding through the HTPG initiative.

Genomic prediction

Trait-linked markers that were discovered and deployed by way of marker assisted selection (MAS) require prior knowledge of the precise location and effect size of QTLs and germplasm specificity. This also requires that the innate genetic architecture of the species supports such interventions by way of having large effect genes/ QTLs controlling agronomically important traits. As discussed before, having very few of such loci identified in maize, genomic prediction becomes an important tool to improve genetic gains. "Genomic prediction" is a form of marker assisted selection where genome-wide markers are used to estimate the breeding value of individuals (Meuwissen et al., 2001). The concept that was originally developed in dairy cattle found its way to crop plants, especially in crops like maize. While the public sector maize breeding programs in the tropics have been slow to make use of genomic prediction, multi-national companies have been routinely practicing genomic prediction in their breeding pipelines. Genomic prediction brought a paradigm shift in the way plant breeding is done, shifting the unit of selection from individual lines to alleles (Lorenz and Nice, 2017). Several factors like heritability, trait architecture, marker density, training population size and relationship between the training and prediction populations are critical for the accuracy of the predicted breeding value (Combs and Bernardo, 2013).

When medium- to high-density genotyping costs and turn-around times decrease sufficiently, to at least partially replace resource-intensive field-based phenotyping, genomic prediction will be highly beneficial and cost-efficient in driving genetic gains in breeding programs. Recently, two medium-density genotyping options have been proposed and deployed with reduced cost; rAmpSeq (Buckler et al., 2016), and rhAMPSeq from Integrated DNA technologies, the costs of which are expected to stabilize at USD 5 per sample. These advancements have huge scope for deploying genomic prediction in public sector breeding programs in the tropics. Practical haplotype graph (PHG), which represents a simplified pan-genome graph of maize is currently under development. Low cost sequencing technologies, coupled with the PHG, facilitate the genotyping of large numbers of samples to increase the size of training populations for genomic prediction models (https://bitbucket.org/bucklerlab/practicalhaplotypegraph/wiki/Home). Other than low-cost marker systems, implementing whole genome prediction models in routine breeding pipelines require careful development of relevant training sets and their phenotyping at high precision, to have an impact on continued and enhanced genetic gains (Cooper et al., 2014; Lorenz and Nice, 2017).

Genomic prediction could be applied to source population improvement by way of rapid cycling and could lead to improvement in genetic gains primarily due to changing allele frequencies through use of markers in a time-efficient manner. In a study of rapid cycle genomic selection (RCGS) in eight biparental populations in eastern Africa, the average gain from genomic selection per cycle across eight populations was 0.086 t ha^{-1}. The average grain yield of Cycle 3-derived hybrids was significantly higher than that of hybrids derived from Cycle 0. Hybrids derived from C3 produced 7.3% higher grain yield under drought than those developed through the conventional pedigree breeding method (Beyene et al., 2015). In two biparental RCGS for deriving improved stress tolerant lines in the CIMMYT-Asia breeding program, a gain of 10-20% in grain yield under drought was observed after two cycles of genomic selection, compared to phenotypic selection (Vivek et al., 2017). RCGS is also applied in multi-parent synthetic populations in CIMMYT breeding programs to increase the efficiency of line derivation. In a multi-parent population, a 7.74% increase in genetic gains was observed under optimal conditions for grain yield (Zhang et al., 2017). CIMMYT maize breeding program in Asia is currently working with RCGS of six biparental populations for deriving doubled haploid (DH) lines from improved cycles (Sudha Nair, unpublished).

Apart from population improvement, genomic prediction based on early stage yield testing (Stage 1) is an important tool in the modern maize breeding pipeline, enabling increased selection intensity and reduced cost and time. In a proof of concept study in a set of 22 biparental populations evaluated for grain yield and other agronomic traits, moderate to high prediction accuracies were obtained with higher heritability and with a training population size that was at least 50% of the total population (Zhang et al., 2017). CIMMYT has started routine breeding program-wide genomic predictions in biparental maize populations in 2017, represented by 15,000 breeding lines entering Stage 1 testing; at least 50% of the CIMMYT breeding pipelines are expected to be based on genomic prediction by 2021-2022 (Zhang, X., unpublished). Toward this, CIMMYT maize breeding program in Asia genotyped about 3000 DH lines using rAmpSeq and developed training populations of approximately 1500 DH lines, which were test-crossed and are being phenotyped under various stresses like drought, high temperature and excess moisture apart from optimal conditions (Sudha Nair, unpublished). Based on the phenotypic data of the training set and genomic estimated breeding values of the prediction set, lines will be selected for advanced stage evaluations. A multi-institutional initiative (funded by BMGF) called GOBii (Genomic and Open-source Breeding Informatics Initiative) guides in main-streaming these applications in the tropical breeding programs. There are many other avenues like hybrid prediction that could be achieved through genomic predictions, but the technology is at a nascent stage in the tropical breeding programs.

Enabling technologies to complement genomic tools in maize breeding

Seed chipping and genotyping

Maize is highly amenable to non-destructive seed-based genotyping, owing to its large seed size and clear separation of the endosperm from the embryo. Seed (endosperm) chipping, DNA extraction and genotyping of maize seeds by manual methods was reported (Gao et al., 2008) and has been used routinely in the CIMMYT breeding programs. By using manual methods, the throughput could not be enhanced beyond certain limits and the process was highly labor-intensive. Monsanto Technology LLC holds the patent for automatic seed chipping and extraction process (US Patent No. US007591101B2), which enables handling of large volumes of seeds. CIMMYT is currently pilot testing this application in collaboration with Monsanto, which when optimized could prove to be a game changer in application of genomic technologies in maize breeding programs in the tropics. From available publications, it is apparent that the breeding programs in the tropics have not yet warmed up to this technology thus it is not being used routinely. The advent of seed DNA genotyping has the capacity to increase genetic gains many-fold due to its impact on manipulating selection intensity and reduction in time and farm and associated costs of leaf DNA extraction. This capacity to enrich the seed sources submitted for DH induction with favorable alleles has made this tool one of the most impactful in enhancing genetic gains.

Doubled haploids

Derivation and use of doubled haploid (DH) lines, compared to conventionally-derived inbred lines, offers several advantages to the maize breeding programs in terms of reduced time taken to develop and deploy superior maize varieties, simplified logistics and reduced costs in line development and maintenance (Prasanna et al. 2012). Use of DH lines in conjunction with molecular markers significantly improves genetic gains and breeding efficiency, by reducing cycle time and enhancing selection intensity. The *in vivo* haploid induction using temperate haploid inducers (genetic stocks with high haploid induction capacity) has been adapted by commercial maize breeding programs in Europe and North America for over a decade, and more recently in Asia (especially China), but the lack of tropically adapted haploid inducer lines for several decades impeded the application of DH technology in the tropical maize breeding programs (Prigge et al. 2012). Tropically adapted first-generation haploid inducers with a haploid induction rate (HIR) of 5-8% were first developed by CIMMYT, in collaboration with the University of Hohenheim (Prigge et al. 2011; Prasanna et al. 2012) by transferring the maternal haploid induction trait from the temperate haploid inducers developed by University of Hohenheim. These tropicalized haploid inducers with better agronomic performance than the temperate haploid inducers in tropical conditions, were released in 2012, enabling the NARS and SME private sector maize breeding programs in sub-Saharan Africa, Asia and Latin America to adopt DH technology.

Recognizing the scope to further improve the first-generation TAILs for various traits, CIMMYT initiated the development of second-generation haploid inducers for the tropics by transferring the haploid induction trait from first-generation TAILs to elite CIMMYT Maize Lines (CMLs), marker-assisted selection for higher haploid induction rate, and phenotypic selection for superior agronomic performance. The CIM2GTAILs showed high haploid induction rates (~10-13%) under CIMMYT-tested (sub)tropical conditions in Mexico and Kenya, besides better agronomic performance in terms of plant vigor, synchrony with tropical source populations, better standability, and resistance to important tropical foliar diseases and ear rots.

While DH technology is the primary mode of deriving new inbred lines by several large private sector breeding programs, NARS and SMEs seed companies in several Asian countries are yet to fully derive the benefits out of maize DH technology for various reasons. CIMMYT, in partnership with Kenya Agricultural and Livestock Research Organization (KALRO) established a centralized maize DH facility at Kiboko (Kenya) in 2013. The facility is now producing nearly 80,000 DH lines each year, serving the maize breeding programs of CIMMYT, national partners and SME seed companies in sub-Saharan Africa. A similar centralized maize DH facility is being planned for Asia, in partnership with Indian institutions.

High throughput and reasonably precise phenotyping

Breeding programs of majority of the NARS and SME seed companies in the tropics have limited capacity for undertaking high-throughput and reasonably precise phenotyping, particularly under repeatable and representative levels of abiotic and biotic stresses in the field. This is indeed a major constraint for increasing genetic gains, and the capacity to breed better cultivars with higher grain yield and stress resilience (Prasanna et al. 2013). Appropriate trial management and spatial variability handling, definition of key constraining conditions prevalent in the target population of environments, and the development of more comprehensive data management, including crop modelling, are all integral components of phenotyping (Araus et al. 2018).

In partnership with advanced research institutions, CIMMYT Maize Program has made significant progress in terms of validating and deploying proximal and remote sensing tools in measuring some of the key traits relevant for maize breeding programs, including plant height, ear height, and ear traits using proximal sensing, and stand count, leaf senescence, canopy cover, etc. through remote sensing (Mainssara Zaman-Allah et al., in this publication).

Intensive efforts are required to build the capacity of the institutions on methods to characterize and control field site variation (for improving repeatability), adopting appropriate experimental designs, selection of "right" traits for phenotyping, proper integration, analysis and application of heterogeneous datasets, and increasing the genetic signal-to-noise ratio to detect real differences between genotypes (Prasanna et al. 2013). There is also a distinct need for the public and private institutions to come together and establish "phenotyping networks" for comprehensive and efficient characterization of breeding materials for important target traits.

Breeding informatics

The increasing availability of breeding related information, including pedigree, phenotypic and genotypic, coupled with environmental data, brings both opportunities and challenges in effectively managing and utilizing such information in breeding programs (Xu et al. 2010). This necessitates development of integrated platforms or one-stop data portals that can effectively bring together high-density genotyping, high-throughput and precision phenotyping and multi-dimensional environment profiling along with a suite of decision support tools to drive modern breeding programs. Data integration from multiple sources is one of the key components in developing breeding informatics systems. Efficient breeding informatics systems will need to include data curation tools, automated quality control workflows, data processing pipelines, visualization tools and simple and user-friendly data analytical and mining tool kits. This is one area where the tropical maize breeding programs are clearly lagging behind, since most of the data accumulated as part of the breeding cycles are maintained as flat files which are not in queriable databases, hence the breeding programs are not able to make the maximum use of the data developed through multiple breeding cycles. This is especially true in cases of using genomic and enabling tools, as the quantum of data produced is too high and converting them into selection units requires breeding informatics support.

Utilizing genomics and enabling technologies to enhance genetic gains

Molecular markers in the public sector maize breeding programs in Asia have so far been largely limited to use of trait-specific markers, majority of which are for quality traits deployed through marker-assisted backcross programs (Babu et al., 2005; Gupta et al., 2009; Muthusamy et al., 2014; Zunjare et al., 2018; Sarika et al., 2018). Though this strategy is helpful in improving the elite breeding materials for specific traits, they do not lead to an improvement of the genetic gains in the overall breeding program. This is possible only by targeted use of available trait-specific markers, wherever possible, in the forward breeding pipeline and a breeding program-wide streamlining of genomic prediction schemes. We will examine in this section how molecular marker-based selection improves genetic gain. Considering the genetic gain equation (discussed before), molecular marker-based breeding can have significant impact on several parameters in the famous breeders' equation (Lynch and Walsh, 1998).

Selection intensity: Selection intensity is described as the proportion of the total population selected for advancement or for further recombination. In conventional breeding process, the number of plants/families handled during early generations is severely limited due to the paucity of land, labor and money. On the other hand, using early generation screen with trait-specific markers, breeders could select for quality and adaptive traits (disease resistance, secondary traits for abiotic stress tolerance etc.), for which trait-linked markers are validated in a large population to improve their favorable allele frequency before various stages of yield testing. Larger population sizes allow greater selection intensity and increase the probability of identifying superior progenies (Moose and Mumm, 2008). Similarly, genomic prediction in early yield testing stage (Stage 1) in a fraction of the total lines available for testing helps in increasing selection intensity, considering the resources required for test crossing and field evaluations. Doubled haploids and seed DNA genotyping are some of the most potent accompanying tools that can be effectively integrated with genomic tools for increasing genetic gains through selection intensity.

CIMMYT Maize Program routinely deploys markers in the forward breeding pipeline for resistance to diseases like MSV and MLN in Africa (Nair et al., 2015; Gowda et al., 2018), and for quality traits like provitamin-A (Babu et al., 2013). In Asia, markers for forward breeding shall soon be deployed for resistance to TLB, after ongoing pilot testing. This has been made possible by high throughput, low-cost, low-density genotyping platforms like HTPG. Genomic prediction within biparental populations on lines entering Stage 1 testing has also been deployed in a large scale from 2017, through coordinated efforts among maize breeders, biometricians, software developers and innovative high throughput medium-density genotyping efforts (Buckler et al., 2016).

Selection accuracy: Selection accuracy in the genetic gains equation could entail multiple facets of accuracy, such as (1) heritability (repeatability) of the trial; (2) accuracy of MAS depending on linkage of the marker with the gene/ QTL; (3) accuracy of genomic prediction; and (4) accuracy of correlation between the tested locations to the target populations of environments (TPEs). Among these, molecular marker-based interventions are directly related to the second and third aspects, related to marker-assisted forward breeding and genomic prediction, respectively. The accuracy with which trait-specific markers can be used in forward breeding depends on the diagnostic nature of the marker, where factors like linkage of the marker with the causal polymorphism and the effect size associated with the marker under selection are important. Diagnostic markers for traits in the strict sense would mean the functional polymorphism itself which is responsible for a large effect size and are identified after fine mapping and cloning (Bortiri et al., 2006). There are very few cloned genes for agronomically important traits in the public domain in maize, except for quality traits like the maize starch pathway genes (reviewed in Whitt et al., 2002), *opaque 2* (Schmidt et al., 1990), fatty acid composition (Zheng et al., 2008) and provitamin-A (Harjes et al., 2008; Yan et al., 2010), resistance to leaf blight (Johal and Briggs, 1992), rust (Collins et al., 1999), NCLB (Hurni et al., 2015), head smut (Zuo et al., 2015), SCMV (Tao et al., 2013; Liu et al., 2017) Anthracnose stalk rot (Frey et al., 2006), and haploid induction (Kelliher et al., 2017; Gilles et al., 2017; Liu et al.,2017). Due to the genetic architecture of the crop, some of the major genes like *htn1* for TLB resistance act as a QTL with low to moderate effect in certain genetic backgrounds and environments (for e.g., race constitution of pathogens) (Hurni et al., 2015). Hence there are not many high-effect haplotypes that could be used as diagnostic markers in the strict sense. Still, marker-assisted forward breeding can be successful depending on the informativeness of the haplotype under selection for the trait in question in the specific breeding pool. The relative efficiency of marker-only selection compared to phenotypic selection depends on the proportion of the total additive genetic variance due to the known loci relative to the heritability of the trait (Smith, 1967).

The selection accuracy factor in the genetic gains equation is also applicable in the context of genomic prediction, where the prediction accuracy shows the relationship of the genomic estimated breeding value to the true breeding value of the breeding lines. One of the most important factors that influences this is the relationship between the training population and the breeding populations. If breeding programs create relevant training sets for the breeding populations and recalibrate training models by dynamically updating the training populations to ensure high prediction accuracy, improved genetic gains can be achieved by genomic predictions. The prediction accuracy was found to be the highest within biparental families, followed by half-sib families and almost none in unrelated families (Riedelsheimer at al., 2013). For achieving high selection accuracy, it is of utmost importance to have high-precision phenotyping technologies, experimental designs, and biometrical analyses that can effectively handle field experimentation errors, if any. For creation of dynamically calibrated training sets which are predictable for breeding populations, it is also important to have a very strong breeding informatics management system to connect breeding pedigrees, their genotypes and phenotypes; this is currently a huge gap in the public sector maize breeding programs in the tropics.

Genetic variance: Molecular markers guide introduction of regulated and useful variation into the breeding programs. They offer an excellent opportunity to accelerate breeding by selecting either for known genes or quantitative trait loci using genetic markers, rather than effects on phenotypes. Many genebank accessions or exotic germplasm carry rare alleles that could greatly improve various traits breeders work with. Marker-based approaches like MABC have been proven to carefully introgress such favorable alleles without disturbing the overall constitution of the breeding pool. In tropical maize, this has been proved in case of transferring of favorable alleles for provitamin-A (Menkir et al., 2017) and MLN resistance (Michael Olsen, unpublished). Also, marker-assisted pyramiding assists in accurately transferring many favorable loci for a trait, without altering the genomic background of elite lines in the breeding pool. Genebank accessions could be used more effectively by a combination of speed breeding and genomic selection (Li et al., 2018). Apart from specific donor germplasm for improving desired traits, genetic diversity analyses within breeding pools guide decisions regarding the diverse founder lines within the genetic pools that are crossed to develop progeny having high additive genetic variance, from which progenies could be selected in such a way that they move the population mean in a significant positive direction in ensuing generations.

Cycle time: When genetic gain is measured by unit time, accelerating the breeding process becomes critical as it will shorten the cycle time and thus increase the total genetic gain per unit time. When markers are available for traits of interest, MAS for target loci through forward breeding or backcross introgression has particular advantages, especially in terms of saving time and resources in introgression recessive traits like *opaque 2* (Babu et al., 2015), quality traits like provitamin-A which are phenotyped after harvesting (Muthusamy et al., 2014). Rapid-cycle genomic selection for population improvement (as against recurrent selection) helps to save every intervening cycle between intermating cycles, where test cross progenies are evaluated (Beyene et al., 2015; Vivek et al., 2017), and enhances genetic gains per unit time. When used in combination with enabling tools like seed DNA genotyping and doubled haploidy, they will have an enormous impact on reducing the breeding cycle time, thereby increasing genetic gain.

Conclusions

Several advances in recent years have changed the whole landscape of maize breeding. Maize is one of the crop plants where the basics of genetics, quantitative genetics, breeding methodologies, and all possible advances in genomic and enabling technologies in breeding, have been well-elucidated. The public breeding programs in the tropics are yet to take maximum advantage of all the new developments in maize breeding due to various factors. The availability of genomic tools, when provided at a cost manageable by the public breeding programs, creates the opportunity for breeders to accurately select and predict genotypes at all stages of the breeding program starting from parental selection, to breeding cross design, to segregating population selection in seeds, to DH evaluation and advancement, and finally to hybrid prediction and creation, selection and characterization (Cooper et al., 2014). This requires developing and implementing the complete spectrum of enabling technologies needed to increase genetic gains in a long term and sustainable way.

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Tropical Maize Genome: What Do We Know So Far, And How to Use That Information

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Introduction

Maize has a number of characteristics that make it an economically important crop and suitable as an experimental model, including (1) an intermediate genome size compared to rice and wheat; (2) typical outbreeding system with flexibility for inbreeding; (3) existence of multiple breeding products (inbreds, hybrids, synthetic varieties, open pollinated varieties and improved landraces); (4) wide adaptability, especially for stressed environments; and (5) a multiple-purpose crop that can be used for food (grain), feed (grain and stalk), fuel (grain and stalk), forage (young grain and stalk) and fruit (sweetcorn, baby corn, fresh corn) (Xu and Crouch 2008a).

Maize has been adapted to diverse environments. Temperate maize is grown in cooler climates beyond 34°N and 34°S, while tropical maize is grown in warmer environments located between 30°N and 30°S latitudes. An intermediate type, subtropical maize, is grown between the 30° and 34° latitudes. The tropical maize can be further classified into lowland (sea level to ≤1000 masl), mid-altitude (1000 to 1600 masl) and highland (≥1600 masl). Tropical maize is grown in over 60 countries, occupying about 60% of the area harvested and representing 40% of the world production.

Maize genetic variation is largely hosted by genetic resources of tropical maize, including wild relatives (teosinte, Tripsicum), landraces, open-pollinated varieties, synthetic varieties, inbreds, hybrids, germplasm complexes, pools and populations, and various genetic stocks (mutant, permanent populations, near-isogenic lines, introgression lines, etc). However, most of the tropical germplasm may not be considered as manageable resources, as they could be too diverse to be used directly and have to undergo a pre-breeding process. Genetic variation can be unlocked from tropical maize germplasm through genetic approaches such as large-scale and systematic identification and characterization of quantitative trait loci (QTL) (Xu et al 2017b).

Why tropical maize genomics

In maize genomics, single temperate genotypes such as B73 have been selected for sequencing with relatively high resolution and precision. With such reference genomes, however, only 50–80% of the original resequencing reads from different ecotypes can be mapped to a specific reference genome. For example, the genome-wide comparison between B73 and Mo17 (Springer et al., 2009) and within an expanded panel including teosinte (ancestral maize) lines (Swanson-Wagner et al., 2010) demonstrated that a considerable portion of the genome (about 50%) was not shared; while only 50% of Hi-seq reads from tropical maize can be mapped properly, about 80% of the SNPs from landraces cannot be mapped (Wenzel, 2014). Transcriptome sequencing of 503 maize inbred lines identified 8681 representative transcript assemblies (RTAs), 16.4% of which were expressed in all lines, compared with 82.7% expressed in line subsets, and about 50% being absent in the B73 reference (Hirsch et al., 2014). Single-genome based references provide only a partial genome coverage, which results in the loss of target genes in map-based cloning, missing of 40% or more important QTL/genes in association mapping, biased estimation (ascertainment bias) of genetic diversity, population structure, LD and IBD and haplotypes, and inefficient procedures and unpredictable results in MAS (Xu et al 2017b). Therefore, a multiple genome-based reference, pangenome, is needed for unlocking genetic variation that is hidden in diverse tropical maize collections, to provide a complete profile of genetic variation, including favorable alleles and haplotypes, at various omics levels across elites, landraces, and wild relatives (Xu et al., 2012; Golicz et al., 2016).

There are significant differences in applied genomics between temperate and tropical maize. Technology transfer from temperate maize to tropical maize and capacity building in tropical countries are needed for improvement of tropical maize. Comparative genomics across tropical maize germplasm and temperate maize will help identify novel genes and alleles required for improvement of both temperate and tropical maize (Xu and Crouch 2008a).

What do we know so far about tropical maize genomes?

To chart and utilize the genetic diversity in maize, a large-scale resequencing has been undertaken for a huge number of germplasm accessions, by which the first, second and third generations of maize hapmaps have been constructed using 27 (7 tropical inbreds), 103 (25 tropical inbreds, 23 maize landraces and 19 wild relatives (17 *Z. mays* ssp. *parviglumis* and 2 *Z. mays* ssp. *mexicana*) and 1218 lines (including those used in HapMap2 and additional 37 tropical CML maize lines), respectively, with 3.3M, 55M and 83M variants identified to capture the whole genome variation (Gore et al 2009, Chia et al 2012, Bukowski et al 2018). Based on the hapmaps, sequencing data from a large number of maize lines, and population relationship, a pangenome could be assembled via sequence alignment. By reduced representation sequencing of 14 129 maize inbred lines, 26 million tags were generated, 4.4 million of which were accurately mapped as sequence anchors (Lu et al. 2015), providing a foundation for pangenome construction. With the availability of whole genome sequences from two temperate inbreds (B73 and Mo17) and several tropical inbreds, a comprehensive maize pangenome is being constructed.

All pangenomic information including SNPs, indels, non-coding RNAs, and transposable elements, needs to be incorporated by databases. By integrating genomic and gene expression data, the core genome, variable genome, and the expression levels can be linked (Golicz et al., 2016). On the other hand, the exome represents a region where mutations are likely to affect protein structure and function, and is many times smaller than that of the whole genome, making exome sequencing data more easily manageable and applicable in plant breeding (Warr et al., 2015). These efforts can be integrated with other functional genomics approaches, including insertional mutation, EST development, gene cloning, transcriptional profiling, transformation, tilling, and used to discover genes and their functions.

Through large scale resequencing of maize germplasm including over 30 tropical maize lines, the 55K ANP array was developed with improved genome coverage, containing over 4000 SNPs that do not exist in the B73 reference genome (Xu et al 2017a). Based on the 55K SNP array, a set of high throughput marker panels, containing 20K, 10K, 5K and 1K SNP markers, were developed through genotyping by target sequencing (GBTS), by which genotyping cost can be significantly reduced by genotyping using the same 20K marker panel at different sequencing depths (Z. Guo and Y. Xu, personal communications), overcoming one of the most important bottlenecks in MAS (Xu and Crouch 2008b)

What can do with the genomics information from tropical maize

Large multi-national seed companies are now routinely using applied genomics information and tools to (i) dissect the genetic structure of their germplasm to understand gene pools and germplasm (heterotic) groups, (ii) provide insights into allelic content of potential germplasm for use in breeding, (iii) screen early generation breeding populations to select segregants with desired combinations of marker alleles associated with beneficial traits (in order to avoid costly phenotypic evaluations), and (iv) establish genetic identity (fingerprinting) of their products.

Molecular markers have been used in genetic diversity studies of tropical maize for diverse purposes (Xu and Crouch 2008a), including: examination of genotype frequencies for deviations from Hardy-Weinberg equilibrium at individual loci; test for linkage disequilibrium (LD) between pairs of loci; construction of "phylogenetic" trees or classification of germplasm accessions based on genetic distance; characterization of molecular variation within populations and/or between populations; determination of heterotic groups; analysis of correlation between the genetic distance and hybrid performance, heterosis, and special combining ability; and comparison of genetic diversity among different groups of maize germplasm including those from temperate and tropical areas.

Marker-trait association can be identified for gene discovery and molecular breeding by using genomics information including hapmaps and pangenome. Wang et al (2017) proposed that the resequenced maize inbreds can be used to cross with each other to develop multiple hybrid populations for GWAS, by which a large number of hybrids can be produced, and their genotypes inferred from their parental lines that have been resequenced. The resequenced parental lines can be easily shared and used to produce a subset of multiple hybrids as required based on the objectives of gene target and discovery. An example has been provided for GWAS of flowering time using 55K SNP markers and 724 hybrids. Similarly, two approaches, geographic associations and F-one association mapping (FOAM), were integrated to characterize the diversity of 4,471 maize landraces, with 1,005 genes identified across 22 environments (Navarro et al 2017). The former powerfully identifies adaptive loci, which are common across populations and are unlikely to be deleterious given their high minor-allele frequency. The latter helps differentiate the adaptive overlapping mutations from the potentially private deleterious mutations.

Marker-assisted recurrent selection and genomic selection has been largely applied for improvement of yield and heterosis, quality (e.g., QPM from tropical maize), abiotic stresses (with tropical maize as donors) and biotic stresses. Taking disease resistance as an example, diseases that are of a global nature and occur in most maize growing environments include leaf blights, leaf rusts, leaf spots, stalk rots and ear rots. Diseases that are of regional economic importance in the tropics include: Asia - downy mildews, which are also spreading to some parts of Africa and the Americas; Africa – MLN, maize streak virus and the parasitic weed Striga; Latin America - maize stunt and tar spot.

Maize breeding can be now accelerated by integration of doubled haploid (DH) breeding and various MAS procedures such as marker-assisted backcrossing, MARS and GS. The DH breeding can be facilitated by molecular markers and gene editing procedures for haploid induction and chromosome doubling. The individuals selected by MAS can be fixed quickly by DH procedure. A highly efficient breeding pipeline can be established with strong conventional breeding programs supported by molecular breeding, breeding informatics and decision support tools. CGIAR has established an *Excellency in Breeding* platform to support molecular breeding activities in small- and medium-size seed companies and developing countries. The open source breeding proposed by CIMMYT scientists combined with molecular breeding networks at national, regional and international levels will help build up a highly efficient molecular breeding system by sharing genotypic, phenotypic and envirotypic information, germplasm resources and breeding materials. Several maize molecular breeding initiatives have been established in China for GS-assisted breeding through sharing training populations, genetic models, simulation results, testcrosses and even parental lines.

Molecular breeding driven by big data and artificial intelligence

The transition of plant breeding from art to science has been driven by big data and will be driven further by artificial intelligence (AI) in the coming years (Xu 2018). Breeding has been evolved for thousands of years from selection based on single phenotypes to selection based on a three- dimension profile determined by genotype, phenotype and envirotype across different developmental stages and environments (Xu 2016). A huge amount of data has been generated through several high throughput platforms of genotyping, phenotyping and envirotyping. Breeding informatics has been revolutionized with significant changes in data generation, storage, scale, dimension, throughput, and precision, distinctly different from other big data in data properties, collection, treatment, analysis, mining and utilization. Now breeders are making contributions to breeding programs by generating, creating, and collecting big breeding-related data.

Modern breeding is now becoming increasingly integrated with program d breeding pipeline, agricultural engineering, facility agriculture with artificial or controlled environments and biological modeling/simulation, to meet human demands for high yielding, improved quality, resource-use efficiency and environment-friendly. Such tendency is expecting a big support from AI-guided agriculture to complete the conversion of breeding from big data-driven to AI-driven. Among the four major factors that affect AI, data and knowledge are the two that shape the distinct properties of AI-assisted breeding. On the one hand, AI will have significant influence on breeding information system because AI-equipped robots will interact with all the processes relevant to data collection, storage, analysis, sharing and utilization. On the other hand, historical experience and relevant knowledge achieved and accumulated in breeding programs need to be incorporated into AI system. Therefore, future breeding will become an AI system governed by breeding informatics and breeders' knowledge. Such AI-assisted breeding system will play significant roles in theoretical study, evaluation, selection, breeding procedure development, and field management. Combined with major breeding platforms, breeding programs in developing countries and small- and medium-size seed companies will be largely benefited by two additional systems, breeding informatics system for data treatment, analysis and mining, and decision support system for breeding simulation, prediction and selection. The breeding system driven by big data and AI will have great capacity of designing and predicting in breeding programs with improve breeding efficiency and enhanced genetic gain, through machine learning, optimization and simulation.

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Herbicide-Induced Chromosome Doubling of Maize Haploids in Tropical Environments

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Introduction

Maize is the most important crop for food, forage, fuel and industrial raw materials in the world. Maize hybrid breeding and application plays an important role in maize production (Dai Jet al. 2010). However, breeding pure maize lines is a long and tedious process (Eder and Chalyk 2002). Doubled haploid technology significantly reduces hybrid breeding time and expenses, essentially improving efficiency. Doubled haploid lines can be produced within one year using this technology (Melchinger et al. 2016). However, there are some obstacles to dedicated application of DH technology.

Maize haploid chromosome doubling is the most critical step in the doubled haploid maize breeding procedure. Colchicine, a chemical reagent, is used to increase the efficiency of doubled haploid lines achievement (Barnabás et al. 1999). However, colchicine's hypertoxicity is a hazard for operators and the environment, while tedious operational steps involving the reagent are a barrier for most researchers.

Numerous studies showed low efficiency and significant genetic background influence in spontaneous doubling of maize haploid plants (Liu and Song, 2000). Finding effective agents for maize haploid chromosome doubling with low toxicity and high efficiency have been top priority for researchers in recent years. Researchers have found that anti-microtubule herbicides induced maize haploid chromosome doubling function with considerable efficiency and could be a potential alternative for colchicine. Previous research results indicated that Orazylin, Trifluralin, Pronamide and Amiprophos-methyl (APM) could cause chromosome doubling in fodder beets, watermelon, cucumber, and maize (Hansen, 1998; Han et al. 2006; Zhao et al. 2008; Ci et al. 2012, Hui et al. 2012). The pollen-shedding rate of maize haploid plants ranged from 3.42% to 35.7%, and concentration ranged from 20 umol/L to 100 umol/L in temperate maize germplasms.

More herbicides Penoxsulam and Quinclorac (Hui et al. 2017), Bispyribac-sodium, and Acetochlor (Lian et al. 2018) were selected to test their capacity on maize haploid chromosome doubling efficiency. These experiments showed different results, and the effects of tropical environments were also unknown; the different climatic conditions which influence the behavior of chromosome mitosis are not well elucidated, especially temperature (Liu and Song, 2000).

Our objectives were to screen out the best herbicides, explore new ones for maize haploid chromosome doubling, and evaluate their efficiency in tropical environments.

Materials and Methods

Materials and experimental location

The inducer line GI1 is an improved and tropical adapted line via temperature inducer line CAUHOI, and its average haploid induction rate (HIR) is 4.64% when applied to tropical maize germplasms (Jiang, 2015).

Maternal materials for these experiments are local hybrid Guidan 0810 that was recently released, and developed by Maize Research Institute (MRI), Guangxi Academy of Agriculture Science (GXAAS).

The experiments were conducted at two breeding stations; Mingyang station in Nanning city, Guangxi province, and off-season station in Sanya city, Hainan province. Both locations have tropical climates. The hybrid was planted in Mingyang station for maize haploid production in autumn season, 2017. The chromosome doubling experiments were carried out in the winter season of 2017 in Hainan and spring season of 2018 in Guangxi.

Haploid production and chromosome doubling experiments

Treated haploid seeds in chromosome doubling experiments were produced from hybrid Guidan 0810, a single cross hybrid that is mainly planted in tropical environments. We conducted haploid production in the autumn season (late July to early August) in Guangxi. The inducer line was planted in three stages to make sure flowering times were synchronized. Haploid seeds were identified by the R-nj markers system as the seed with purple pigment at the top of maize kernels and colorless in the top of embryo, whereas both the top of endosperm and embryo of diploid seeds are purple or colorless. Haploid seeds were mixed, and a required number of seeds were sub-packed separately in bags.

Reagents and treatments

Reagents Oryzalin (95% pure), Trifluralin (98% pure), Butralin (99% pure), and accessory ingredient of DMSO and Tween (analytically pure) were used. The reagents were dissolved in accessory ingredients and attenuated to target concentrations with ultrapure water. The target treatment concentration was 60umol/l, 80umol/L and 100umol/L, regulated with 2% of co-solvent ingredient tween and 2.5% of infiltrate adjuvant DMSO, whereas the control was treated with ultrapure water only without any herbicides. Haploid seeds were sown in seedling float trays with 8*4 holes that fill up with nursery substrate by single seed. Haploid seedlings were treated with herbicides via drop-in method at 5 to 6 leaf stage in the afternoon of non-rainy days. The dew in the interior leaf was removed before drops of the herbicide solutions were applied.

Experimental design and data statistical analysis

A completely randomized experiment design was employed in the chromosome doubling experiments. Microsoft Office Excel 2016 and IBM SPSS statistics (64-bit version 24) was used for data processing and ANOVA analysis. To analyze efficiency of haploid seeding chromosome doubling of each treatment, we calculated the following rate:

N1: number of haploid plants with pollen shedding (NPS). N2: number of survival plants (SP) in the experiments of each plot. Where survival plants refer to the plants with normal character of haploid plants alive until pollination time. N3: number of seedlings treated in the experiments. one of each plot. N4: number of seedlings treated in the experiments, two of each plot. N3 and N4 is 40 and 50 respectively. Pollen shedding rate (PSR, %) = N1*100/N2. Survival rate (SR, %) = N2*100/N3 or (N4). APSR (%): Average of PSR.ASR (%): Average of SR. Over success rate (OSR, %):N1*100/N3*3 or (N4*3).

Results

Results showed that treatment with Butralin at concentration of 100umol/L has the highest pollen-shedding rate (PSR) of 14.18%, and the highest over success rate (OSR) of 12.67%. Additionally, the result of the three-concentration treated with Butralin ranged from 9.29 % to 14.18 % on average, whereas the control was less than half (Table 1).

Table 1. Pollen shedding rate (PSR), survival rate (SR) and overall success rate (OSR) for seedling treatments using herbicides for haploid chromosome doubling in tropical environments.

Herbicides	Concen-tration (umol/L)	Experiment 1 (2017 winter)				Experiment 2 (2018 Spring)			
		No. of seedlings treated	Rate (%)			No. of seedlings treated	Rate (%)		
			A-PSR	A-SR	A-OSR		A-PSR	A-SR	OSR
Orazylin	60	120	7.66^{b*}	86.67^{a}	6.67	150	10.42 b	90.00^{a}	9.33^{b}
	80	120	10.37^{a}	87.50^{a}	9.17	150	12.40^{a}	91.33^{a}	11.33^{a}
	100	120	10.09^{a}	90.83^{a}	9.17	150	11.54^{a}	86.67^{a}	10.0^{a}
Control		120	3.56^{c}	92.50^{a}	3.33	150	5.96^{c}	89.33^{a}	5.33^{c}
Trifluralin	60	120	8.39^{a}	90.00^{a}	7.50	150	7.09^{b}	85.33^{a}	6.0^{b}
	80	120	9.42^{a}	88.33^{a}	8.33	150	11.13^{a}	84.00^{a}	9.33^{a}
	100	120	9.19^{a}	81.67^{a}	7.50	150	10.86^{a}	92.00^{a}	10.0^{a}
Control		120	4.46^{b}	93.33^{a}	4.17	150	5.59^{c}	95.33^{a}	5.33^{b}
Butralin	60	120	9.29^{a}	90.00^{a}	8.33	150	9.45^{c}	90.67^{a}	8.67^{b}
	80	120	11.07^{a}	90.83^{a}	10.0	150	11.89^{b}	95.33^{a}	11.33^{a}
	100	120	12.07^{a}	90.00^{a}	10.83	150	14.18^{a}	89.33^{a}	12.67^{a}
Control		120	4.68^{b}	89.17^{a}	4.17	150	7.17^{d}	92.67^{a}	6.67^{b}

Note: A-PSR: Average of pollen shedding rate; A-SR: Average of Survival rate; OSR: Over success rate.
*: Values followed by the same letter are not significantly different at the 0.05 probability level.

The PSR of control ranged from 3.56% to 4.68% in winter season, and 5.96 % to 7.17 % in spring season; that is almost less than half compared to the herbicide treatments in the two experiments. The ANOVA analysis showed that the efficiency of chromosome doubling is different between herbicide treated seedlings and the control. Apparently, the efficiency of herbicides from highest to lowest is Butralin, Orazylin and Trifluralin as shown in Figure 1.

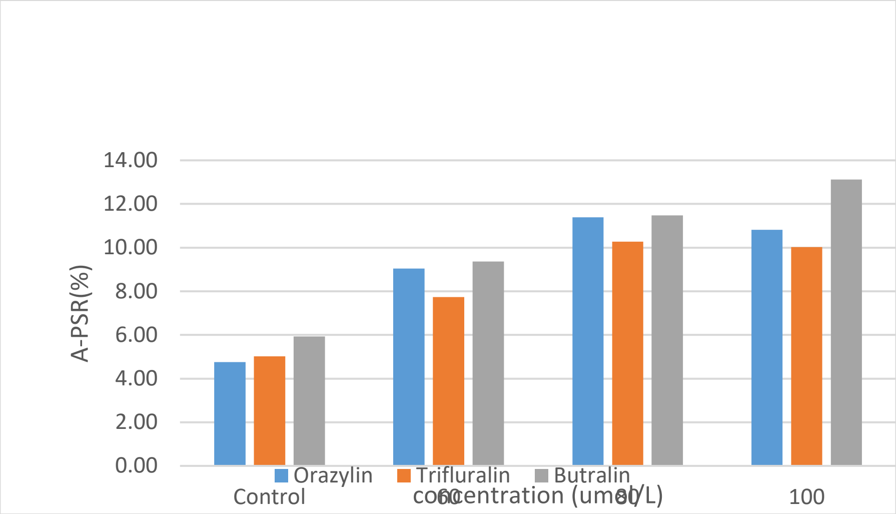

Figure 1. Pollen shedding rate contrastive analysis shows the haploid chromosome doubling efficiency by using herbicides treated haploid seedling in tropical environment.

We observed the phytotoxic effect of herbicides on maize haploids seedlings. The survival rate of herbicide treated seedling is slightly lower than control and showed no significant difference. The SR ranged from 86.67% to 95.33%s by herbicide treatments, and 89.17% to 95.33% by control in the experiments. The OSR shared similar trend of PSR due to the SR, as there are no significant differences.

Discussion

There are many kinds of herbicides that could interfere with chromosome behaviors, especially when microtubules are combined with herbicides in high concentration. Researchers found that lower dosage of herbicides can induce chromosome doubling and are widely used for ploidy breeding procedure. Doubled haploid lines development is the goal of haploid induction for commercial maize hybrids improvement. Maize breeders pushed for efficient chromosome doubling methods since the DH technology had been developed. Labor and other costs are limited in large-scale programs, and the complicated operating procedures are not suitable for companies that do not have skilled workers. Herbicides have similar effects to colchicine, but with low toxicity and cost, and are extremely easy to use.

Orazylin and Trifluralin showed relatively high effects in haploid doubling in maize and other crops. However, Butralin working similarly to Trifluralin has not been reported. In the experiments, the efficiency of chromosome doubling using herbicides is twice that of spontaneous method in general and showed significant difference. The pollen-shedding rate was 14.18% in spring season and 12.07% in winter season, with a significant improvement over the control of 7.17% and 4.68% respectively. The success rate of Butralin is 12.67 in spring season and 10.83% in winter season, which is close to the result of colchicine according to A. E. Melchinger [Albrecht E. Melchinger,2016]. We also found that PSR can reach up to 28.5%, or as low as zero, in some materials; this signifies that the efficiency of herbicide-induced chromosome doubling of maize haploid may be influenced by the genetic background of breeding materials. The efficiency of Amiprofos-methyl and propyzamide in tropical environments is not as high as that of previous studies in temperate environments (Hui guo-qiang, 2012), which means that herbicides may have different application effects in different environments. The survival rate of herbicide treated seedlings is slightly lower than control and have the obvious hypertoxicity phenomenon with yellow leaf after application of the solutions but recovered gradually and seem not to affect growth of seedling.

In conclusion, results suggested that Butralin and Orazylin could be promising alternatives to colchicine in tropical environments. Further exploration is needed to develop reagents and methods for haploid chromosome doubling, to increase the chances of getting DH lines in target environments more efficiently.

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New Horizons of Sustainable Intensification in Maize Systems in South Asia

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Introduction

Agriculture remains central to the economy of south Asian nations providing livelihood to the majority of their population. Though agriculture have made spectacular progress for food self-sufficiency through significant increase in crop yields over past few decades, production still requires an increase by another 60-70% by 2050 to meet the expected demand. These production increases need to be achieved from less land, water, energy and other critical inputs and constrained natural resource base. Climate change poses additional challenges to agriculture in south Asia. Since 1980, climate change is estimated to have reduced global yields of maize and wheat by 3.8% and 5.5%, respectively. Climate change not only affects crop yields but also affects the availability and productivity of natural resources including land and water (Jat et al., 2016; Lal, 2016). The natural resources of South Asia are 3-5 times more stressed due to population, economic and political pressures compared to rest of the world. Further, changing land uses, urbanization and increasing pollution could affect the rice-wheat niche directly and indirectly through their impacts on climate change variables (Lal, 2016). For example, about 51% of the IGP may become unsuitable for wheat crop, a major food security crop for India, due to increased heat-stress by 2050 (Ortiz et al., 2008). Similarly, water table in western IGP being depleted at 13 to 17 km^3 yr^{-1} (Rodell et al., 2009) due to over-pumping for rice will also have serious impacts on regional agro-ecosystem and rice production (Yadvinder-Singh et al., 2014).

With no scope for horizontal expansion of farming, the future food and nutrition demand of growing population has to be met mainly through increasing yield per unit area with lesser external inputs (labor, water and energy) while protecting the environment (Gathala et al., 2013; Choudhary et al., 2018a). The soil organic carbon (SOC) contents in most cultivated soils of India is less than 5 g/kg compared with 15-20 g/kg in uncultivated virgin soils (Bhattacharya, et al., 2000), attributed to intensive tillage, removal/burning of crop residues, mining of soil fertility and intensive monotonous cropping systems. Fertility fatigue, multiple nutrients deficiency and poor-quality ground water in intensively cultivated area of rice-wheat system in South Asia is a common phenomenon (Kakraliya et al., 2018). This adds to the challenge of making farming system more and more resilient to climatic risks.

To sustainably increase the food production while conserving precious natural resources, we need a multi-pronged strategy that includes (i) bridge the management yield gaps, (ii) diversify the resource intensive & less efficient crops/cropping systems with resource use efficient production system and (iii) transition from a commodity centric technology to infusion of market inclusive system-based management innovations. Maize has emerged as the most produced grain in the world and its annual rate of production increase is twice compared to rice and thrice compared to wheat (Fischer et al., 2014). With the changing economy, increasing wealth and dietary patterns leading to higher consumption of animal-based foods and growth in the poultry industry, the demand for maize is likely to increase. Maize being an important crop for food and nutritional security and grown in diverse ecologies and seasons, serves the basis for diversification through intensification management paradigm.

Increasingly, sustainable intensification is being considered as “an important component of the overall strategy for ensuring food security, poverty alleviation, health for all, rural development, enhancing productivity, improve environmental quality and preserve natural resources”. The sustainable intensification in irrigated ecologies of IGP can be achieved through diversification of RW system and integration of mungbean in rice/maize systems. However, diversifying rice with maize in IGP, especially western IGP can only be attained through new horizons of sustainable intensification rather than simply replacing rice with maize. Layering precision water, nutrient & energy management with adapted genotypes with key elements of conservation agriculture (CA) could have multiplier effects on different performance indicators of the production systems and provide basis for sustainable farming future in IGP. In this paper, we provide evidence base of sustainable intensification and discuss the new horizons through several strategic research trials on new generation agronomy in cereal based systems of south Asia.

With limited scope for further expansion of area under agriculture, production gains can be accomplished through intensification of agriculture by pursuing one or more strategies including: (i) increasing productivity per unit of land; (ii) increasing cropping intensity per unit of land and (iii) changing land use (diversification).

Methodology

Strategic research platforms were established all across the IGP to develop sustainable intensification options involving portfolio of practices and management strategies in maize based production systems having potential for future food security in South Asia while protecting natural resources and environment. A total of six strategic research platforms have been established across western and eastern IGP. A summary of the characteristic features of experimental platform sites as well as the sustainable intensification factors studied at these platforms are given in Table 1. Standard management protocols were used for all the trials except the factors under investigation, which were used as per the experimental treatments.

Table 1. Characterization of experimental platforms on various aspects of sustainable intensification across Indo-Gangetic plains (IGP).

Research Platform	**Key factors studied**	**Year of start**	**Location**	**Geo-coordinates**	**Soil**	**Climate**	**Rainfall (mm)**
Developing portfolios for resource use efficient and climate smart future cropping systems in reclaimed sodic lands of Western IGP	Cropping system, tillage, residue, water	2009	Karnal, India	29.705749N, 76.955496E	Silty loam	Semi-arid Sub-tropical	700
Diversification options for cereal systems of Western IGP through conservation agriculture and precision water management	Cropping system, tillage, crop establishment, residue, water	2012	Taraori, India	29.80879107N, 76.92043490E	Clay loam	Semi-arid Sub-tropical	650
Precision water and nutrient management using sub-surface fertigation in CA based maize-wheat systems in North-West IGP	Tillage, residue, nutrient (rate, method), water	2015	Ludhiana, India	30.9877177N, 75.74285788E	Sandy loam	Semi-arid Sub-tropical	680
Developing water and energy smart portfolio for sustainable intensification of cereal based systems in North-West IGP	Cropping system, tillage, establishment, water, nutrient, energy source	2015	Ludhiana, India	30.99024708N, 75.74530406E	Silty loam	Semi-arid Sub-tropical	680
Assessment of genotype x management interaction under futuristic sustainable intensification regimes	Tillage, water, nutrient, genotypes	2016	Ludhiana, India	30.9877177N, 75.74285788E	Sandy loam	Semi-arid Sub-tropical	680
Precision nutrient application rate and placement methods in conservation agriculture based maize-wheat system of Eastern Gangetic Plains of India	Tillage, nutrient rate, method, time of application	2014	Samastipur, India	25.955705N, 85.668865E	Clay loam	Sub-humid Sub-tropical	1344

Results

The results of sustainable intensification options evaluated from six strategic research platforms across IGP were analyzed on various performance indicators that includes yield, income, water, energy, nutrient use efficiency, environmental foot prints, soil health etc and are described in this section.

Innovative packaging with maize for sustainable intensification portfolio for sustaining the food bowl: Conventional management practices of cereal based systems are not only labor, energy, water and capital intensive but also leads to deterioration of natural resources. It is therefore imperative to develop a sustainable intensification portfolio of conservation agriculture (CA) agriculture-based management coupled with precise water and nitrogen (N) management using innovative practices including sub-surface drip irrigation/fertigation (SSDI) and replacing rice with maize were studied to ensure food security while protecting natural resources in Green Revolution corridors, so as to achieve evergreen revolution. A research platform with five portfolio of management scenarios: i) conventional-till (CT) rice-CT wheat (Scenario I; farmers' practice; FP); ii) ZT rice-ZT wheat with flood irrigation (Scenario II; full CA); iii) ZT rice-ZT wheat with SSDI (Scenario III; full CA+SSDI); iv) ZT maize-ZT wheat with flood irrigation (Scenario IV; full CA); v) ZT maize-ZT wheat with SSDI (Scenario V; full CA+SSDI) were evaluated during 2016-2018 for their effect on crop and water productivity as well as other parameters. On 2-years mean basis, MW system with full CA+SSDI recorded 11% higher system productivity (rice equivalents), saved 88% (204 cm/yr) of irrigation water and increased irrigation water productivity (WP_I) by eight times compared to conventional RW system/FP (Table 2). Also, RW system with full CA+SSDI recorded 3% higher system productivity, saved 57% (133 cm/yr) of irrigation water and increased irrigation water productivity (WP_I) by 141% compared to FP. Application of nitrogen through SSDI saved 20% of nitrogenous fertilizer in both rice and maize based systems with same or even higher yields. Layering of SSDI with CA based management has a potential to increase the productivity while minimizing the ground water pumping and lowering N use. CA based MW system with SSDI is a potential game changing portfolio of practices for ensuring food security through cereal production while protecting natural resources and minimizing environmental externalities in western IGP.

Table 2. Grain yield and irrigation water use in different sustainable intensification scenarios (2 year mean).

Scenarios	Grain yield (t ha^{-1})			Irrigation water use (mm ha^{-1})		
	Rice/Maize	Wheat	System	Rice/Maize	Wheat	System
I: Conventional based RW system	7.04a	5.68b	13.36b	1886a	435a	2321a
II: Full CA based RW system with flood irrigation	5.87b	6.47a	13.06b	1447b	385a	1832b
III: Full CA based RW system+ SSDI	6.30b	6.70a	13.75a	785c	207b	992c
IV: Full CA based MW system with flood irrigation	7.14a	6.51a	14.38a	110d	372a	482d
V. Full CA based MW system+ SSDI	7.48a	6.59a	14.81a	85d	198b	283e

Where: CA- conservation agriculture; RW- rice-wheat; MW- maize-wheat; SSDI- sub surface drip irrigation

Green solutions coupling maize for addressing the food-energy-water (FEW) nexus in western IGP: A combination of cropping systems, tillage/ crop establishment, residue & water management and energy sources for irrigation have been researched in western IGP to develop sustainable solutions for different resource circumstances and production environments. Research results revealed that layering of CA+sub-surface drip fertigation + solar energy for water pumping in rice-wheat systems resulted in higher (0.35t/ha) productivity with almost half (107 cm/year) water use and zero water footprints as against 3077 kg of CO_2-eq/year/ha under conventional RW system and also with higher (INR ~30000; US$ 440/ha/year) income (Table 3). Whereas, diversifying RW system with maize-wheat with layering of CA+sub-surface drip fertigation + solar energy for water pumping has shown a potential of higher system productivity by further (> 1 t/ha/year) with significantly lesser water use (< 35 cm/year) and significantly higher income (INR ~47000 (US$ 690)/ha/year) and zero water foot prints as against 3077 kg of CO_2/year/ha under business as usual. These research evidence suggest that maize with innovative agronomic management not only has potential to diversify rice with maize for sustaining water reservoir but also form a sound economic competitive basis for sustaining the food bowl through protecting natural resource and environment.

Table 3. Effect of different management portfolios on system productivity (crop and water) and profitability.

Scenario	System yield (rice equivalent) (t/ha)	System irrigation water use (cm)	System irrigation water productivity (kg grain m^{-3} water)	Net return, INR/ha (US$/ha)
RWZT-SSDI	12.36bc	107.30b	1.15d	138274 (2033)
RWZT-Fl	11.99c	188.32a	0.63e	118522 (1743)
RWCT-FP	12.02c	202.09a	0.59e	108897 (1601)
MWPB-SSD	13.19a	32.87d	4.19a	156571 (2303)
MWPB-Fu	12.79ab	56.62c	2.37b	149164 (2194)
MWCT-FP	12.04c	68.14c	1.86c	133403 (1962)

Where; RW: rice-wheat; MW: maize-wheat; ZT: zero till; CT: conventional till; PB: permanent beds; SSDI: subsurface drip irrigation; Fl: flood irrigation; Fu: furrow irrigation; FP: farmer's practice

Smallholder precision nutrient management holds the key for intensification of maize systems

Experiences from western IGP

To address the growing challenges of deteriorating soil health and multi nutrient deficiencies in intensive cereal systems of western IGP, a research trial was initiated during 2015 at BISA farm, Ladowal, Ludhiana, India. The treatments consisting of two residue management (with and without residue retention), four N management in permanent beds with sub-surface drip irrigation; PB-SSDI (0, 60, 90 & 120 kg N/ha) and one absolute control (conventional tillage and furrow irrigation in maize and flood irrigation in wheat) were evaluated. Grain yield of maize under PB-SSDI was significantly higher than conventional tillage and flood irrigation. Grain yield of wheat with PB-SSDI (120 kg N/ha) was significantly higher by 7% compared to conventional flood irrigation system with similar N rate. In maize, PB-SSDI saved ~ 65% irrigation water compared to CT-FI (conventional till-furrow irrigated). Similarly, amount of irrigation water applied to wheat was ~45% less under PB-SSDI compared to CT-FI. Irrigation water productivity (WP_I) of maize in PB-SSDI (11.36 kg m-3) was 3 times higher compared to CT-FI treatment (3.68 kg m-3). WP_I of wheat in CT-FI was 64.5% lower compared to PB-SSDI. The partial factor productivity of applied N (PFPN) was significantly higher by 41.0, 86.4 and 83.3% under PB-SSDI (60 kg N/ha) compared to PB-SSDI (90 kg

N/ha), PB-SSDI (120 kg N/ha) and CT-FI with 120 kg N/ha, respectively in maize during first year. Similarly, PFPN in maize during year 2 was significantly higher by 35.3, 58.6 and 76.9% under PB-SSDI (60 kg N/ha) compared to PB-SSDI (90 kg N/ha), PB-SSDI (120 kg N/ha) and CT-FI with 120 kg N/ha, respectively. PFPN in wheat during year 1 was significantly higher by 50.9, 70.2 and 81.8% under PB-SSDI (60 kg N/ha) compared to PB-SSDI (90 kg N/ha), PB-SSDI (120 kg N/ha) and CT-FI with 120 kg N/ha, respectively. Similarly, PFPN in wheat during year 2 was significantly higher by 25.0, 53.8 and 50.9% under PB-SSDI (60 kg N/ha) compared to PB-SSDI (90 kg N/ha), PB-SSDI (120 kg N/ha) and CT-FI with 120 kg N/ha, respectively.

Experiences from eastern IGP

Conservation Agriculture (CA) based management practices have demonstrated multiple benefits under different agro-ecologies by reducing the risks of aberrant weather abnormalities. In eastern Indo-Gangetic plains of India, maize is grown under conventional-till management system with sub-optimal nutrient management (rate, method, time etc). Therefore, CA- based management system may address the probability of maize crop failure due to poor crop establishment, inappropriate input use and sub-optimal agronomic management under monsoonal risks. Moisture stress under *kharif* season/ monsoon season maize followed by wheat under CT- based systems has very low productivity. A strategic research trial on precision nutrient management practices in CA based (on permanent beds: PB) maize-wheat system was conducted under CIMMYT (International Maize and Wheat Improvement Centre)-CCAFS (Climate Change Agriculture and Food Security) at BISA, Pusa, Bihar, India for two consecutive years (2014–15 and 2015–16). Nutrient management includes i) farmers fertilizer practices (FFP); ii) state recommended dose of fertilizer (SR); iii) precision nutrient management using Nutrient Expert®; iv) NE + Green Seeker (GS) based nitrogen rates applied with two methods; broadcasting and drilling. Nutrient management through NE, NE+GS and SR along with drilling method significantly increased yield, nutrient use efficiency as well as net returns compared to broadcasting method under respective management practices. Cultivation of MW system on permanent beds (PBs) with NE+GS-drilling increased the system productivity and net returns by 31.2% and 49.7%, respectively compared to FFP. Significantly, higher (11-18%) NUE was observed under NE+GS treatment with drilling method compared to FFP. Global warming potential (GWP) of maize and wheat production was lower with NE-drilling compared to FFP-broadcasting. NE-drilling recorded 15.2 percent (2 yrs' mean) carbon sustainability index compared to FFP-broadcasting.

Going energy efficient sustainable intensification of cereal rotations through maize systems management

Open field burning of crop residues in IGP not only repose to use the indirect renewable source of energy but also impaired the soil and environment quality. Conservation agriculture (CA) based management practices in cereal (rice/maize) systems helps in utilizing the carbon rich crop residues (renewable energy source) and their beneficial effect on soil and crop micro-climate to achieve higher crop productivity and profitability in North-West India (Choudhary et al., 2018a, b). A long-term (5-yrs) experiment was conducted to evaluate the effect of CA- based management practices on energy budget, water productivity and economic profitability in rice-wheat (RW) and maize-wheat (MW) systems in comparison to CT- based management (farmer's practice). The treatments for RW systems included: i) conventional till rice-wheat (RW/CT); ii) RW/CT + mungbean (RWMb/CT); iii) Zero-till RW with residue retention (+R) (RW/ZT+R); and iv) RW/ZT+R + mungbean (RWMb/ZT+R). A similar set of treatments were evaluated for MW systems, except the crops were raised on raised fresh beds (CT beds) and permanent beds (ZT beds).

In CA-based systems, crop residues contributed maximum (~76%) in total energy input (167995 MJ ha^{-1}). However, fertilizer application (non-renewable energy source) contributed maximum (43%) to the total energy input (47760 MJ ha^{-1}) in CT- based systems. In RWMb/ZT+R, source-wise input energy shared 76, 10 and 7% under residue, fertilizer and water respectively, of total energy (5 yrs' mean). Whereas, the corresponding mean values for MWMb/ZT+R were 79, 13 and 3%. The operation-wise input energy utilization of total energy was 44, 31 and 11% and 60, 15 and 9% under fertilizer, irrigation, and land preparation & sowing, respectively, for RWMb/ZT+R and MWMb/ZT+R, (Figure 1). CA- based cereal (rice/maize) systems recorded higher energy output and energy intensiveness (EI) by 251 and 300% respectively and recorded 21% lower net energy which decreased the energy use efficiency (EUE) and energy productivity (EP) by ~67% compared to CT- based rice (RW/CT), irrespective of mungbean integration. MWMb/ZT+R utilized 204% more input energy and resulted in a higher net energy by 14% and EI by 229% compared to RW/CT. However, EUE and EP were only 53 and 52% of the RW/CT system (6.45 and 0.48 kg MJ^{-1}). CA-based R/M-W system enhanced the crop productivity by 10 and 16%, water productivity by 56 and 33%, and profitability by 34 and 36% while saving in irrigation water by 38 and 32% compared to their respective CT-based systems, respectively. CA- based maize-wheat-mungbean system improved net energy, crop productivity and profitability, therefore reduced EUE and EP can be ignored as open field burning of crop residues lost the energy and deteriorates the soil and environmental quality in NW India.

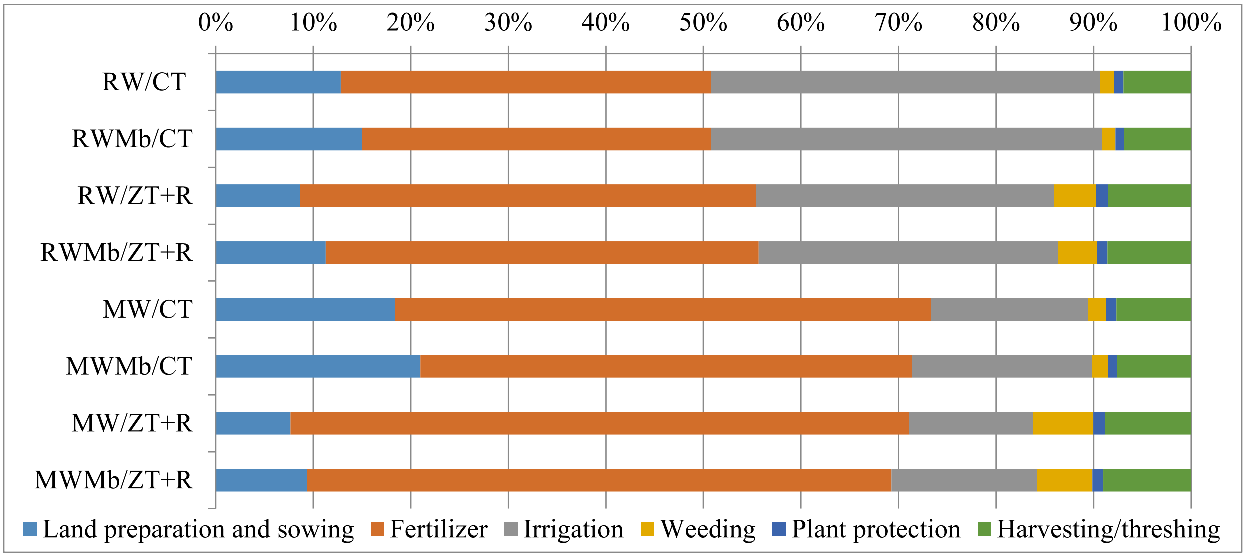

Figure 1. Operation-wise input energy-use (%) under different management scenarios of rice/maize based cropping system

Capturing genotype x environment x management (GEM) interactions for making maize more productive through realizing high potential:

As described in above section it is evident that for addressing the emerging challenges of natural resource degradation, CA-based sustainable intensification practices are central to future food security while conserving natural resources. However, the uptake of these management practices is still slow due to variable outputs of various CA- based management under different production paradigm. One of the key factors is variable yield responses of crop cultivars under CA- based management as they are tailored under conventional tillage-based management and may respond differently under CA. Therefore, the emphasis is now being laid to tailor genotypes suited to specific agronomic management. CA based practices (e.g. zero tillage, residue retention) can help timely planting, reduce the cost of cultivation, and improve crop productivity, water use efficiency and soil health. Recently, layering of the micro-irrigation system with CA have demonstrated further complementing opportunities to improve water and nutrient use efficiency as described in previous section of the paper. Therefore, it is imperative to develop varieties responsive to a particular agronomic management environment to realize the maximum yield potential.

In south Asia, maize has been transitioned from a subsistence to commercial crop and hence being grown in diverse ecologies across the year. Traditionally, maize (and other crops) genotypes are bred under conventional tillage (CT) management system. However, performance of genotypes varied with the management system because of regulated micro-climatic conditions. Recently, subsurface drip irrigation (SSDI) system is being promoted to improve water and nutrient use efficiency under CA. Therefore, it is imperative to evaluate the performance of advanced breeding lines/genotypes under different agronomic management systems to realize the maximum yield potential. A field experiment was conducted for two consecutive years to evaluate 15 maize genotypes (pre-release and recently released hybrids) from diverse genetic background under three management scenarios: (i) conventional tillage with flood irrigation (CT-Flood), (ii) zero tillage with flood irrigation (ZT-Flood) and zero tillage with subsurface drip irrigation (ZT-SSDI) at Borlaug Institute for South Asia (BISA)-CIMMYT farm, Ladhowal, Punjab, India. Maize grain yield was influenced with management scenarios, genotypes and their interactions in both years.

The maize yield was higher by 6.3–21.7% in ZT-SSD compared with the other two management scenarios (Figure 2). While, maize genotypes CAH 153 and CAH 1414 in CT, VH 13079, VH 141733, PMH 3, CAH 1511 and CAH 162 in ZT-SSD were out yielded than other genotypes in 2016. Mean grain weight was significantly higher in ZT-SSDI compared with ZT-flood, irrespective of genotypes. Total amount of irrigation applied under ZT-SSDI was ~50% less compared with conventional and ZT-flood irrigated maize. Results from this study revealed that the hybrids performed differently under varying management systems showing genotype x management interaction.

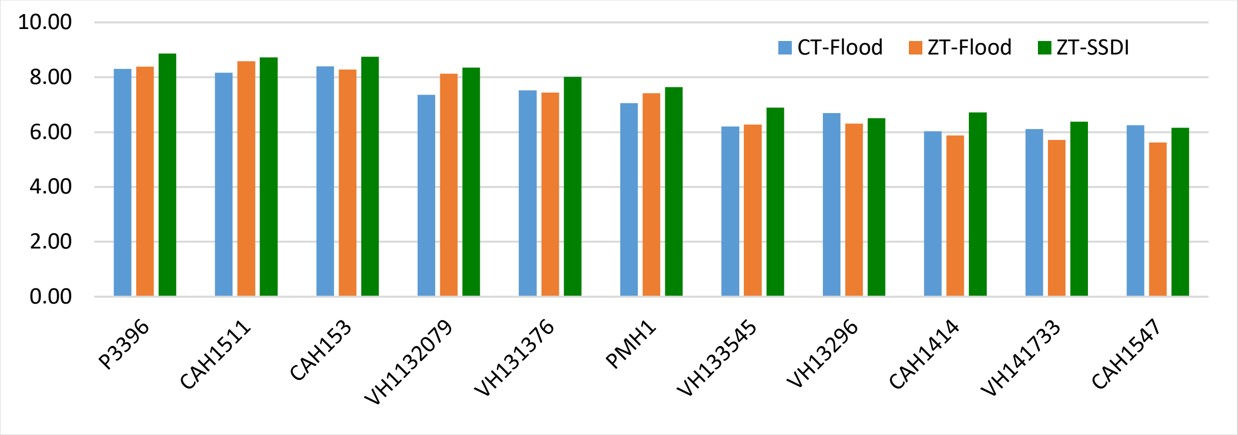

Figure 2. Yield performance of different maize hybrids under contrasting management systems at Ladowal, Punjab, India

Conclusions

One of the great challenges in agricultural development and sustainable intensification is the performance and acceptance of portfolios of technologies in diverse bio-physical and socio-economic domains. Many technology introductions fail because they are promoted in the wrong environments or because insufficient attention paid to the enabling conditions that are necessary for success. Developing portfolio of sustainable intensification practices integrating basic elements of conservation agriculture and adapted component technologies for systems' optimization, water, nutrient, energy, genotypes etc for maize systems provides sound basis for future food security while protecting natural resources and minimizing environmental externalities of farming.

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Maize Intensification in Major Production Regions of the World: Evidence from a Global Multi-location Study

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Introduction

Historically, the global average yield for maize has been increasing steadily over time. Since the 1960s, yields have been improving at a rate of 65 kg/ha/yr (FAO, 2017). In terms of the world's total maize production, it has been increasing at a steady rate of 10 million (M) t/yr until 2004, after which it shifted to a steeper production of 31 M t/yr (FAO, 2017). The shift in production closely follows the most recent trend for maize harvest area expansion, which had been increasing at a rate of 0.9 M ha/yr prior to 2007, has now been increasing at the more rapid pace of 4.7 M ha/yr. The United States, China and Brazil have contributed to most of this area expansion, and in 2014 these three countries accounted for 47% of world's maize production. In sub-Saharan Africa (SSA), maize production accounted for 36% of the cereal production in the region, and has increased by 500% between 1961 and 2014, mainly due to area expansion (Garcia et al., 2017).

These global trends clearly show that the rapid increase in maize production are associated more with the expansion of maize growing areas than with rapid increases in yield. However, with an estimated global population of 9.7 billion by 2050, the global community must find a way to ensure food security to the growing population. This is possible through achieving maximum possible yields on every hectare of currently used arable land through maize intensification in major production regions of the world, to meet the economic, environmental and social objectives of sustainable production. The Global Yield Gap Atlas (GYGA), developed by the researchers from University of Nebraska-Lincoln (USA) and Wageningen University (The Netherlands), reported wide gaps between actual and attainable maize yields (http://www.yieldgap.org). However, Cassman (1999) indicated that ecological intensification of maize systems could be achieved through closing exploitable yield gap, improving soil quality, and by practicing precision agriculture.

International Plant Nutrition Institute (IPNI), has launched Global Maize Project (GMP) with an objective to use Ecological Intensification practices to improve yields over time at a faster rate than farmer practice (FP) while minimizing adverse environmental effects. The IPNI Global Maize Project (GMP) provides data from over 20 sites that can be used to compare typical farmer practice (FP) to what scientists and local agronomists believe to be improved practices aimed at sustainably improving yields and meeting the standards for environmental quality – a goal termed Ecological Intensification (EI). These EI practices differ from region to region but include strategies for better cultivars, balanced nutrition, and improved soil and crop management. The initial EI studies in the GMP were estimates of an ideal set of practices for accomplishing the objectives of EI at a given site. However, the long-term aspect of the GMP provides opportunities for the local agronomy team to make adjustments in the practices as observations and measurements suggest and to accommodate improved technologies or genetics as needed during the experiment. The current summary discusses some of the results of the EI studies conducted across the major maize production regions of the world.

Opportunities for Ecological Intensification approaches when yield gaps are narrow

There are many maize growing areas in the world where farmers have been steadily increasing management intensity, already producing what are considered high yields in their respective regions. The difference between attainable yield and yields under current farmer practices, or the exploitable yield gap, is believed to be narrow in these areas. Three research sites in the states of Iowa and Minnesota in the USA, as well as one research site in southern Russia, located in areas thought to have narrow exploitable yield gaps demonstrate that management practices assembled to achieve ecological intensification produced comparable or greater maize yields than those achieved with standard farmer practices. All four sites considered improvements to existing nutrient management practices with improvements to other agronomic practices.

Study conducted at two research sites, one on a rainfed, clay loam Mollisol in south-central Minnesota near Waseca and the other on an irrigated loamy sand Mollisol in central Minnesota near Becker compared FP to EI management systems. At Waseca, EI produced most consistent economic returns by making changes only in agronomic practices, while at Becker, EI produced the greatest yield and was consistently profitable due to changes in both agronomic and nutrient management practices (Table 1). EI system considered better residue management, in combination with a longer-season hybrid and a 14% greater planting density, while advancing nutrient management for P and K applied at rates of grain removal and splitting N application at pre-plant, planting and at the six leaf-collar maize stage.

Table 1. Comparison of ecological intensification (EI) and farmer practice (FP) management systems at Waseca and Becker, Minnesota, USA.

Agronomic management	Nutrient management	Yield (kg/ha)	AEN, kg/kg	REN, kg/kg	Change in net return (US $/ha)	No. of years when management changes were profitable
Waseca						
FP	Standard	9,550c	62bc	0.40c	-	-
	Advanced	10,410b	57c	0.46b	79	0 of 4
EI	Standard	10,730b	71a	0.53a	69	3 of 4
	Advanced	11,690a	66ab	0.50ab	-20	1 of 4
Becker						
FP	Standard	9,170a	80c	0.42c	-	-
	Advanced	10,090b	92b	0.58a	92	2 of 3
EI	Standard	10,460b	93b	0.50b	116	3 of 3
	Advanced	11,750a	101a	0.59a	101	3 of 3
Data given in the table is average for 2013-16 at Waseca and for 2014-16 at Becker. Within a column for a given location, means followed by the same letter are not significantly different. (Source: Murrell et al., 2017)						

In maize-soybean rotation at Iowa, United States, strip-till maize and no-till soybean were used in the EI system instead of more intensive, full-width conventional tillage in the FP. Over the 2011 to 2016 duration of the experiment, the EI system used maize seedling rates 19 to 27% higher than the FP, with rates ranging from 84,000 to 100,000 seeds/ha, keeping the planting dates same for both the systems. Detailed description of nutrient management in both the systems were discussed in Murrell et al. (2017). The EI system reported a greater agronomic efficiency of N (AE_N), producing 35 kg dry matter (DM)/ha per 1 kg N/ha applied. The FP system produced 10 kg DM/ha less per 1 kg N/ha applied. The greater AE_N of EI was due to lower unfertilized yields in EI, lower N application rates in EI, and the greater grain yield response to N in EI. A similar study conducted in maize-soybean-chickpea rotation in a clay loam soil of Rostov Oblast in Tselina district of southern Russia, compared EI management system with FP. The study revealed that EI increased maize yield by 9%, soybean yield by 25%, and chickpea yield by 27% over FP, resulting in greater overall system productivity. Right rate of N, P, and K and right timing of application coupled with seed treatment with Zn proved to be the promising interventions under EI at this study site.

Ecological intensification management when yield gaps are wide

Wide yield gaps in maize are still common in several regions of the world. Maize trials conducted as part of the GMP in Eastern Kenya showed the strong influence of ecological intensification on maize yields (Figure 1). The results highlighted the need to change the blanket recommendations and tailor sources of fertilizer to account for the multiple nutrient deficiencies associated with low inherent soil fertility and long-term N+P application. The study also suggested the need to address K, secondary, and micronutrient deficiencies in maize production as a result of significant maize responses to K, S, Zn, B, Mg, and Mo observed across the continent (Garcia et al., 2017).

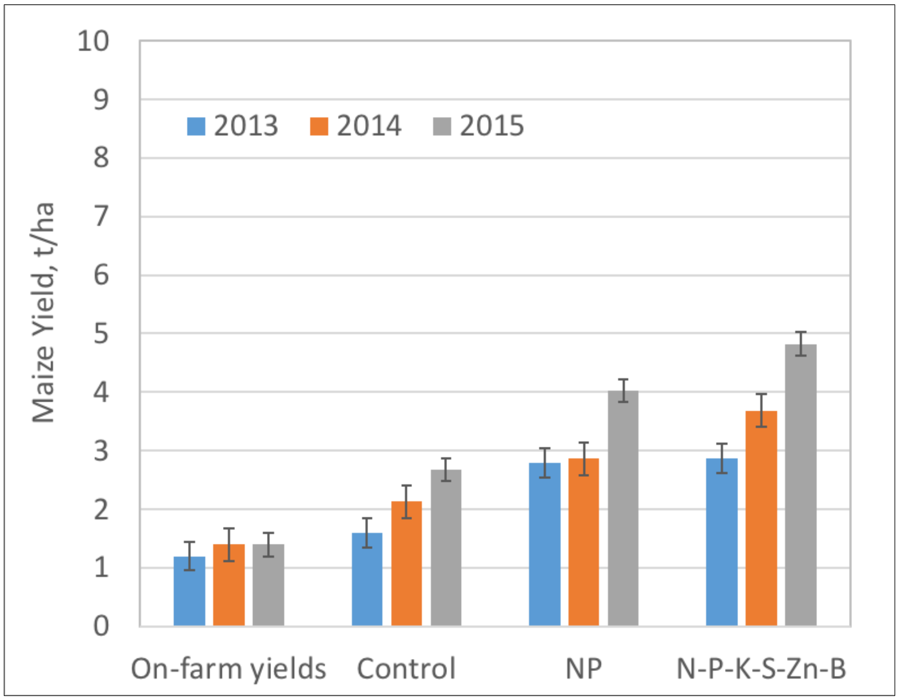

Figure 1. Maize grain yields over three cropping seasons of the Global Maize Project at Kambiyamwe in eastern Kenya. Bars indicate standard errors of the mean. (Source: Garcia et al., 2017)

The GMP in India compared EI with FP at two locations, one at Dharwad (Karnataka) and the other at Ranchi (Jharkhand). EI considered 4R principles of nutrient management combined with other best management practices such as planting time, planted population, hybrid selection, and residue management. EI recorded a significantly higher maize yield (6.5 t/ha) at Dharwad, which was 26% higher than FP. A net return of US $ 1,080/ha was obtained with EI, which was 22% higher than that obtained with FP. Long-term evaluation of EI within a maize-wheat rotation in Ranchi, with red and lateritic soil produced a six-year average grain yield of 6.2 t/ha, amounting to 123% more than the FP average (Figure 2). Applying 240 and 150 kg N/ha (in maize and wheat) split between three applications based on Leaf Color Chart-based N assessment proved to be most beneficial.

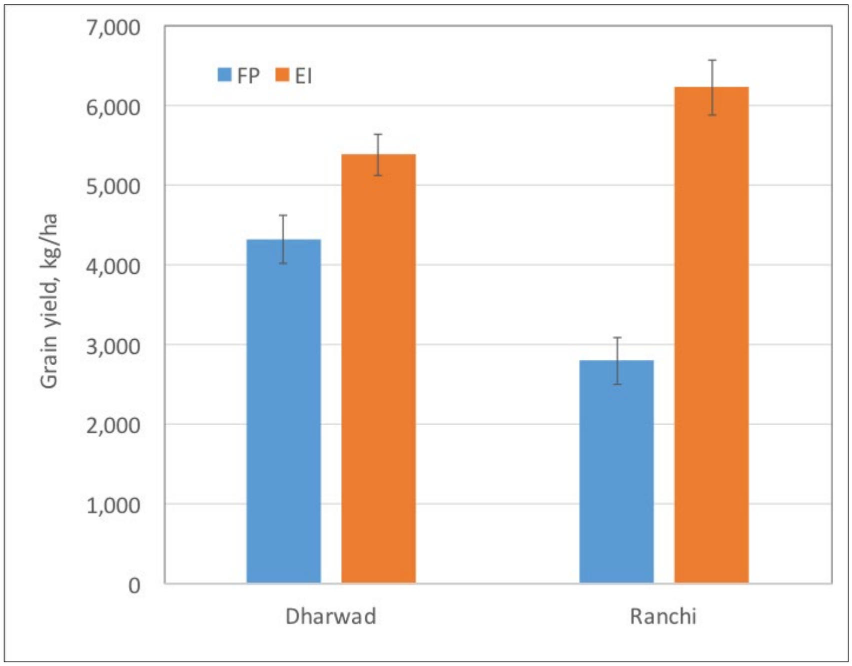

Figure 2. Average grain yields of maize under FP and EI at the GMP sites in India. The data represents an average of six years (2009-14). Vertical bars show standard errors of the means (Source: Garcia et al., 2017).

Data from six years of the GMP at Argentina found that improved soil and crop management increased grain yields by 22% at the Balcarce site and by 43% at the Parana site. Differences in management between FP and EI were related to hybrid, plant population, row spacing, and NPS fertilizer management (Garcia et al., 2017). The study indicated that 4R nutrient management is a key set of practices among the several management practices involved in getting higher yields. Extension work, public policies, and improved economic and political scenarios could greatly contribute to sustainably narrowing the maize yield gap in the study region.

Ecological intensification to increase nutrient use efficiency while maintaining yield levels

In China, the GMP found very interesting results. Results from EI study conducted in a spring maize cropping system in Jilin province found significantly greater grain yield in three of five years and higher nutrient use efficiency for all years under EI. Optimized planting density, reduced fertilizer N rate, and improved application timing were implemented to improve maize yield while significantly improving nutrient use efficiency (Zhao and He, 2017). Agronomic efficiency, which measures how much grain yield has increased per unit of N applied, was 32% lower in FP than in EI. The study indicated that widespread adoption of EI practices will bring sustained benefits to maize cropping systems in northeast China.

Ecological intensification when maize is not the primary crop

In Brazil, farmers of Midwest region began to grow maize more intensively in the fall, as a 2nd crop following soybean harvest. In the 2016 season, 2nd crop maize occupied 66% of the total 15.9 M ha planted to maize and represented 62% of Brazil's total maize production. Intercropping of maize with cover crops, either legumes or grasses, showed benefits related to soil quality, such as better aggregation, increased soil organic carbon and water holding capacity, more N availability through indigenous fixation with legumes, and others. The GMP carried out for more than six years in the states of Parana and Mato Grosso of Brazil considered introduction of forage pea into the EI copping system and offered solution for needed adjustments to N management for both high yield and improved soil quality. The soil and climatic conditions in the study region do not favor accumulation of soil organic matter. The GMP study looked at different cropping system options and tested their abilities to accumulate higher levels of soil organic matter to make the cropping systems more sustainable.

Conclusion

The use of EI practices represents a more sustainable and economic way of employing knowledge and technologies in maize intensification than current farmer practices and aims to address food and environmental security. The Global Maize Project of IPNI helped in measuring the impact of ecological intensification over farmer practice. It offered solutions to intensify maize production in regions where yield gaps are narrow or wide. EI practices improved net return in the majority of sites in USA, which were already recognized to have high yields and narrow yield gaps. EI practices also confirmed the possibility to bridge wider yield gaps through improving maize yield in sub-Saharan Africa, India and Argentina. In China, EI practices significantly improved the nutrient use efficiency while maintaining maize yields at par with FP. Such important information generated from GMP sites in China can be used to optimize nutrient use in the country and offer solutions to reduce excessive nutrient use in the region. Increasing maize yield around the world is a critical goal for EI and right nutrient and agronomic management practices identified in the GMP would help to intensify maize systems around the world. Educating farmers and crop advisers about the impacts of ecological intensification over farmer practice is critical for sustainable intensification of maize systems.

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Conservation Agriculture, Mixed- and Full-tillage Systems in Coastal Bangladesh: A Multi-criteria Analysis of Three Years of Farmer-managed Rice-Maize Trials

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Introduction

Conservation agriculture (CA) is widely advocated as a key method for sustainable crop intensification (FAO, 2011). CA is based on three principles: (1) establishing crops with reduced tillage, (2) residue or live mulches on ≥ 30% of the soil surface, and (3) use of diversified crop rotations (Derpsch et al., 2011). Studies in South Asia report improved production efficiencies and environmental advantages accrued from CA (cf. Hobbs et al., 2008; Jat et al., 2014). Meta-analyses conversely show negative yield effects comparing zero to full-tillage – particularly for maize in the tropics – although use of all three principles reduces losses (Pittlekow et al., 2014; 2015). Evidence also indicates that key biophysical and socioeconomic problems limit smallholder uptake (Giller et al., 2015).

In South Asia, the full suite of CA principles are practiced on 1.5 M ha (FAO, 2018), largely on irrigated fields with good water control in intensive rice-wheat (R-W) rotations in India's western Indo-Gangetic Plains (IGP). Here, use of four-wheel tractors and zero-till drills are common. Conversely, in the eastern IGP and Bangladesh in particular, development initiatives have refocused attention on risk-prone and impoverished coastal regions, where more than 3.5 million families generate livelihoods from agriculture (MoA and FAO, 2013). As an alternative to R-W, rice-maize (R-M) rotations are practiced on 3.5 M ha in Asia, with >0.31 M ha in Bangladesh (Timsina et al., 2010). While acreage is low, R-M rotations have considerable potential for high system-level yields and profits, thereby addressing household rice consumption needs and income generation from maize.

Compared to the western IGP, there is considerably less research on CA and R-M rotations in the eastern IGP. This is especially the case in Bangladesh's coastal region where maize is promoted as an income-generating cash crop. In this zone, two-wheel tractors (2WTs) are commonly used for land preparation. Work on CA has however been mainly focused outside the coastal zone in the country's north, where studies of 2WT attachable planters used for strip tillage, bed planting, and zero tillage are common (Johansen et al., 2012; Gathala et al., 2015; 2016). Of these crop establishment and machinery options, the Chinese-origin power tiller operated seeder (PTOS) can be used for strip tillage and is increasingly commercially available in Bangladesh (Krupnik et al., 2013). Farmers' preferences for repetitive wet tillage and difficulties with field water control however present challenges for establishing monsoon season *aman* rice under CA. An alternative to puddled transplanted rice (TPR) is unpuddled TPR, in which seedlings are manually or mechanically transplanted into uncultivated fields. High capital costs and difficulties with field leveling and precise water management however indicate that machine transplanters are unlikely to be widely suitable for *aman* rice in coastal areas; this necessitates a focus on unpuddled and manually established TPR. Farmers can also puddle TPR and then strip till maize by PTOS, which may confer agronomic and cost-saving advantages by adapting CA principles when and where appropriate. Combined with stress tolerant varieties suited for saline or tidally flood prone coastal areas (Ismail et al., 2013), these practices may further reduce risks for marginal farmers with limited investment capacities.

We implemented multi-season and multi-locational farmer-managed trials over three years across a suite of locations in coastal Bangladesh where farmers either had access to limited irrigation resources or practiced rainfed cropping. Our objective was to assess the agronomic, socio-economic, and environmental impact of R-M rotations under a range of tillage and crop establishment methods, including CA and its adaptations. Drawing upon insights from this research, this paper provides suggestions and implications for agricultural development and extension organizations working to popularize maize and CA in South Asia's stress-prone coastal environments.

Materials and Methods

Site description

Researcher designed and backed but farmer-managed field trials were conducted for three years from the 2011-2012 winter *rabi* season to the 2014 *aman* rice season. Trials were placed in both "rainfed" and "partially irrigated" environments where water access, financial and/or ground and canal water salinity constraints meant that farmers could supply only a limited number of irrigations (maximum two) to maize. Two rainfed (Bhatia Ghata (89°31'38.617"E 22°40'51.422"N) and Kalapara (90°10'40.552"E 21°56'18.812"N) and partially irrigated sites were selected in Babuganj (90°19'52.828"E 22°47'37.573"N), Kaliganj (89°1'26.064"E 22°28'29.126"N), and also in a nearby block of fields in Kalapara (Table 1).

Temperatures followed similar trend across environments, with the highest maximum and minimum temperatures in March-June and lowest in December-January. Precipitation during the winter *rabi* season was unevenly distributed, ranging from as low as 1 mm (Kalapara) to as high as 325 mm (Babuganj) in 2013. Soil salinity increased during the season and was highest in April-May in Kaliganj and Bhatia Ghata, and lowest in Babuganj. Soil salinity was not notable during the monsoon. Rainfall during the monsoon was also variable, though cumulative rainfall ranged from 514 mm in 2014 (Bhatia Ghata) to 1,587 mm in 2013 (Kalapara).

Table 1. Description of the environments and soils (soil C %, total N (%), available P (mg kg^{-1}), exchangeable K (meq 100 g^{-1}), pH and ECa (dS m^{-1}) for each study location in coastal Bangladesh[a].

Environment and location	*Rabi* season Irrigation details					Soil characteristics (0 – 20 cm depth) [c]						
	Type[b]	Ec range (dS m^{-1})[c]		*Aman* season water details		Texture	Soil C	Total N	Avail. P	Exc. K	pH	ECa[e]
Rainfed												
Bhatia Ghata	–	–		Rainfed only		Silty clay	1.47	0.15	3.75	0.37	6.52	4.59 (0.89)
Kalapara	–	–		Rain + tidal water		Silty clay loam	1.13	0.11	3.60	0.32	6.82	1.61 (0.74)
Partially irrigated												
Babuganj	STW	0.24–0.33		Rain + tidal water		Sandy clay	1.28	0.12	3.65	0.31	5.63	0.40 (0.30)
Kaliganj	STW	0.40–4.61		Rainfed only		Silty clay	1.59	0.15	7.09	0.33	7.49	5.55 (0.91)
Kalapara	Canal	2.87–5.68		Rainfed only		Clay	1.28	0.12	3.05	0.37	5.24	1.79 (0.78)

[a.] Mean values for continuous variables except irrigation salinity. [b.] STW indicates shallow tube well. [c.] Five composited samples sub-plot across treatments for each farmer before trials. Exchangeable K was analyzed by atomic absorption spectroscopy after extraction in 1 M NH_4OAc, pH 7. Other soil parameters were measured following SRDI (2014). [e.] Mean ECa (values in parentheses are SD) measured at 0-5 cm depth every two weeks from sowing to harvest in the *rabi* season only using WET Sensors (Delta-T Devices Ltd., Cambridge, UK).

Experimental design

Experiments in all locations were laid out in a split-plot design in the 2011-2012 *rabi* season with maize hybrid NK40 planted to all 28.1 m^2 sub-plots. Three tillage and crop establishment treatments were applied to main plots: (1) CA, (2) “Mixed” tillage, (3) full-tillage across all sub-sub-plots (Table 2). During the *aman* season in 2012, sub-plots were established with two new rice genotypes popularized as high yielding and stress tolerant, including BRRI 41 (salinity-tolerant) and BRRI 52 (submergence tolerant). NK40 was planted subsequently to all sub-plots in *rabi*. Farmers were considered as dispersed replicates. Participants were selected if they (a) had land tenure to maintain trials, (b) had attended trainings on CA and maize crop management, (c) were able and willing to use their own labor to manage treatments, and (d) in case of partially-irrigated locations, were able to supply at least one irrigation. Fifteen farmers in rainfed environments (five in Kalapara and 10 in Bhatia Ghata) and 20 farmers in partially-irrigated environments (five each in Babuganj and Kalapara and 10 in Kaliganj) were selected.

Crop management

In all treatments, fertilizer rates for *rabi* maize were held constant, though they differed for partially-irrigated and rainfed locations. In rainfed environments, N, P and K were applied at 150, 25 and 85 kg ha^{-1}, respectively, while in partially-irrigated environments, 200, 35 and 130 kg N, P and K ha^{-1} were applied. In rainfed environments, half of N was applied basally and the remaining near V8-10 coinciding with precipitation. In partially-irrigated environments, 30% of N was applied basally, with the remaining applied equally at V6 and V10, with a light flood irrigation (~5 cm depth) to incorporate fertilizer into the soil. All P fertilizer was applied basally. In rainfed environments, all K was applied basally while in partially-irrigated environments, 50% was applied basally and 50% at V8-10. In CA and mixed-tillage maize, all basal fertilizers were PTOS drilled, with splits broadcast.

A*man* rice in all locations was rainfed. In Babuganj and Kalapara, fields also experienced freshwater tidal inflow and outflow in the monsoon season. Fertilizer rates for rice in all treatments were the same. N, P, K and S was applied at 90, 24, 41 and 60 kg ha^{-1}, respectively. One-third N was applied basally, with the remaining two-thirds applied equally by broadcasting at 20-25 days after transplanting and at panicle initiation. All P, K and S were applied basally. Rates were the same across locations, exempting Babuganj and Kalapara, where Zn (5 kg ha^{-1}) was also applied basally to overcome known Zn deficiencies in soil. For both maize and rice, N, P, K, S, and Zn were applied through urea, TSP, MOP, gypsum, and $ZnSO_4$ heptahydrate, respectively.

Table 2. Descriptions of tillage, crop establishment, and weed management methods for rice and maize in coastal Bangladesh.

Treatment	Management of maize-rice rotations
Conservation agriculture	Maize was directly drilled using the PTOS for strip tillage by skilled machinery service providers. The PTOS is a 1200 mm wide single-pass shallow tillage implement with a seed and fertilizer drill. It is compatible with a 2WT (Dongfeng company, Wuhan, China). Fluted rollers were used for seed and fertilizer metering. The PTOS can be modified for strip tillage by removing selected rotary blades. Strip tilled furrows are usually < 5 cm width, and therefore disturb < 10% of the soil surface and conform to CA recommendations (cf. Derpsch et al., 2011). Seeds were sown at 6-7 cm depth by the same operator in each site. 35-40 cm standing rice residue height was retained on the soil surface. In the first trial year, straw was retained from the previous *aman* crop managed by farmers. A target spacing of 60 cm between rows and 20 cm between plants was used. Glyphosate with was applied within five days before planting. Pendimethaline, a pre-emergence herbicide, was applied two days after planting. Both were applied at 1 kg a.i. ha^{-1}. For the rice crop, fields were permitted to soak until the soil was sufficiently soft to support unpuddled transplanting. 35-40 cm height of standing maize residue was retained on the soil surface before transplanting. Three to four 35-40 day old seedlings per hill were transplanted at 25 × 15 cm. Glyphosate was applied at 900 g a.i. ha^{-1} by sprayer before land soaking. Pretilachor at 500 g a.i. ha^{-1} at 1-3 days after transplanting. For all glyphosate applications, urea surfactant was simultaneously applied at 1.5–2.0 kg ha^{-1}.
Mixed-tillage	Maize was established using strip tillage with a rice residue mulch as described for CA. Land preparation for rice involved 2-4 wet tillage passes using a 2WT-driven power tiller that incorporated 35-40 cm height of standing maize

	stover residue into the soil as an organic amendment. Tillage was approximately 10 cm deep. Transplanting was done as in CA. Because soils were puddled, herbicides were not applied. Hand weeding was done as needed as determined by farmers, with emphasis on weeding before fertilizer application.
Full-tillage	For maize, 3-4 tillage passes were made by the same 2WT power tiller followed by manual seeding with all rice residues removed. The same planting depth and configuration was used as described for CA and mixed-tillage. Rice was managed as described for mixed-tillage above, though with all maize residues removed and no organic amendments applied. No herbicides were used in either crop. Any weeds were manually removed by farmers at their discretion to avoid crop losses.

Data collection, greenhouse gas emissions modeling, and analysis

Maize population density was measured from three 4 m long randomly selected rows approximately 18 d after sowing. Soil apparent electrical conductivity was measured in eight locations plot^{-1} at 0–5 cm depth *in situ* every 15 d after seeding using WET Sensors (Delta-T Devices Ltd., Cambridge, UK). Maize was harvested from 10.08 m^2 in the center of each plot to determine grain yield (15.5% moisture content) after air drying to a constant weight. Stover yields were obtained by drying 20 plants in the same way, with ~350 g fresh sub-samples used to determine moisture content gravimetrically after oven drying (70° C for 72 h). Rice grain yield (14% moisture content) was measured from 10 m^2 after the same drying process. Straw yield was recorded similarly from a 1.8 m^2 surface in each harvest plot. In CA and mixed-tillage, residues retained as mulch or incorporated were measured separately from those exported.

Input costs and labor use data were collected from farmers through surveys 3-4 times season^{-1} and after harvest. Prices for inputs and outputs for each season were monitored from local markets. Fuel use for land preparation and seeding, as well as irrigation, were measured following Gathala et al. (2016). These data were used for partial budgeting and were converted to megajoule (Mj) equivalents for energetic analysis following Gathala et al. (2016) and Nassiri and Singh (2009). Net income was calculated by dividing all variable costs from gross returns from grain and exported stover or straw. Energy inputs and outputs were calculated for grain and straw or stover recycled, and for total biomass exported. Energy use efficiency (EUE) was computed by dividing total energy output for both rice and maize (Mj ha^{-1}) by total energy inputs (Mj unit^{-1}). Specific energy (SPE) was computed as total energy inputs (Mj inputs^{-1}) divided by grain + straw or stover yield (kg ha^{-1}). Lastly, energy productivity (EP) was defined as grain yield (kg ha^{-1}) divided by total energy input (Mj ha^{-1}).

We also used the CCAFS Mitigation Options Tool (CCAFS-MOT; Feliciano et al., 2016) to estimate greenhouse gas (GHG) emissions. CCAFS-MOT comprises a generic set of empirical models and estimates full farm-gate level emissions. Soil and climatic information, production inputs and management information from trials were fed into a version of the CCAFS-MOT scripted in "R". Emissions from rice were estimated following Yan et al. (2005), which estimates CH_4 from rice under different water regimes as a function of soil pH, climate and organic amendments. Background and fertilizer-induced N_2O emissions were calculated based on Stehfest and Bouwman (2006). Emissions from fertilizer production and transport were calculated from the Ecoinvent Center (2007) database. Changes in soil C from residue management were based on Ogle et al. (2005) and Smith et al. (1997). Those due to tillage were based on Powlson et al. (2016). Similarly, soil CO_2 emissions from fertilizers and water regime were estimated following the IPCC (2006). All GHGs were converted into CO_2-equivalents (CO_2e) using 100-year global warming potentials of 34 and 298 for CH_4 and N_2O, respectively (IPCC, 2013). Yield-scaled emissions for each treatment was determined by dividing total GWP by Mg grain ha^{-1}.

Data were averaged across years and analyzed separately for partially irrigated and rainfed environments. A split-split ANOVA considering location, tillage, and rice genotype plots as the main, sub-, and sub-sub sources of variation was performed using the restricted maximum likelihood (REML) option in JMP 14 (SAS Institute Inc., San Francisco). Soil moisture measurements were compared using least squares means contrasts. Farmers were treated as a random effect.

Results and Discussion

Cropping systems productivity

In environments with partially irrigated maize, a significant effect ($P<0.001$) of location on maize yield was observed, with the greatest yields (7.6 Mg ha^{-1}) found in Kaliganj (Table 3).

Table 3. Agronomic performance of tillage and crop establishment in rice-maize systems in two environments in coastal Bangladesh.

Variation source	Partially irrigated environments					Rainfed environments			
	Rice yield (Mg ha^{-1})	Maize yield (Mg ha^{-1})[a]	Maize density (plants ha^{-1})	System yield (Mg ha^{-1})[a]		Rice yield (Mg ha^{-1})	Maize yield (Mg ha^{-1})	Maize density (plants ha^{-1})	System yield (Mg ha^{-1})[a]
Location									
Kaliganj	4.7 a	7.6 a	78,525 a	12.3 a		—	—	—	—
Kalapara	4.6 a	7.1 b	77,634 ab	11.7 b		4.4 b	4.2 b	68,587 b	8.6 b
Babuganj	3.5 b	6.0 c	76,775 b	9.5 c		—	—	—	—
Bhatia Ghata	—	—	—	—		4.8 a	6.0 a	77,995 a	10.8 a
Tillage									
CA	4.4 a	7.2 a	78,525 a	11.6 a		4.8 a	5.3 a	74,582 a	10.2 a
Mixed-tillage	4.2 ab	7.1 a	77,634 ab	11.3 a		4.6 b	5.2 a	73,717 ab	9.8 a
Full-tillage	4.2 b	6.4 b	76,775 b	10.6 b		4.4 c	4.7 b	71,574 b	9.1 b
Rice genotype									
BRRI 41	4.0 b	6.9	77,651	10.9 b		4.4 b	5.0	73,324	9.4 b
BRRI 52	4.6 a	6.9	77,638	11.5 a		4.9 a	5.0	73,258	9.9 a
Probability									
L (L)	***	***	***	***		***	***	***	***
Tillage (T)	***	***	***	***		***	***	***	***
Genotype (G)	***	ns	ns	***		***	ns	ns	***

[a] R-M system yields in this column may slightly differ from the sum of rice and maize columns due to rounding. CA refers to conservation agriculture (unpuddled transplanted *aman* rice – strip tilled maize), 'mixed-tillage' refers to puddled transplanted (PTR) rice– strip tilled maize, full-tillage entails PTR *aman* – fully tilled, hand planted maize. Means in columns not separated by a blank row space not sharing the same letter are significantly different according to Tukey's HSD at alpha = 0.05. ***, ** and * indicates significance at $P < 0.001$, 0.01 and 0.05. NS indicates non-significance. L × T, L × G, T × G, and L × T × G effects were generally not significant and are not shown.

Tillage treatments also showed significant differences ($P<0.001$). Both CA and mixed-tillage yielded 0.8 and 0.7 Mg ha^{-1} more than full-tillage. In rainfed environments, significant effects of location and tillage were also observed (both $P<0.001$). Kalapara had yields 1.8 Mg ha^{-1} less than Bhatia Ghata. Relative to mid-range yields in partially irrigated environments, these results indicate that at least some irrigation is likely to be required to increase yield. As in irrigated environments, rainfed maize yields were highest under CA and mixed-tillage, each 0.6 and 0.5 Mg ha^{-1} higher than full-tillage. In both CA and mixed-tillage, maize was drilled by PTOS into standing rice residue. Under full-tillage, it was sown by hand after 2-4 power tiller passes, as is commonly practiced throughout Bangladesh (cf. Gathala et al. 2015; 2016). Significant ($P<0.001$) effects of tillage and crop establishment method on maize stand density were found in both partially irrigated and rainfed environments. CA and mixed-tillage resulted in 1,750 and 859 more plants ha^{-1}, respectively, than full tillage in partially irrigated environments. In rainfed environments, 3,008 and 2,143 more plants ha^{-1} resulted from CA and mixed-tillage relative to full-tillage. These trends can be partly explained by higher soil moisture levels from sowing through the reproductive phase that result from rice residue retention and strip-till drilling of maize seed under CA and the mixed-tillage treatments. Although our measurements were only at limited depth (0-5 cm), we found consistent and significant differences ($P<0.01$ or 0.001) found using least square means contrasts between treatments (data not shown). This suggests an improved soil environment for maize crop establishment and early growth under CA and mixed-tillage. Higher soil moisture and yields may have also resulted from earlier crop establishment using strip tillage in these treatments. Ali et al. (2009) observed declining yield potential in maize grown in Bangladesh when *rabi* season sowing is delayed. Farmers managing trials achieved maize crop establishment using strip tillage techniques (used in the CA and mixed-tillage treatment for maize) that were on average 6 and 5 days earlier than with full-tillage in partially irrigated and rainfed environments, respectively. Combined with improved soil moisture storage and population density, this may have affected the higher yields observed in farmers' fields. Importantly, our results contrast with meta-analyses conducted by Pittlekow et al., (2014; 2015) that indicate poor performance of CA in the humid sub-tropics, particularly for maize. These studies however did not consider the potential advantages of earlier sowing under CA or adapted CA systems, nor did they include strip-tillage as a means of CA crop establishment.

Similar yield patterns were observed in *aman* rice. The lowest yields in the partially irrigated environments was found in Babuganj, which experiences prolonged and sometimes deep (up to 1 m) tidal flooding stress. For this reason, BRRI 52, which has submergence tolerant qualities (Ismail et al, 2013), yielded 0.6 Mt ha^{-1} above BRRI 41. In the rainfed environments, tidal flooding was less of a concern, but BRRI 52 still yielded 0.5 Mg ha^{-1} above BRRI 41. Considering rice establishment, yields in partially irrigated and rainfed locations were highest with unpuddled transplanting in CA relative to full-tillage. This was most consistent and significantly different (P<0.001) in rainfed environments, where unpuddled transplanting yielded 0.2 and 0.4 Mg ha^{-1} above mixed- and full-tillage, respectively. This effect may have been due to transplant laborers' more careful transplanting efforts in CA treatments, and/or shallower transplanting in unpuddled soils relative to a tendency to plunge seedlings deeply into the soil of puddled fields. Maintenance of residues under CA may also contribute to improved soil N availability over time (Gathala et al., 2017). Such practices may reduce transplant shock and stimulate early growth, though this hypothesis requires further verification. It is worthy to note, however, that while laborers tended to transplant more carefully in unpuddled CA fields, they did so more because of difficulty experienced transplanting rather than by choice or intent. Laborers in all locations for example complained of the difficulty experienced with unpuddled transplanting, indicating that it be painful to one's hand to transplant without tillage.

Combining rice and maize yields at the systems level, location effects in partially irrigated environments followed those observed for maize and rice. In both partially irrigated and rainfed environments, rice-maize systems established using CA or mixed-tillage significantly (P<0.001) out-yielded full-tillage treatments by between 0.7–1.1 Mg ha^{-1}. Given the constraints experienced by transplant laborers, and the lack of statistically significant differences between mixed and full-tillage when considered from a cropping systems perspective, mixed-tillage may be an appropriate CA adaptation in Bangladesh's costal region. This however requires further analysis considering additional criteria, as discussed below.

Energetic performance

In both partially irrigated and rainfed environments, site differences in energetic inputs to maize were observed as a result of variation in tillage number (in full-tillage) and human manual labor. Significant differences (P<0.001) were found between tillage and crop establishment treatments in both partially irrigated and rainfed environments, with CA and mixed-tillage utilizing fewer energy inputs for maize than full-tillage (Tables 2 and 4). While significant, differences were however not exceedingly large, partially due to the relatively limited number of tillage passes in full-tillage, and the high energetic costs for herbicides (713 Mj ha^{-1} across all trials) used to establish maize by strip tillage. In rice, unpuddled transplanting in the CA treatments also resulted in significantly lower (P<0.001) energy inputs, but only in partially irrigated environments.

Table 4. Field energetic performance of tillage and crop establishment systems in partially irrigated environments in coastal Bangladesh.

Variation source			Rice output (MJ ha^{-1})[a]				Maize output (MJ ha^{-1})[a]			System energy performance components (rice + maize)[a]		
		All rice inputs (MJ ha^{-1})	Grain + recycled straw[b]	Total biomass (grain + all straw)		All maize inputs (MJ ha^{-1})	Grain + recycled stover[b]	Total biomass (grain + all stover)		EUE[c]	SPE[c]	EP[c]
Location	Kaliganj	9,611 a	85,754 b	154,587 a		36,671 a	140,541 a	236,383 b		8.4 b	1.8 b	0.27 a
	Kalapara	9,381 b	90,358 a	152,741 a		36,644 a	120,798 b	207,668 c		7.8 c	1.9 a	0.25 b
	Babuganj	9,398 b	72,861 c	122,638 b		36,172 b	139,960 a	311,156 a		9.6 a	1.8 b	0.21 c
Tillage	CA	8,620 b	95,021 a	144,613		36,199 b	155,364 a	260,873		9.1 a	1.7 b	0.26 a
	Mixed till	9,885 a	92,307 a	142,795		36,203 b	151,302 a	253,569		8.6 ab	1.8 a	0.24 b
	Full till	9,884 a	61,645 b	142,559		37,085 a	94,633 b	240,765		8.2 b	1.9 a	0.23 c
Rice	BRRI 41	9,461	77,895 b	137,560 b		36,498	134,091	252,340		8.5	1.9	0.24 b
genotype	BRRI 52	9,465	88,087 a	149,085 a		36,493	133,442	251,132		8.7	1.8	0.25a
Probability	L (L)	***	***	***		***	***	***		***	***	***
	Tillage (T)	***	***	***		***	***	ns		***	***	***
	Genotype (G)	ns	***	***		ns	ns	ns		ns	ns	***

[a.] All calculations consider grain + total straw or stover produced. [b.] For full till, values in this column are for grain energy only. [c] EUE, SPE and EP refer to Energy Use Efficiency, Specific Energy, and Energy Productivity, respectively For descriptions of tillage treatments, refer to Table 2. For descriptions of tillage treatments and significance levels, refer to Table 2. Interactive model effects were generally NS and are not shown.

No differences were observed in rainfed locations, a consequence of the trade-off in herbicide use in CA relative to a lower fuel consumption rate and lower number of tillage passes applied by farmers in mixed and full-tillage for puddling rice. At the cropping systems level, 106,141-155,101 Mj ha^{-1} of energy were recycled as retained or incorporated rice and maize residues across trials. Systems-level energy use efficiency (EUE, Mj biomass ha^{-1} / Mj inputs ha^{-1}) in partially irrigated environments was significantly different ($P<0.001$). The highest and lowest EUE was found with CA and full-tillage, respectively.

Table 5. Field energetic performance of tillage and crop establishment systems in rainfed environments in coastal Bangladesh.

Variation source			Rice output (MJ ha^{-1})				Maize output (MJ ha^{-1})			System energy performance components (rice + maize)[a]		
		All rice inputs (MJ ha^{-1})	Grain + recycled straw[b]	Total biomass (grain + all straw)		All maize inputs (MJ ha^{-1})	Grain + recycled stover	Total biomass (grain + all stover)		EUE[c]	SPE[c]	EP[c]
Location												
	Kalapara	9,365	86,862	149,015 b		26,686 a	74,234 b	132,317 b		7.8 b	1.9 a	0.24 b
	Bhatia Ghata	9,438	87,350	156,480 a		26,221 b	108,413 a	167,510 a		9.1 a	1.7 b	0.30 a
Tillage	CA	8,710	100,471 a	156,258		26,264 b	103,976 a	158,616		9.0 a	1.7 c	0.29 a
	Mixed till	9,748	95,715 b	151,854		26,264 b	100,948 a	151,223		8.4 b	1.8 b	0.27 b
	Full till	9,746	65,131 c	150,131		26,833 a	69,047 b	139,902		7.9 c	1.9 a	0.25 c
Rice	BRRI 41	9,401	83,712 b	150,728		26,459	91,178	149,885		8.4	1.8	0.26 b
genotype	BRRI 52	9,401	90,499 a	154,766		26,448	91,470	149,942		8.5	1.8	0.28 a
Probability	L (L)	ns	ns	***		***	***	***		***	***	***
	Tillage (T)	ns	***	ns		***	***	ns		***	***	***
	Genotype (G)	ns	***	ns		ns	ns	ns		ns	ns	***

[a] For details of abbreviations, treatments, and measurements, readers may refer to the footnote in Table 4.

From the agronomic perspective, energy productivity (EP, kg grain ha^{-1} / Mj external input ha^{-1}) is however arguably a more important metric. EP was also significantly different ($P<0.001$) and greatest under CA followed by mixed and full-tillage (Gathala et al. 2016). In rainfed environments, the lack of irrigation for maize lowered overall energy use. EUE was greatest with CA, followed by mixed- and full-tillage. EP followed the same trend. Considering rice genotypes, BRRI 52 showed consistently and significantly ($P<0.001$) higher EP than BRRI 41, regardless of environment.

Economic performance

Across environments and trial locations, maize production requires more investment costs for farmers than rice (Tables 6 and 7). This is largely due to higher seed costs and elevated fertilizer rates relative to less intensively produced *aman*, regardless of variety tested. Significant differences (all $P<0.001$) between locations within rainfed or irrigated environments were found for rice, maize, and at the cropping systems level, a result in local differences in input prices and variation in slight differences in manual labor requirements.

Table 6. Profitability, global warming potential (GWP), and yield-scaled emissions of rice-maize tillage and crop establishment in rice-maize systems in partially irrigated environments in coastal Bangladesh[a].

Variation source	Costs ($ ha^{-1})			Net benefit ($ ha^{-1})			Total GWP (kg CO^{2}e ha^{-1})			Yield-scaled (kg CO^{2}e ha^{-1} Mg grain)		
	Rice	Maize	System	Rice	Maize	System	Rice	Maize	System	Rice	Maize	System
Location												
Kaliganj	566 b	683 c	1,249 b	646 a	1085 a	1,731 a	3,971 b	227 b	4,217 c	863 c	34 c	897 c
Kalapara	581 a	781 a	1,365 a	530 b	804 b	1,331 b	6,294 a	1,156 a	7,441 a	1,382 a	178 b	1,560 b
Babuganj	585 a	756 b	1,344 a	253 c	610 c	861 c	3,880 c	1,133 a	5,004 b	1,042 b	211 a	1,242 a
Tillage												
CA	469 a	702 b	1,173 c	588 a	933 a	1,520 a	4,602 b	360 b	4,961 c	1,022 b	60 b	1,082 c
Mixed till	633 b	704 b	1,338 b	385 c	903 a	1,286 b	5,210 a	355 b	5,565 b	1,223 a	60 b	1,283 b
Full till	631 b	814 a	1,447 a	456 b	664 b	1,118 c	4,334 c	1,802 a	6,136 a	1,041 b	303 a	1,344 a
Rice genotype												
BRRI 41	574	737	1,313	412 b	840	1,250 b	4,882 a	842	5,724 a	1,184 a	142	1,319 a
BRRI 52	581	743	1,326	540 a	827	1,366 a	4,549 b	835	5,384 b	1,007 b	141	1,147 b
Probability												
L (L)	***	***	***	***	***	***	***	***	***	***	***	***
Tillage (T)	***	***	***	***	***	***	***	***	***	***	***	***
Genotype (G)	ns	ns	ns	***	ns	***	***	ns	***	***	ns	***

[a] All calculations consider grain + total straw or stover produced. For descriptions of tillage treatments, refer to Table 2. Interactive model effects were generally NS and are not shown.

Large and significant (P<0.001) cost reductions for CA and mixed-tillage were found in irrigated environments comparing CA ($112 ha^{-1} less) and mixed ($110 ha^{-1} less) to full-tillage. In rainfed locations, differences were modest ($67 and 76 ha^{-1} less, respectively), though still significant (P<0.001). In rice, significant cost differences (both P<0.001) for *aman* crop establishment were found in both environments. Unpuddled transplanting in CA reduced overall costs by $162–169 ha^{-1} relative to puddled transplanting with mixed- and full-tillage.

Table 7. Profitability, global warming potential (GWP), and yield-scaled emissions of rice-maize tillage and crop establishment in rice-maize systems in rainfed environments in coastal Bangladesh[a].

Variation source	Costs ($ ha^{-1})			Net benefit ($ ha^{-1})			Total GWP (kg CO^{2}e ha^{-1})			Yield-scaled (kg CO^{2}e ha^{-1} Mg grain)		
	Rice	Maize	System	Rice	Maize	System	Rice	Maize	System	Rice	Maize	System
Location												
Kalapara	578 a	585 a	1,164 a	493 b	409 b	902 b	3,392 b	629 a	4,016	782	158 a	938 a
Bhatia Ghata	561 b	471 b	1,033 b	655 a	844 a	1,499 a	3,762 a	270 b	4,032	797	56 b	852 b
Tillage												
CA	458 b	509 b	967 c	708 a	698 a	1,407 a	3,331 c	-25 b	3,304 c	698 c	3 b	701 c
Mixed till	627 a	500 b	1,127 b	487 c	686 a	1,173 b	3,945 a	-32 b	3,910 b	870 a	3 b	872 b
Full till	624 a	576 a	1,200 a	526 b	495 b	1,022 c	3,454 b	1,405 a	4,857 a	799 b	314 a	1,113 a
Rice genotype												
BRRI 41	569	528	1,097	525 b	626	1,152 b	3,670 a	449	4,117 a	852 a	107	958 a
BRRI 52	570	529	1,099	623 a	626	1,249 a	3,483 b	450	3,931 b	727 b	107	833 b
Probability												
L (L)	***	***	***	***	***	***	***	***	***	ns	***	***
Tillage (T)	***	***	***	***	***	***	***	***	***	***	***	***
Genotype (G)	ns	ns	ns	***	ns	***	***	ns	***	***	ns	***

[a] For details of abbreviations, treatments, and measurements, readers may refer to the footnote in Table 6.

At the cropping systems level, costs were significantly different in both irrigated and rainfed locations ($P<0.001$). CA reduced overall costs by \$274 and \$233 ha^{-1} in irrigated and rainfed environments, relative to full-tillage. When comparing mixed-tillage to CA, costs were \$160-165 greater, although mixed-tillage still reduced costs by \$109 and \$73 ha^{-1} in partially irrigated and rainfed environments, respectively. Both the CA and mixed-tillage treatments therefore clearly have cost-saving advantages.

Farmers in coastal Bangladesh also tend to be cash constrained prior to the cropping season. CA and mixed-tillage were \$169 ha^{-1} and \$80 ha^{-1} less costly to establish at the cropping-systems level than full-tillage in partially irrigated environments. In rainfed environments, trends were very similar (\$169 and \$87 ha^{-1} lower establishment costs, data not shown). Rural Bangladesh is also experiencing increasing urban and international migration as laborers leave agriculture in search of more remunerative employment (Zhang et al., 2014). This results in increasing rural labor scarcity and costs, thereby mounting pressure on farmers working to balance their food and income generation needs with growing production costs. Partial substitution with mechanized crop establishment for maize under CA and mixed-tillage reduced cropping systems-level labor requirements by 60 and 38 person-days ha^{-1} relative to full-tillage in partially irrigated environments, and 59 and 35 person-days ha^{-1} in rainfed environments (data not shown).

Lower costs and a tendency towards higher yields with CA and mixed-tillage led to higher profits with each treatment relative to full-tillage across sites. In partially irrigated environments, net profits were significantly different ($P<0.001$) and \$402 ha^{-1} and \$168 ha^{-1} greater than full-tillage, even with foregone profits for rice straw or maize stover retained or incorporated. In rainfed environments, significant ($P<0.001$) differences were also found, with \$385 ha^{-1} and \$151 ha^{-1} greater profit from the same treatments, respectively. It is also important to note that while no differences were found between rice cultivation costs, BRRI 52 yielded consistently and significantly ($P<0.001$) higher net benefits across tillage treatments regardless of environment (Tables 6 and 7).

Greenhouse gas emissions

Simulation modeling highlighted significant ($P<0.001$) location differences in total global warming potential (GWP, kg CO^{2}e ha^{-1}) within partially irrigated and rainfed locations, both for maize and rice production (Tables 6 and 7). These differences result from variation in soil physical and chemical qualities, and as a result of tidal flood water ebb-and-flow patterns that affect soil-water status in simulations for *aman* rice in Babuganj and Kalapara. Comparing tillage treatments across partially irrigated locations, significant differences ($P<0.001$) were found for rice and maize individually, and also at the cropping systems level. In rice, full-tillage had the lowest total GWP (4,334 kg CO^{2}e ha^{-1}), followed by CA (4,602 kg CO^{2}e ha^{-1}) and mixed-tillage (5,201 kg CO^{2}e ha^{-1}). These results come from higher reactive CH_4 emissions when maize residue was retained or incorporated in CA and mixed-tillage, respectively, resulting in a trade-off between yield, profitability, and energetics with total GWP. It should however be noted that emissions arising from farmers' postharvest use of residues taken off the field was not accounted for in our simulations. In maize, CA and mixed-tillage however had GWP 1,442 and 1,447 kg CO^{2}e ha^{-1} less than full-tillage, respectively. Trends were similar in rainfed environments, although the lack of energy consumed for irrigation and residue retention under strip tillage resulted in net C sequestration of -25 and -32 kg CO^{2}e ha^{-1} under CA and mixed-tillage. Full-tillage in rainfed environments, in which all residues were exported, conversely resulted in much higher GWP (1,405 kg CO^{2}e ha^{-1}). At the cropping systems level GWP was significantly different in both partially irrigated and rainfed environments at the $P<0.001$ level. Total GWP followed the trend CA < mixed-tillage < full-tillage in both environments.

Yield-scaled emissions (kg CO^{2}e Mg^{-1}grain) on the other hand provide an additional and useful measure of agronomic performance by integrating production with mitigation goals (Pittelkow et al. 2014). At the cropping systems level in partially irrigated environments, very significant differences were observed ($P<0.001$). CA had the lowest yield-scaled emissions (1,082 kg CO^{2}eMg^{-1} grain), followed by mixed and full-tillage (1,283 and 1,334 kg CO^{2}eMg^{-1} grain), respectively. In rainfed locations, highly significant differences ($P<0.001$) were also found. Yield-scaled emission under CA and mixed-tillage were 412 and 241 kg CO^{2}e Mg^{-1} grain lower than full-tillage, respectively. These results should however be treated with caution. While they are indicative of the likely pattern of GWP across the environments and treatments observed, our data are derived from simulation modeling rather than direct measurements. They are therefore subject to some degree of imprecision (Richards et al., 2016), although are arguably a 'good-bet' approach to determining emissions given the infeasibility of *in-situ* GHG measurements from a large number of dispersed farmer-managed trials over multiple environments and years. We were also unable to model the GWP of fallow periods between the end of *aman* and start of the *rabi* season, nor were we able to assess the transition from *rabi* into *aman.*

Integrated multi-criteria assessment of cropping systems performance

Sophisticated changes in crop management and cropping systems require multi-criteria assessments to identify potential trade-offs and offer solutions to resolve conflict between agronomic, economic, environmental, and cultural criteria. Over three years of farmer-managed rice-maize rotational trials comparing different tillage and crop establishment methods, we observed that CA and mixed-tillage tended to have slightly higher relative yields, lower manual labor and energy requirements, reduced production costs – particularly for those incurred early in the season – and large economic labor productivity and value-cost ratio benefits (data not shown). Similar responses were found in rainfed environments, though with a tendency for larger relative benefits exempting value-to-cost ratios. CA tended to perform better across these criteria compared to mixed-tillage.

Farmer surveys at the conclusion of three years of experimentation however indicated strong preferences against CA, particularly because of the difficulty farmers faced in convincing hired laborers to manually transplant their fields without having puddled them. In partially irrigated locations, for example, 72% of farmers responded to hypothetical questions on technology uptake by saying they would not adopt CA based rice-maize systems on any of their fields. Twenty-eight and 94% conversely said they would not adopt mixed or full-tillage, with the same pattern repeated in rainfed locations. Sixty-one and 56% of the farmers who participated in experiments in partially irrigated and rainfed environments said they would consider adopting the mixed-tillage treatment on all of their fields if service providers could reliably offer strip till seeding services with the PTOS for maize. No farmers however showed any interest in adopting full-tillage on all their fields – largely a consequence of their observation of superior maize performance using strip tillage. Only 22 and 31% of farmers in partially irrigated and rainfed environments however indicated an interest in adopting CA on all their fields.

Farmers in South Asia have been wet puddling rice fields for thousands of years, largely to control weeds and maintain required levels of water control (Greenland 1997). Our data also indicate that puddling has important implications for the adoptability of principled production practices like CA that ask farmers to forgo tillage. Farmers preferences for mixed-tillage over CA resulted largely from their dislike of unpuddled manual transplanting, despite the yield-enhancing benefits observed in the environments detailed in this study. Furthermore, large-scale adoption of mechanized transplanting – which requires shallow water during machine operation – is unlikely in many of the studied environments because farmers have limited control over floodwater depth in the early *aman* season. Farmers in all locations faced problems with laborers refusing or arguing against manually transplanting rice in unpuddled trial plots. Although we did not include transactions costs associated with farmers who negotiated with laborers to convince them to implement CA to maintain trial validity, further research or farm surveys examining these practices may consider this generally unreported but important consideration. Cofre-Bravo et al. (2018) also pointed out the importance of farmer-workforce relations and the need for laborers to support transitions to, and adoption of, new technologies and practices. Our study provides some support for these observations while helping to underscore the need for broader-measures of farmers' transactions costs and ability to negotiate with laborers as active actors in technology adaptation and adoption.

Conclusions

We studied rice-maize tillage and crop establishment systems performance using multiple agronomic, environmental, and economic criteria in the context of three-years of farmer managed experiments across water-resource scarce and rainfed environments in coastal Bangladesh. At the cropping systems level, both CA and mixed-tillage, in which maize was established with machine-aided strip tillage followed by puddled transplanting of rice, tended to have significantly higher yields than conventional full-tillage treatments. Energy use efficiency and energy productivity also followed similar trends, as did economic performance, although CA tended to lend the most benefits. CA also had lower GWP and yield-scaled emissions than mixed and full-tillage in partially irrigated and rainfed environments. Yield-scaled emissions were also much lower with mixed-tillage than full-tillage in rainfed sites. Submergence tolerant BRRI Dhan 52 performed consistently better than BRRI 41, indicating its relative suitability in rice-maize systems in coastal Bangladesh. Despite the benefits of CA, farmers however showed considerably less interest in unpuddled transplanting as a component of CA. They broadly opted for mixed-tillage as a more adoptable suite of management practices in coastal environments. Our results therefore point to the importance of adapting CA, as strict application is unlikely to be appropriate given current cultural and labor workforce constraints, and without availability of environmentally suitable mechanized transplanters. The mixed-tillage system conversely exhibited many of the benefits of CA and is likely a better-bet for rice-maize rotational systems managed by smallholder farmers in coastal Bangladesh.

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Efficiency of Slow/ Controlled-Release Fertilizers or Nano-Endophyte on Agronomy and Yield of Maize Hybrids in the Mekong Delta of Vietnam

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Introduction

Grain maize production in Vietnam not only faces biotic and abiotic stresses, but also high cost of production due to low levels of mechanization, postharvest losses and little application of advanced technological fertilizers. Survey data by (FAOSAT, 2018) showed that cost of production for one ton of maize grain was USD 138 in Brazil, USD 142 in the USA, USD 225 in Thailand, USD 275 in the Philippines, USD 282 in Indonesia and USD 329 in Vietnam. As for components involved in maize production in Mekong delta of Vietnam (Ho Cao Viet *et al*, 2014), the average cost of fertilizers takes up of 30-35.5% of total costs; labor costs 38.2%; mechanization (hired machines) is between 5.0 to 8.7% and pesticides vary from 4.9-12.2%. To increase efficiency of fertilizer and lower the cost of production, study of Slow/Controlled-Release Fertilizers (SCRF), combined with micronutrient Bioplant Flora originating from Ukraine, was conducted to investigate their effects on agronomy and yield of MN585 maize hybrid in the Mekong delta of Vietnam in 2016 to 2018. Five experiments in winter-spring (2016-2017), summer-autumn (2017) and winter-spring (2017-2018) in alluvial soil with pH of 6.5 in Dong Thap province, and two others in spring-summer (2017) and winter-spring (2017-2018) in minor sulphate acid soil (pH of 4.5-5.0) in Long An and Hau Giang provinces were conducted.

Materials and Methods

Materials

1. MN585, which was released in 2017, is an early to medium maturity (98-100 days) maize hybrid in Southern Vietnam. It has good lodging resistance, minor infection of foliar diseases (2.0 scores), good plant and ear aspects (2 to 2.5 scores) and high yield potential (8 t/ ha in South Eastern region, and over 12 t/ ha in intensive farming region of Mekong delta).
2. Slow/controlled-release fertilizers (SCRF) with pure N:P:K formula being 22:8:12 TE from Kingenta Ltd, China.
3. Complex particles of Nano-endophyte (< 100 nanomet) under particle (<100 nm) including Fe, Cu, Zn, Mn, B, Mo and Co, combined with micronutrient Bioplant Flora (namely Bio in this paper) under liquid solution including acid Humic, Fulvic, N, P_2O_5, K_2O, Cu, Zn, Co, Mn, Mo, Fe, Mg with pH from 7-9, originated from Ukraine (Nguyen Tien Dung, 2018).
4. Amistar Top 325SC (Syngenta fungicide) with composition of Azoxystrobin 200 g/l + Difenoconazole 125 g/l (Syngenta, 2018).
5. Fungicides of Syngenta: Anvil 5SC with composition of 50g/L Hexaconazole; Kasumin with composition of kasugamycin 2%.
6. Urea (46%N), Super phosphate (16 % P_2O_5) and Kaliclorua (60 % K_2O).

Methods

1. All experiments were conducted following randomized complete block design with three replications; eight rows per plot (21 m^2); each row 5m long; two rows per bed, 0.3m in height; 0.7m between rows and 0.2m between plants on rows.
2. Control treatments were no fertilizer at all (Table 2 and 3) or spraying water only (Table 4 and 6). Farmers practice of fertilizer varied from (kg per hectare) $200N:106P_2O_5:48K_2O$, $233N:161P_2O_5:48K_2O$, to $265N: 147P_2O_5:120K_2O$. SCRF was applied with compatible doses (kg per hectare) of $154N:56P_2O_5:84 K_2O$. The check for these experiments was formula (kg/hectare) of $220N:90 P_2O_5: 60 K_2O$ which was standardized and concluded from previous experiments.
3. Nano-endophyte particles and Bio Plant Flora (liquid) were applied following guidelines by Vuagro company (Nguyen Tien Dung, 2018).
4. Technical packages for maize production in Mekong delta of Vietnam were applied following (IAS, 2016).

v. Data of agronomical characters such as root lodging, stem borer, banded leaf spot blight, plant and ear aspects and grain yields were collected from two middle rows of each plot according to guidelines by national technical regulation on testing for value of cultivation and use of maize varieties in Vietnam (QCVN, 2016). Criteria using score of 1-5, with one being best and five being worst, was used.

vi. Data from all experiments was analyzed by IRRISTAT 5.0, using LSD0,05 or Duncan multiple test.

Results and Discussion

Agronomical characters and yield of MN585 maize hybrid affected by different fertilizer doses and SCRF

Mean yield of MN585 maize hybrid (Table 1) across all kinds of fertilizer varied from 3.7 MT/ha (summer-autumn 2016-2017, Long An), 6.8 MT/ha (spring 2016-2017, Long An) to 8.7 MT /ha (summer-autumn 2017, Dong Thap). The SCRF treatment attained high yield (7.3 MT/ha in Long An 2016-2017; 5.2 MT/ha in Long An winter-spring 2017 and 10.4 MT/ha in Dong Thap summer-autumn 2017) which is statistically similar to the yield of recommended formula of fertilizer (8.1; 5.1 and 10.8 MT/ha respectively).

Table 1. Effects of different fertilizer doses and slow controlled release fertilizer on agronomical characters and yield of MN585 maize hybrid in Mekong delta of Vietnam in 2016-2017.

Treatments	Long An spring 2016-2017					Long An summer-autumn 2017					Dong Thap summer-autumn 2017			
	Root lodge (%)	Green death (%)	BLSB (%)	Stem borer (1-5)	Yield (MT/ha)	Root lodge (%)	Green death (%)	BLSB (%)	Stem borer (1-5)	Yield (MT/ha)	Root lodging (%)	BLSB (%)	Stem borer (1-5)	Yield (MT/ha)
90 P_2O_5:60 K_2O	6.67	16.67	7.33	3.17	1.9 c	14.0	20.7	7.33	3.00	0.8 d	0.00	1.33	2.33	3.1 c
220N: 60 K_2O	3.33	4.67	6.67	3.00	6.9 b	19.3	7.3	8.67	3.67	3.5 b	2.00	2.00	2.67	8.9 b
220N: 90 P_2O_5	5.33	6.00	4.67	3.00	6.6 b	30.7	6.7	10.00	4.00	2.9 c	6.00	3.33	3.50	8.6 b
220N:90P_2O_5:60K_2O	2.67	5.33	3.33	2.33	8.1 a	24.7	4.7	8.00	3.33	5.1 a	3.33	2.00	2.83	10.8 a
SCRF	3.33	5.33	4.00	2.50	7.3 ab	22.7	7.3	8.67	3.67	5.2 a	4.00	2.67	2.83	10.4 a
Farmer's formula	2.00	4.00	5.33	2.67	8.2 a	28.0	6.0	9.33	4.00	4.9 a	4.67	4.00	2.83	10.1 a
Mean	3.89	7.00	5.22	2.78	6.5	23.2	8.78	8.67	3.61	3.7	3.33	2.56	2.83	8.7
LSD(0.05)					0.88					0.7				1.0
CV (%)					7.5**					10.5**				6.8**

220N:90 P_2O_5:60 are recommended formula from previous results. SCRF being 154N:56P_2O_5:84 K_2O (kg/ha); **Presented significantly different at P<0.01); Similar characters in a column presented no statistical significance at P>0.05; Different characters in a column showed statistical significance at P<0.05.

The trend of data shown in Table 1 has also been seen in Table 3 and Table 4.

Table 2. Effects of different doses of nitrogen, SCRF and endophyte product on agronomical characters and grain yield of maize hybrid MN585 in Dong Thap province, winter-spring 2017-2018.

Treatments	Plant aspect (1-5)	Ear aspect (1-5)	Stay green (1-5)	Stem borer (1-5)	BLSB (1-5)	Root Lodging (%)	Yield (MT/ha)
Control (without fertilizer)	4.3	5.0	4	2.0	1.0	2.0	1.51 h
110N-90P-90K+ Nano-BIO	2.2	2.7	3	2.7	2.0	1.3	5.88 f

165N-90P-90K+ Nano-BIO	2.5	2.7	2	2.3	2.0	2.0	7.39 d
220N-90P-90K (Recommended)	2.0	2.2	2.7	2.7	2.0	2.7	9.22 bc
SCRF	1.8	1.7	2.5	2.8	2.0	4.0	9.07 c
Mean	2.7	3.0	2.8	2.5	1.9	1.7	6.12
LSD (0.05)							1.13
CV (%)							11.0**

Control: Maize planted with fertilizer, nano-endophyte includes nano-particles and bioflora (liquid)

Table 3. Effects of different doses of nitrogen, SCRF and endophyte product on agronomical characters and grain yield of maize hybrid MN585 in Hau Giang province, winter-spring 2017-2018.

Treatments	Plant aspect (1-5)	Ear Asp (1-5)	Stalk Lodg (1-5)	Rood Lodg (1-5)	Stay green (1-5)	Stem borer (%)	H. Tur. (1-5)	H. Maydis (1-5)	BLSB (1-5)	Rotten kernel (1-5)	Yield
Control (without fertilizer)	4	4	2	1	9	15.3	1.0	2.3	2.7	2.0	1.40
110N-90P-90K+ Nano-BIO	2	3	2	1	8	10.0	1.3	2.0	2.3	2.0	4.85
165N-90P-90K+ Nano-BIO	2	3	2	1	9	15.3	1.0	2.7	2.0	2.0	5.54
220N-90P-90K (Recommended)	2	3	2	1	8	15.3	1.7	1.7	2.0	1.7	6.20
SCRF	2	3	2	1	9	11.0	1.0	2.0	2.7	2.0	6.47
Mean	2.7	3.3	2	1	8.5	11.4	1.4	2.1	2.2	2.0	4.63
LSD (0.05)											7.01
CV (%)											9.04

Control: Maize planted with fertilizer, nano-endophyte includes nano-particles and bio-flora (liquid).

Agronomical characters and yield of MN585 maize hybrid affected by different chemical fungicides and nano-endophytes

During winter-spring 2016-2017 in Long An province (Table 4), use of nano-endophyte (Nano particles + Bioplant flora - liquid) produced high yield (8.1 MT/ha), statistically equal to treatment of Amistatop325SC (8.1 MT/ha), nano (particle only) (7.8 MT/ha) and farmers' practice (7.6 MT/ha). This trend can be seen in all other agronomical characters such as root lodging (3.3-5.3%), stay green (score of 2.5-2.8), plant aspect (score of 2.5-2.8), ear aspect (score of 2.8-3.0), banded leaf spot blight (*Rhizoctonia solani) (4.7-60%),* rotten ear (score of 2.0). However, dried death of farmers' practice (6.0%) - like that of control (7.3%) - is higher than using Nano (particle)+ Bio (liquid), Nano (particle) (4.0-4.7%). Using Amistatop325SC (Table 4) showed the lowest level of stalk lodging (1.3%), dried death (2.7%), H. Turrcicum (score of 2.7) and *H. Maydis* (score of 1.3). The control treatment (spraying water only) showed all characters infected by abiotic stress (stalk and root lodging, stay green, plant and ear aspect) and biotic stresses (BLSB, dried death, *H. Turrcicum* and *H. Maydis*) which resulted in the lowest grain yield (7.2 MT/ha).

Table 4. Effects of different chemical and nano-endophyte products on agronomical characters and grain yield of maize hybrid MN585 in Long An province, winter-spring 2016-2017.

Treatments	Stalk Lodge (%)	Root Lodge (%)	Stay green (1-5)	Plant Asp (1-5)	Ear Asp (1-5)	BLSB *(%)*	Dried death (%)	Rotten Ear \ (1-5)	H. tur (%)	H. maydis (%)	Yield (MT/ha)
Control	4.7	7.3	3.3	3.0	3.3	16.7	7.3	2.7	6.0	6.7	7.2 b
Amistatop325SC	1.3	3.3	2.5	2.5	2.8	4.7	2.7	2.0	2.7	1.3	8.1 a
Nano (particle)+ Bio (liquid)	4.7	4.7	2.8	2.7	3.0	5.3	4.0	2.0	3.3	2.7	8.1 a
Nano (particle)	3.3	5.3	2.8	2.7	3.0	6.0	4.7	2.0	4.0	3.3	7.8 a
Farmer's practice	2.7	4.7	2.7	2.8	3.0	7.3	6.0	2.0	4.0	2.0	7.6 ab
Mean	3.3	5.1	2.8	2.7	3.0	8.0	4.9	2.1	4.0	3.2	7.8
LSD (0.05)											0.57
CV (%)											3.9

Control: Spraying with water only; Farmer's practice: Spraying Anvil + Kasumin of Syngenta; nano-endophyte includes nano-particles and bio-flora (liquid).

The trend which is shown in Table 5 and Table 6 is similar to that in Table 4.

Table 5. Effects of different chemical and nano-endophyte products on agronomical characters and grain yield of maize hybrid MN585 in Long An province, summer-autumn 2017.

Treatments	Stalk Lodge (%)	Root Lodge (%)	Stay green (1-5)	Plant Asp (1-5)	Ear Asp (1-5)	BLSB *(%)*	Dried death (%)	Rotten ear (1-5)	H. tur (%)	H. maydis (%)	Yield (MT/ha)
Control	9.3	29.0	2.5	4.0	4.0	27.3	8.0	3.0	6.0	3.3	3.8 c
Amistatop325SC	8.7	25.0	2.5	4.0	3.7	12.7	4.7	2.3	3.3	1.3	4.7 a
Nano (particle)+ Bio (liquid)	8.3	21.3	2.5	4.0	3.7	10.7	5.3	2.5	2.7	1.7	4.7 a
Nano (particle)	8.0	24.3	2.5	4.0	3.7	13.3	4.0	2.2	4.0	1.7	4.1 bc
Farmer's practice	8.3	26.7	2.5	4.0	3.7	14.0	6.0	2.2	4.7	2.0	4.3 ab
Mean	8.5	25.3	2.5	4.0	3.7	15.6	5.6	2.4	4.1	2.0	4.3
LSD (0.05)											0.5
CV (%)											6.2

Control: Spraying with water only; Farmer's practice: Spraying Anvil + Kasumin of Syngenta; nano-endophyte includes nano-particles and bio-flora (liquid).

Table 6. Effects of different chemical and nano-endophyte products on agronomical characters and grain yield of maize hybrid MN585 in Dong Thap province, summer-autumn 2017.

Treatments	Stalk Lodg (%)	Root Lodg (%)	Stay green (1-5)	Plant Asp (1-5)	Ear Asp (1-5)	BLSB (%)	Dried death (%)	Rotten ear (1-5)	H. tur (%)	H. maydis (%)	Yield (MT/ha)
Control	1.33	4.67	2.17	2.33	2.67	3.67	4.67	11.33	2.00	2.0	9.7 b

Amistatop325SC	1.33	3.33	2.00	2.00	2.17	1.67	1.33	6.67	0.00	2.0	10.6 a
Nano (particle)+ Bio (liquid)	1.33	2.00	2.00	2.00	2.00	1.33	2.00	6.00	0.67	2.0	10.5 a
Nano (particle)	2.00	4.00	2.00	2.17	2.50	2.00	2.67	8.67	0.67	2.0	10.4 a
Farmer's practice	1.67	2.67	2.17	2.17	2.50	1.67	3.33	9.33	0.67	2.0	10.3 a
Mean	1.73	3.33	2.07	2.13	2.37	2.07	2.80	8.40	0.80	2.0	10.3
LSD (0.05)											0.57
CV (%)											3.0

Control: Spraying with water only; Farmer's practice: Spraying Anvil + Kasumin of Syngenta; nano-endophyte includes nano-particles and bio-flora (liquid).

Effects of different fertilizer forms, chemical and nano-endophytes on economic efficiency of MN585 maize hybrid in Mekong delta of Vietnam.

Data in Table 7 presented the highest rate of return using SCRF of Kingenta for MN585 hybrid in Dong Thap province (1.1 in winter-spring 2016-2017 and 0.8 in summer-autumn 2017) which is higher than of farmers' formula (0.8 and 0.5, respectively).

Table 7. Economic efficiency of different fertilizer doses and nano-endophytes on MN585 maize hybrid in Dong Thap Province.

Treatments	Dong Thap winter-spring 2016-2017				Dong Thap summer-autumn 2017			
	Total return (VND million)	Total cost (VND million)	Net benefit (VND million)	Rate of return	Total return (VND million)	Total cost (VND million)	Net benefit (VND million)	Rate of return
90 P_2O_5:60 K_2O	37.7	28.2	9.5	0.3	16.3	25.7	(9.3)	-
220N: 60 K_2O	54.5	29.1	25.4	0.9	40.7	26.6	14.1	0.5
220N: 90 P_2O_5	53.0	30.5	22.5	0.7	38.9	28.0	10.9	0.4
220N:90P_2O_5:60K_2O	60.6	31.2	29.4	0.9	48.0	28.8	19.2	0.7
SCRF	60.0	28.3	31.7	1.1	46.4	25.6	20.9	0.8
Farmer's formula	60.5	33.2	27.3	0.8	45.2	29.8	15.4	0.5
Mean	54.4	30.7	23.7	0.8	39.2	28.0	11.2	0.4

Using nano-endophytes had a rate of return similar to that of spraying Amistatop325SC or farmers' practice (Table 8).

Table 8. Economic efficiency of different fertilizer doses and nano-endophytes on MN585 maize hybrid in Mekong delta of Vietnam.

Treatments	Dong Thap winter-spring 2016-2017				Dong Thap summer-autumn 2017			
	Total return (VND million)	Total cost (VND million)	Net benefit (VND million)	Rate of return	Total return (VND million)	Total cost (VND million)	Net benefit (VND million)	Rate of return
Control	54.4	29.1	25.3	0.9	45.2	29.4	15.8	0.5

Amistatop325SC	59.2	30.0	29.2	1.0	49.9	30.3	19.6	0.6
Nano (particle)+ Bio (liquid)	56.9	32.0	24.9	0.8	48.7	32.4	16.3	0.5
Nano (particle)	55.8	31.8	24.0	0.8	48.1	32.1	16.0	0.5
Farmer's practice	57.7	30.6	27.1	0.9	48.9	31.2	17.7	0.6
Mean	56.8	30.7	26.1	0.9		31.1	17.1	0.6

Discussion

Application of slow/controlled release fertilizer (SCRF) of Kingenta gained similar agronomical characteristics, abiotic and biotic tolerance and yield as with recommended formula of fertilizer. The economic efficiency of using SCRF is higher than that of conventional fertilizer application. Use of SCRF only once during planting as a base application saves labor costs and has higher economic efficiency compared to conventional fertilizer doses with three or four applications.

In the Mekong delta of Vietnam, the main crop production season is winter-spring (planted from mid-October to end of November and harvested early January to mid-February), resulted in higher yield (10.4 - 10.8 MT/ha) than other copping seasons. Spring-summer cropping season (planted early March to mid-April) or summer-autumn cropping season (planted end of April to mid-May) are the two adverse cropping seasons due to heavy rainfall, which resulted in lower yield of maize (4.1 - 4.7 MT/ha in summer-autumn season.

Due to small scale of experiments using nano-endophyte, the rate of return is not significantly different from fully recommended formula or farmers' practice. Even efficiency of SCFR treatment is not much higher than that of recommended formula.

Conclusion

Based on the experimental results, it is concluded that using SCRF was more efficient than conventional fertilizer application. It is suggested that large scale demonstrations of slow/controlled-release fertilizers or nano-endophyte be conducted in different maize growing regions to evaluate more precise economic efficiency.

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Intercropping of Maize-Mungbean to Increase Farmer's Income

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Introduction

Maize farmers in several regions in Indonesia generally have limited land resources (<1 ha) and follow monocropping system. So, farmers have low income and have high risks of crop failure. The alternative to increase the income and reduce the risk of crop failure of maize is to increase the land use index by intercropping. In the tropical areas, maize is commonly intercropped with legumes.

Some earlier studies have shown that intercroppinng maize with legumes leads to increase of farmers' income through the increase in land equivalent ratio and maize grain yield equivalent. The increase of farmers' income depends on the legume used and the proportion of the maize-legume in intercropping. Intercropping maize-cowpea inreased income by 332–440% (Midega et al. 2014), maize-soybean with proportions 1:1 and 1:2 increased income, respectively, by 100% and 125%, maize-groudnut with proportion 1:1 increased income by 52%, and in 1:2 increased income by 69% (Kheroar et al. 2013).

Farmers feel easier to sell maize grain as maize planting is more profitable than that of legume; so, for intercropping of maize-legume to be widely adopted not only one must show monetory advantage, but also higher maize productivity or at least the same grain yield as in the monoculture planting system. In order to avoid any possible decrease in productivity of maize, the plant population of maize in intercropping should be the same as the optimal population in monoculture. The twin row planting system of maize is particularly suitable for intercropping with legumes like mungbean, as the maize population remains the same as in monoculture. The present study was aimed to ascertain the profitability of maize-mungbean intercropping (using twin-row maize planting) without reducing the productivity of maize.

Materials and Methods

The experiment was conducted at Gowa, South Sulawesi, Indonesia, during dry season of 2015. The experiment site was located at S 05^0 17' 10.7 "and E 119^0 34' 09.8", at the altitude of 3 m above sea level. The treatments consisted of: 1) Intercropping 1 row of mungbean in maize twin-rows at a plant spacing of 50-100 x 20 cm; 2) Intercropping 2 rows of mungbean in maize twin-rows at plant spacing of 50-100 x 20 cm; 3) Intercropping 1 row of mungbean in maize twin-rows at plant spacing of 40-110 x 20 cm; 4) Intercropping 2 rows of mungbean in maize twin-rows at plant spacing of 40-110 x 20 cm; 5) Monoculture of maize with plant spacing of 75 x 20 cm; 6) Mungbean monoculture with plant spacing of 40 x 20 cm.

Both maize monoculture and intercropping models maintained maize plant population at 66,666 plants ha^{-1}. The plant population in mungbean monoculture was 125,000 plants ha^{-1}, whereas the intercropping with 1 row of mungbean at 33,333 plants ha^{-1} and 2 rows of mungbean at 66,666 plants ha^{-1}. The optimal maize plant population in the tropics area are 65,000-71,000 plants ha^{-1} (IPNI & IAARD 2009). The plot size was 12.6 m x 8 m.

Maize variety used was Pioneer-21, which has a semi-erect leaf type, and the mungbean variety was Kenari. The row of plants in the direction of the sun (East-West) in order to obtain optimum sunlight on crops. The mungbean crop was planted one week after maize planting. The maize plants were fertilized 184 kg N, 60 kg P_2O_5 and 60 kg K_2O ha^{-1}. Half the rate of N and all rate of P and K was applied at 10 DAP, and the remaining N was applied at 40 DAP. Mungbean was fertilized with 45 kg N, 45 kg P_2O_5 and 45 kg K_2O ha^{-}

^{1} for monoculture, and fertilization of mungbean in intercropping was adjusted based on population of the mungbean monoculture. The entire quantity of fertilizer on mungbean crop was applied at 7 DAP.

Data collection and statistical analysis

Grain yield of maize (intercroping and monoculture) and grain yield of mungbean in intercropping was recorded from an area of 4.5m x 4 m for each plot. On mungbean monoculture, yield was recorded in an area of 4m x 2 m. Grain yield data of maize and mungbean were adjusted to 15% moisture content.

To compare the maize-mungbean intercropping to monoculture, we used the following criteria:

a) Maize equivalent yield (MEY) based on productivity and the market price of each commodity.
MEY = Yim + (Yib × Pb)/Pm
where:
Yim = maize grain yield in intercropping system (t ha^{-1})
Yib = mungbean grain yield in intercropping system (t ha^{-1})
Pm = selling price of maize (\$ kg^{-1})
Pb = selling price of mungbean (\$ kg^{-1})

b) Land equivalence ratio (LER) was calculated using an equation suggested by Mead and Willey (1980):
LER = Yim / Ymm + Yib / Yb
where:
Ymm = maize grain yield in monoculture (t ha^{-1})
Yb = Mungbean grain yield in monoculture (t ha^{-1})

If LER>1, it indicates that the efficiency and productivity of land in intercropping is more profitable than monoculture, and if LER<1 it means that monoculture is more profitable than intercropping.

c) Monetary Advantage
To determine the economic feasibility of intercropping pattern, the following calculations were performed: Cost of inputs (seeds, fertilizer, pesticide, and herbicide); Cost of labor of carrying out activities (land preparation, planting, weeding, fertilizer application and harvesting); profit and B-C ratio, as follows:

NR = TR - TC
B-C ratio = TR/TC
TR = Ym x Pm + Yb x Pb
where:
NR = Net return/profit
TR = Total Return
TC = Total Cost (cost of inputs and labor)

If the B-C ratio > 1, it means that intercropping maize with mungbean is profitable; conversely if B-C ratio <1, it means it is not profitable. Recommended for intercropping is B-C ratio > 1 and highest profit.

Result and Discussion

In general, intercropping maize-mungbean using twin rows obtained grain yield of maize 2.0 to 7.9% higher than monoculture. Similar result was obtained for intercropping maize-soybean with twin rows where the grain yield of maize was 4 to 10% higher than monoculture (Syafruddin, 2017). Verdelli et al. (2012) showed that intercropping maize-soybean gave grain yield of maize 13 to16% higher than monoculture. The higher maize grain yields in intercropping was because: 1) twin-rows spacing is far better than single-row; Zubachtirodin *et al.* (2009) and Syafruddin and Biba (2015) demonstrated that twin-rows increased maize grain yield by 2.5 to 20.0% as compared to the grain yield of the single-row, 2) additional nutrients either by fertilizers or from N fixation or by mungbean that are transferred to soil and is absorbed by maize plants.

Grain yield of mungbean in intercropping was lower than monoculture. Intercropping of mungbean in maize gave mungbean grain yield 0.54 to 1.15 t ha^{-1}, while in monoculture it was 2.30 t ha^{-1} (Table 1). The difference in grain yield of mungbean between intercropping compared to monoculture could be attributed to differences in plant population and reduction of grain yield in individual plants. In monoculture, mungbean population have 125,000 plants ha^{-1}, while in intercropping two rows of mungbean have population 66,666 plants ha^{-1} (53% of the population in monoculture) and one row of mungbean have poplation 33,333 plants ha^{-1} (27% of the population in monoculture). Mungbean grain yield of individual plant in intercropping declined by about 6-47 % compared to monoculture; this decrease was perhaps caused by shading of maize. Intercroping two rows of mungbean with maize twin-rows with spacing (110-40) cm x 20 cm gave mungbean grain yield (1.15 t ha^{-1}) higher than other intercropping.

Maize equivalent yield (MEY) in intercropping is largely determined by productivity and price of each commodity (grain maize and soybean price). Intercropping provided higher MEY (7.62 – 9.67 t ha^{-1}) over monoculture (5.99 t ha^{-1}for maize monoculture and 6.44 t ha^{-1} for mungbean monoculture). This means that intercropping maize-mungbean, the farmer will receive additional grain yield of mungbean equal to grain yield of maize 1.51 to 3.22 t ha^{-1} (24-50% of maize grain yield actual). The increased MEY in intercropping was due to the value of the mungbean and an increase in maize yield. The higher MEY in intercropping was also obtained in other studies on maize-legume intercropping (Sahu 2006, Kheroar and Patra 2013, Kheroar and Patra 2014, Shri *et al*. 2014)) and maize-soybean (Paudel *et al*. 2015). Two rows of mungbean intercropping in maize with twin rows (110-50) cm x 20 cm resulted in the highest MEY of 9.67 t ha^{-1} than other intercropping.

Land equivalent ratio (LER) reflects the efficiency and productivity of land use. In intercropping maize with mungbean we obtained LER of 1.25–1.58. This means that intercropping maize-mungbean improved the productivity of land use by 25-58% compared to monoculture. The Increased LER was due to increased yield of maize and additional yield from mungbean. Several experiments showed that intercropping of maize-legumes have higher LER than monoculture (Sabaruddin *et al*. 2011, Kheroar and Patra 2013), maize-sobean (Waktola *et al*. 2014, Paudel *et al*. 2015, Syafruddin 2015), maize cowpea (Yilmaz *et al*. 2008, Shri *et al*. 2014). Intercropping of 2 rows of mungbean had LER higher than intercropping with 1 row of mungbean. If intercropping is applied with 2 rows of mungbean the LER value 1.37 - 1.58, while 1 row of mungbean had LER value 1.25 - 1.44. LER is influenced by productivity of each commodity, while the productivity of each commodity is affected by the ratio of plant population between main crops and secondary crops. Therefore, to improve the efficiency and productivity of land use and obtain higher grain yield of maize is to maintain the plant population of maize in intercropping such as the optimal population in monoculture. In the intercropping maize-mungbean based on the grain yield and LER is it advisable to use twin row at plant spacing of maize (110-40) cm x 20 cm with 2 rows of mungbean.

Table 1. Grain yield, maize equivallent yield, land equivalent ratio under maize-mungbean intercropping at South Sulawesi, Indonesia.

	Grain Yield (t ha^{-1})			
Treatments	Maize	Mungbean	Maize Equivalent	LER
maize twin rows (100-50), one row of mungbean	6.11c	0.54c	7.62	1.25
maize twin rows (100-50), two rows of mungbean	6.50a	0.65c	8.32	1.37
maize twin rows (110-40), one row of mungbean	6.36b	0.62c	8.10	1.33
maize twin rows (110-40), two rows of mungbean	6.45a	1.15b	9.67	1.58

Monoculture maize	5.99c	0	5.99	1.00
Monoculture mungbean	0	2.3a	6.44	1.00
CV (%)	9	5		

Economic advantage

Technologies recommended to farmers must be technically and economically feasible. Production cost (inputs and labor) in intercropping was higher than monoculture. In intercropping, the total cost was \$593.80 – 674.28 ha^{-1}, while in maize monoculture the total cost was \$5026 ha^{-1} and mungbean monoculture had total cost of \$398.28 ha^{-1} (Table 2). Although intercropping had higher total cost, net return or profits was higher than in monoculture. In the intercropping the total and net return were \$1,520.87-1929.52 and \$.927.07–1,255.25, respectively, while in maize monoculture the total and net returns were \$1195.23 and 685.97, respectively, and for soybean these were \$185.02 and 886.74, respectively. Intercropping maize-mungbean with twin row of maize at plant spacing (110-40-110) cm x 20 cm and was planted with two rows of mungbean had the highest profit (\$.1,255.25) and B-C ratio (2.86).

Therefore, this intercropping model is very suitable to be applied by farmers. These results were in agreement with earlier studies by Sahu (2006), Pudel *et al*. (2015), and Cuit *et al*. (2017) that showed intercropping maize-soybean with two rows of maize and two rows of soybean gave higher profit. Intercropping maize-mungbean with twin row of maize at plant spacing (110-40-110) cm x 20 cm and with two rows of mungbean was found to be very suitable for adoption by farmers, because this had higher grain yield, also higher profit and B-C ratio than other models.

Table 2. Total cost, total return, net return, and B-C ratio as influnced by different intercropping models in South Sulawesi, Indonesia.

Treatments	Total Cost	Total Return ($)	Net Return ($)	B-C ratio
Maize twin rows (100-50), one row of mungbean	593.80	1520.87	927.07	2.56
Maize twin rows (100-50), two rows of mungbean	647.34	1660.15	1012.81	2.56
Maize twin rows (110-40), one row of mungbean	603.26	1615.45	1012.19	2.68
Maize twin rows (110-40), two rows of mungbean	674.28	1929.52	1255.25	2.86
Monoculture of maize	509.26	1195.23	685.97	2.35
Monoculture of mungbean	398.28	1285.02	886.74	3.23

Conclusions

Intercropping of maize-mungbean with twin rows of maize showed higher maize yield, maize equivalent yield, land equivalent ratio, B-C ratio, and profit compared to monoculture. Intercropping of maize-mungbean was the best in models of twin rows with plant spacing (110-40) cm x 20 cm with 2 rows of mungbean as it showed B-C ratio >1, with highest productivity and profitability.

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Maize Improvement in India – Status and Prospects

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Introduction

Vagaries of climate change are being felt everywhere. The impact of climate change on agricultural production is projected to be most prominent in the tropics and subtropics. South Asia is projected to be particularly vulnerable for multiple stresses due to low adaptive capacity (IPCC, 2007; ADB, 2009; Rodell et al., 2009; Niyogi et al., 2010). Thus, the challenge before agriculture is to feed this ever-increasing population under changing climate and depleting availability of arable land and water (Rakshit et al., 2014). This can be achieved through higher crop yields per unit area rather than increasing the area under crops (Foulkes et al., 2011) and growing the crops that can adapt well to biotic and abiotic stresses limiting crop yields (Rakshit et al., 2014). Directly and indirectly cereal crops, *viz*., wheat, rice and maize account for approximately 50% of human food calories (Tweeten and Thompson, 2008). Among these top cereals, water requirement of maize is lowest (500 mm) as compared to that of rice (2100 mm) and wheat (650 mm). Maize is the most versatile crop used as food, feed, fodder and in recent past as source of bio-fuel with wider adaptability.

In the year 2016-17, India produced little over 272 million MT food grains. Among cereal grains rice represent 44% of the gross cultivated area followed by wheat (30%), maize (9%), pearl millet (8%) and other millets. Rice and wheat constitute 44% and 39% of cereal production, respectively while maize represents little over 9% of cereal production.

Area and Production Status of Maize and its Uses

In 1950-51, total maize produced in India was around 1.73 million MT, which was doubled to 3.46 million MT by 1958-59 because of 35% increase in area and 48% in yield (Yadav et al., 2015). During the period of 1949-60 the annual increment in maize area was 109 thousand ha, while the productivity enhanced by 24.7 kg/ha/year. In the 1960s the corresponding figures were 168 thousand ha/year and 7.4 kg/ha/year. The maize area during 1970s and 1980s was almost stagnant, while yield increment in 1980s turned significant at 29 kg/ha/year. This figure further increased to 37 kg/ha/year in 1990s, which reached to its peak at over 46 kg/ha/year in the next decade (2000-10). Current yield increment is over 10 kg/ha/year (2011-17). With some slow down in area increase during 1980-90, the area under maize cultivation has increased substantially and reached to historical maximum growth rate at over 200 thousand ha per year during first decade of this millennium. Currently maize area is increasing @ 70 thousand ha annually. The current five yearly average areas under maize is 8.9 million ha and production is 23.0 million MT.

In India, maize was predominantly a rainy season (*kharif*) crop, mainly grown in the states of Uttar Pradesh, Bihar, Rajasthan and Madhya Pradesh. However, after 1980s, the area shifted more towards peninsular region and currently this region represents nearly 40% of the total area under maize and over 52% of production. The major maize growing states are Karnataka (14.8%), Maharashtra (10.9%), Madhya Pradesh (10.8%), undivided Andhra Pradesh (10.4%), Rajasthan (10.6%), Uttar Pradesh (8.3%), Bihar (7.9%), Gujarat (5.0%) and Tamil Nadu (3.6%), accounting for nearly 80% of the total maize area of the country. However, productivity of maize in many of these states like in Rajasthan (1.6 t/ha) and Gujarat (1.6 t/ha) are quite low, while that in Uttar Pradesh (1.7 t/ha), Madhya Pradesh (1.9 t/ha) and Maharashtra (2.3 t/ha) are below the national average of 2.6 t/ha.

Maize in India is grown across the country (except for Kerala) in all the three seasons, i.e. winter or *rabi* (in Bihar and Peninsular India), rainy season or *kharif* across the country (except Goa and Kerala) and summer in Punjab, Haryana and western Uttar Pradesh. In last five years, among all cereals, maize has recorded highest Compound Annual Growth Rate (CAGR) of 0.68% in yield and 0.73% in production. Area under winter and spring maize in the country has registered a steep growth of 270% during the period (Singh, 2017). Average productivity of winter maize is as high as 4.1 t/ha and some farmers are harvesting up to 11-12 t/ha with irrigation and high input condition. However, *kharif* maize dominates the maize scenario, out of which 80% or above is rainfed, which is the main reason for lower productivity of Indian maize program.

Current requirement of maize in India is around 24 million MT, of which roughly 62% is used as feed, 18% for industrial purposes, around 10% for export, 6% as food and 4% for other purposes including seed. The demand growth trend suggests an increase in demand of 7.18%, leading to targeted demand for maize of 50-60 million MT by 2025. International demand for maize is also expected to increase. Currently India is barely able to meet its domestic demand and if the international demand is also clubbed in the projected demand may increase further.

Demand for Maize in India

In poultry production, 60-70% cost is incurred on feed and maize is the principal ingredient used as the main source of calories and crude fiber. In India, the annual demand of maize is expected to increase by 5-9% mainly due to poultry industry alone. The livestock population of 500 million in India is expected to grow at a rate of 1.3% in days to come leading to a requirement of 526 million MT of dry matter, 855 million MT of green fodder and 56 million MT of concentrate by 2022. This accounts for a total requirement of 274 million MT cereals of which maize will be the main component. Dry/stay green maize stover after harvest can be used as fodder. Besides maize stalk of some specialty corns, mainly sweet corn, baby corn and maize for green ears are good source of green fodder and silage. Products of wet milling such as cornstarch, corn oil, corn steep, liquor, gluten etc. have great demand in the domestic food processing, pharmaceutical, leather and textile industries as well as have potential for their export. The present consumption of maize in starch and industrial products at the level of 4.25 million MT is expected to rise to 15 million MT in coming 5 years.

With increased production of maize, the country has been able to meet its domestic need. Besides meeting the domestic need India has been exporting maize for the last fifteen years (since 2003). Maize export reached its peak in 2013-14 and has been dropping since then. India mainly exports maize to South East Asian Countries like Indonesia, Vietnam, Malaysia, etc.

Recent Initiatives of Maize Research and Development in India

Yield and quality improvement

During this period, both QPM research and hybrid maize program received major focus. Maize project scientists developed more than a dozen QPM maize single cross hybrids using hard endosperm QPM inbred received from CIMMYT. Using marker-assisted selection (MAS), the first QPM version of normal maize, Vivek QPM 9 was released in 2008. Recently, essentially derived QPM hybrids through MAS, *viz*., Pusa HM-8 Improved (AQH-8), Pusa HM-9 Improved (AQH-9) and Pusa HM-4 Improved (AQH-4) and improved QPM hybrid, Pusa Vivek QPM-9 Improved (APQH-9) with enhanced vitamin A content were released by IARI. Even though maize hybrid Prakash was being cultivated as baby corn, but the first hybrid identified as baby corn, sweet corn and popcorn namely HM 4, HSC 1 and BPCH 1 were released for commercial cultivation in 2005, 2010 and 2015, respectively. Recently, two more new hybrids namely FSCH 18, a sweet corn hybrid and DMRHP 1402, a popcorn hybrid were also released in 2016 and2017, respectively for commercial cultivation. In addition, several sweet corn hybrids *viz.,* Mishti, Candy, Hy-brix 53, Hi-brix-39 have been released to meet the increased demand for sweet corn.

The protection provided in the PPV&FR Act encouraged private sector to invest more to develop and market single cross hybridsand modified single cross hybrids. Major shift in breeding priorities beyond

2000 was more emphasis on hybrids, more on single cross hybrids. Out of the 220 cultivars released between 2000 and 2017 only 42 (19.1%) are OPVs. Among released hybrids, numbers of public and private bred cultivars are 94 and 84, respectively. Medium to long duration cultivars continue to receive more prominence with 33.6% of the releases to be of late maturity, while 31.8% are of medium maturity. Short duration cultivars constitute 33.7% of total releases since 2000. There are substantial number of cultivars released by both private and public sectors for all major maize growing states.

Rough estimate suggests that around 65% of the maize area in India is covered under various types of hybrids. In terms of single-cross hybrids it is 22-25% of the maize area.

Better production technology for hybrids

For *kharif* irrigated crop, plant population of 74-80 thousand per ha found to be optimum. Nutrient management with 10 t/ha FYM, N-P-K @ 150:75:75 kg/ha proved to be remunerative. Similarly, for rainfed *kharif* season crop, 66 thousand plant population and 10 t/ha FYM along with N-P-K of 120:40:40 kg/ha have been worked out. *Rabi* maize with 80-90 thousand plant population per ha and N-P-K @ 250:105:105 kg/ha reported to give the best return. However, extensive studies suggest site-specific nutrient management gives best results so far nutrient management is concerned. The application of crop residue @ 5 t/ha found effective for enhancing maize productivity under rainfed conditions.

Weed is a major production constraint particularly in *kharif* maize causes 30-60% yield losses. Application of a post-emergence herbicide tembotrione @ 120 ml a.i. /ha at 25 days after sowing has been reported the best for control of second/third flush of weeds in maize. The tank-mix application of atrazine (700 g a.i. per ha) + pendimethalin (700 ml a.i. per ha) as pre-emergence found most effective for providing weed free conditions up to 25 days after sowing. Inter-cropping with vegetables (cabbage, cauliflowers, spinach), legumes (pigeonpea) etc. proved to be very effective to ensure higher and regular income to the farmers, better risk management and mitigating climate changes. Intercropping of specialty corn with vegetables proved to be a boon, particularly in peri-urban ecologies.

Resource conservation technology

Zero tillage (ZT) technology and crop residue incorporation in maize-based cropping system proved to be highly remunerative. Maize system productivity of 11.3–12.9 t/ha with reduced water requirement by 40–65 ha-mm under ZT has been reported in maize (Parihar et al., 2016). It gives up to 31% higher net returns with lower production cost.RCT is gaining momentum in Indo-gangetic region and in peninsular India.Currently in the state of Andhra Pradesh and Tamil Nadu over 100 thousand ha maize is being cultivated under ZT. Drip irrigation under ZT is specifically recommended for spring maize for enhancing water productivity and minimizing irrigation water requirement.

Plant protection technology

Though chemical control measures against insect pests and diseases are established, eco-friendly control measures need to be practiced. Incorporation of pest resistance in the released cultivars has remained in priorities since beginning, which has provided dividend. Biopesticides though is quite effective its extensive use in controlling maize pests is yet to be a matter of regular practice. So far specialty corns like sweet corn, baby corn and maize for green cobs are concerned there should be more judicious application of pesticides. Farmers earn more if specialty corns are produced organically than under chemical control.

Mechanization

Farm mechanization ensures timely operations, labor and natural resources, reducing cost of cultivation, increased per day productivity, quality produce, improved living standard, enhanced per manpower farm income, crop intensification, reduced labor drudgery among others. In India, land preparation and to some extent sowing are mechanized, while harvesting, and postharvest handling is predominantly manual. In Tamil Nadu and other parts, the farmers are using Combined Harvester. To a large extent mechanical and power farmers, particularly in peninsular India, are using operated shelling machines.

Postharvest handling

Postharvest quality of maize is dependent on moisture at harvest, weather and storage conditions. Normally maize crop is harvested at 18-20% grain moisture level. The grain has to be stored at 12% moisture to protect them from store grain pests (rice weevil) and fungal infection (aflatoxin). About 5% losses are estimated during harvesting, shelling, winnowing, transportation and cleaning.

Value addition

Maize grains are processed using three major processes, *viz*., dry milling, wet milling and alkali processing. Series of maize based ready-to-cook (RTC) and ready-to-eat (RTE) products have been developed like Maize-based vermicelli, crisp, noodles and papad developed by UAS, Mandya are being marketed in the brand name of 'Maizy' in the state of Karnataka.Dry milled QPM-based products like roasted flour,dalia, suji, multigrain flour, maize grit, namkeen products etc. offer immense promise to start entrepreneurship. Primary processing of sweet corn, popcorn, baby corn has potential to improve household employment opportunities, profitability to the farmers and most importantly engage them at local level. Such products have international demand.

Challenges and strategies for sustained increase in maize production

The main challenge is to enhance productivity. For this a detailed action plan needs to be developed involving all stakeholders, *viz.*, planners, researchers, farmers, processors and traders in a Public-Private-Producer Partnership (PPPP) mode to address the issues in a holistic manner. Following defined strategies may attain the challenge of increased productivity:

- Enhancing breeding efficiency
- Hybrid seed production
- Production and protection technologies
- Development of maize value chain
- Policy interventions

Strategies for Enhancing Breeding Efficiency

Strengthening the pre-breeding activity

Constitution of heterotic pools under various maturity groups is very important. Available germplasm as well as exotic introductions are to be thoroughly screened for the traits of economic importance as well as heterotic relationship.

Genetic enhancement for stress tolerance

Adverse effect of climate change is being felt across crops including maize. To address these, climate resilient maize germplasm is to be developed by incorporating traits imparting toleranceto drought, water logging, high temperatureetc. Simultaneous selections under combination of stresses should be the strategy to develop cultivars.

Use of frontier technologies for enhancing genetic gains

Advances made in breeding techniques like Doubled Haploid (DH), molecular marker-assisted breeding, high throughput precision phenotyping of traits of interest, decision-support systems/tools offer new opportunity for enhancing genetic gains and breeding efficiency. There is a need to integrate DH techniques and high throughput genotyping with conventional breeding program to improve breeding efficiency. Genetic engineering, RNA interference and CRISPER technique provide us new tools to engineer maize germplasm resistant to biotic and abiotic stresses in long run.

Adoption and development of genetically modified (GM) maize

GM maize is being cultivated in 60.6 million ha in 16 countries (ISAAA, 2016). In India some GM events from private sectors are under field trials but none of them have received permission for large-scale cultivation. Research program should be strengthened to develop GM maize able to control insect pests and diseases and tolerate herbicides.

Strengthen Hybrid Seed Production

Seed production of single cross maize hybrid is to be taken up in a mission mode to bridge the productivity gaps. A rolling plan for seed production for at least five years should be prepared to outscale better SCHs suitable for a specific region.

Production and Protection Technologies

Agronomic recommendations need to be revisited towards sustainable intensification by adopting conservation agriculture for reducing cost on inputs, improving soil health, water and nutrient use efficiency towards improved production and farmers' income and reducing environmental foot print. Organic mulching, on farm soil and water management including micro irrigation where ever possible are to be practiced. Specialty corns particularly in the *peri*urban areas need to receive special attention. In plant protection, priorities are to be given to control biotic stresses by emphasizing host plant resistance, IPM approaches and biological control methods. This will help to reduce loss of beneficial insects/soil biota, less pesticide in the food chain, reduced human health hazards and environment pollution, and improved farm income.

Development of Value Chains

While developing maize value chains, focus should be given towards improvement of efficiency, consumer preferences/demands, industrial supply and benefits to consumers. Processing of maize for value addition involving local women and youth should receive priorities. This can offer considerable off-farm job opportunity for better profitability to the farmers. Also, more wet milling industries are required to be established to meet demand for starch and its derivatives.

Farmers should be sensitized about newly developed QPM SCHs and the advantages associated with QPM. Poultry feed producers also need to be educated on the superiority of QPM over normal maize. QPM should be procured and provided though public distribution system (PDS) in the states predominated by tribal and poor masses where maize is directly consumed as food to ensure nutritional security.

Key policy inputs for improvement of proven technologies of maize crop in India

- Frontier technologies like DH, MAS, GS, GWAS and CA should be integrated in the R&D strategies.
- Sustainable practices like ZT should receive subsidies so that such practices are encouraged to ensure significant social, economic and environmental benefits.
- Maize-based value chains are to be developed at village level in a PPPP mode through self-help groups (SHGs), producer companies, and farmers' cooperativesetc.
- The yield potential and realized yield gaps in maize can only be bridged up by disseminating the improved location-specific production technologies in a PPPP mode involving local agriculture graduates as paid technology and input agents.
- Contract farming by major processors will ensure better return to the farmers, which will encourage them to adopt better production technologies.

Challenges and way forward for maize research and development in India

The productivity of *rabi*/spring maize (4.1 t/ha) is almost double that of *kharif* maize (2.3 t/ha). However, the *kharif* maize represents 82.3% of total maize area. To enhance maize production, the productivity of *kharif* maize needs to be increased substantially. About 75% *kharif* area is under rainfed condition, while *rabi* and spring maize is predominantly grown in favorable ecologies. However, tropical and sub-tropical environmental conditions during *kharif* season like shorter day length, early maturity, hot night temperature, poor quality sunshine, cloudy weather *etc*. prevents realization of potential productivity during *kharif* season. Further, under climate change extreme weather events like uneven rainfall, drought, flooding, high temperature, high wind *etc*., also adversely affectparticularly *kharif*maize productivity. Heat stress at flowering and grain filling stages in spring maize causes substantial yield losses.

Biotic stresses such as post flowering stalk rot (PFSR), leaf blights, banded leaf & sheath blight (BLSB), downy mildews (DM), ear rots (ER), borers, and weeds adversely affect maize productivity. Among insect pests, stem borer (*Chilo partellus* Swinhoe) is a common problem across year. Pink borer (*Sesamia inferens* Walker) is of major concern during *rabi* season, particularly in the southern peninsular region. Spring maize is gaining popularity in northern parts of the country, particularly in the states of Punjab, Haryana and western Uttar Pradesh. With this shoot fly (*Atherigona spp.*) is becoming a major problem, particularly when the crop is sown late. Recent reports of the Fall Army Worm (*Spodoptera frugiperda*) in India needs an integrated approach for its management. In recent past reporting of fall armyworm (*Spodoptera frugiperda*) from India has added to the worries, which needs a concerted effort to address the challenge.

Non-availability of quality seed of single cross hybrids is another important factor contributing low productivity of rainfed *kharif* maize. Mostly the private sector is focusing on development and production of single cross hybrids suitable for low risk-high potential irrigated ecologies of *rabi* season. This calls for immediate attention to address above-mentioned challenges to increase productivity of *kharif* maize. Labor is another issue affecting maize production. Beside these challenges credit availability, postharvest processing including poor storage, low bargaining power, poor transport, lack of technological inputs are some of the constraints being faced by the maize growers.

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Maize for Changing Climate - Chasing the Moving Target

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Introduction

The average annual growth rate of harvested maize area from 1993 to 2013 was 2.7% in Africa, 3.1% in Asia, and 4.6% in Latin America (FAOSTAT, 2018). Maize has emerged as the cereal with largest global production, which surpassed rice in 1996 and wheat in 1997, and its production is increasing at twice the annual rate of rice and three times that of wheat (Fischer et al., 2014). Among cereals, including rice, wheat and other coarse cereal, maize has recorded highest increase in area and productivity during 2006-2015 and is projected to keep the momentum during 2016-2025 (OECD/FAO, 2016). Asia, with its 31% share in global maize production from about 34.0% of the total global area harvested, is the second largest maize producer in the world. The current decade continued impressive growth in maize production, as all the sub-regions showed significant increase in maize production (Figure 1), including Southeast Asia - 10.8%, Southern Asia - 27.3% and East Asia - 30.6%, which resulted in an overall 27.7% maize production increase in Asia within a short period of 2010-2016 (FAOSTAT, 2018). These gains in maize production were contributed by increase in productivity per unit area and increase in maize growing areas in some countries.

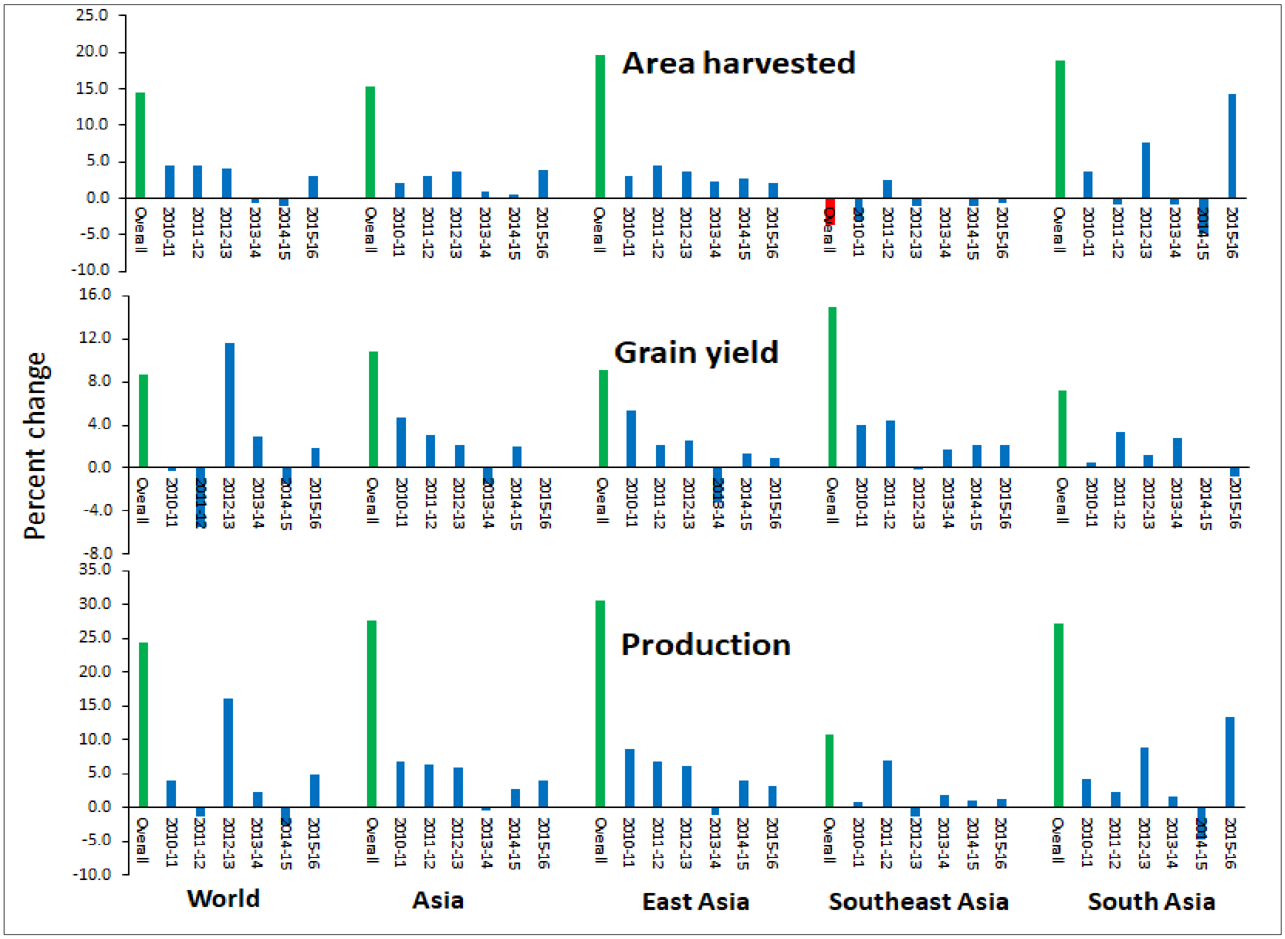


Figure 1. Maize trends in Asia – progress in current decade (2010-2016).

There has been an unprecedented increase in global maize demand at a rate faster than increase in global maize production. Maize has been identified as number one in the estimated global demand for cereals by 2020, with 45% increase in its demand (compared with 30% for wheat and 32% for rice). Increase in maize demand is projected to be acute in Asia, i.e. 87% rise by 2020 as compared with its demand in 1995 (IFPRI, 2003). Within Asian countries, the highest increase in demand for maize by 2020 is projected for the countries of East Asia, dominated by China that alone would require 252 million MT, followed by Southeast Asia requiring 39 million MT, and South Asia requiring 19 million MT (Figure 2, James, 2003). This has specific implications on Asian maize, where an array of factors contributing to a sharp increase in maize demand, including growth rate in per capita gross domestic product (GDP), changing diets, and a significant rise in feed use driven largely by a rapidly growing poultry sector (Shiferaw *et al*., 2011). This indicated quite a challenge for most of the maize growing countries in the developing world which, except Latin America, all had to import maize to meet their demand as their net trade is projected to be in negative (IFPRI, 2003), ranging from about -1.0% in case of South Asia to as high as -43.0% for East Asia (IFPRI, 2003). By 2020, the global area of maize is expected to increase by only 12% compared to maize area in 2000. Thus, 88% of the necessary increase in maize production will have to be met from increased productivity per unit area of land (James, 2003). Meeting the projected maize demand is a daunting challenge for developing world maize farmers, who grow about two-thirds of the global maize area.

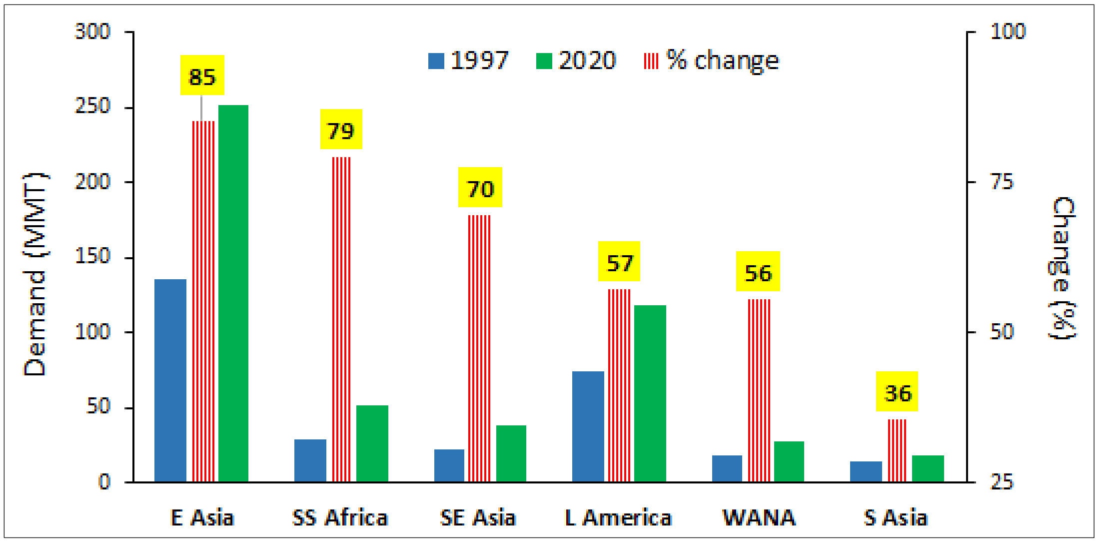


Figure 2. Maize demand projection during 1997-2020.

Maize in Asian tropics - a rainfed crop prone to array of stresses

Most of the maize in Asian tropics (about 70%) is grown in lowland tropics (<1000 masl), including both dry and wet lowlands, followed by sub-tropical/mid-altitudes and tropical highlands (Zaidi *et al*., 2014). Maize is largely (about 80%) grown as a rainfed crop, which is prone to the vagaries of monsoon rains and associated with an array of abiotic and biotic constraints. This is clearly reflected in the productivity of the rainfed system, which is usually less than half of the irrigated system (Zaidi *et al*., 2014). In general, there is considerable pressure on irrigation water, resulting in increased irrigation intervals thus subjecting the maize to stress and a consequent reduction in yield. Moisture availability is seldom adequate for rainfed maize. Erratic or un-even distribution pattern of monsoon rains occasionally causes drought or excessive moisture/ waterlogging at different crop growth stage(s) within the same crop season, which is probably the main factor responsible for relatively low productivity of rainfed maize. Due to the uncertainty of assured returns, farmers are often hesitant to invest in recommended cropping management practices, which results in low soil fertility, and eventually poor yields. Also, in recent years Asian tropics have experienced frequent and widespread severe drought years, for example - seven drought years in South Asia since 2000, coupled with increased day/night temperatures during major maize growing season (monsoon season) covering about 80% of the total maize area, apart from scattered drought/heat almost every year in one or the other country in South Asia (Zaidi *et al*., 2016).

Maize is highly vulnerable to reproductive stage drought and/or high temperature stress. Spring maize in Asian tropics grown during the hot summer period of the year (Feb-May) is invariably exposed to high temperature regimes during most of the critical crop growth period, starting from late vegetative stage until early grain filling. Also, in drought years in summer-rainy season (major maize crop season in Asia) the temperature (both T_{max} and T_{min}) increased close to or beyond their threshold limit, which resulted in even more severe stress condition to maize crop due to combined drought and heat stress at same time (Figure 3). Assessment of the impact of current and future heat stress on maize in South Asia clearly showed that heat stress affected areas will significantly increase under the future climates, particularly in the pre-monsoon (spring) and monsoon (rainy) seasons. The study also highlighted the potential yield advantage of heat tolerant maize varieties in both the spring and rainy seasons, relative to the current heat-vulnerable maize varieties that are extensively grown in the region (Tesfaye *et al*., 2016).

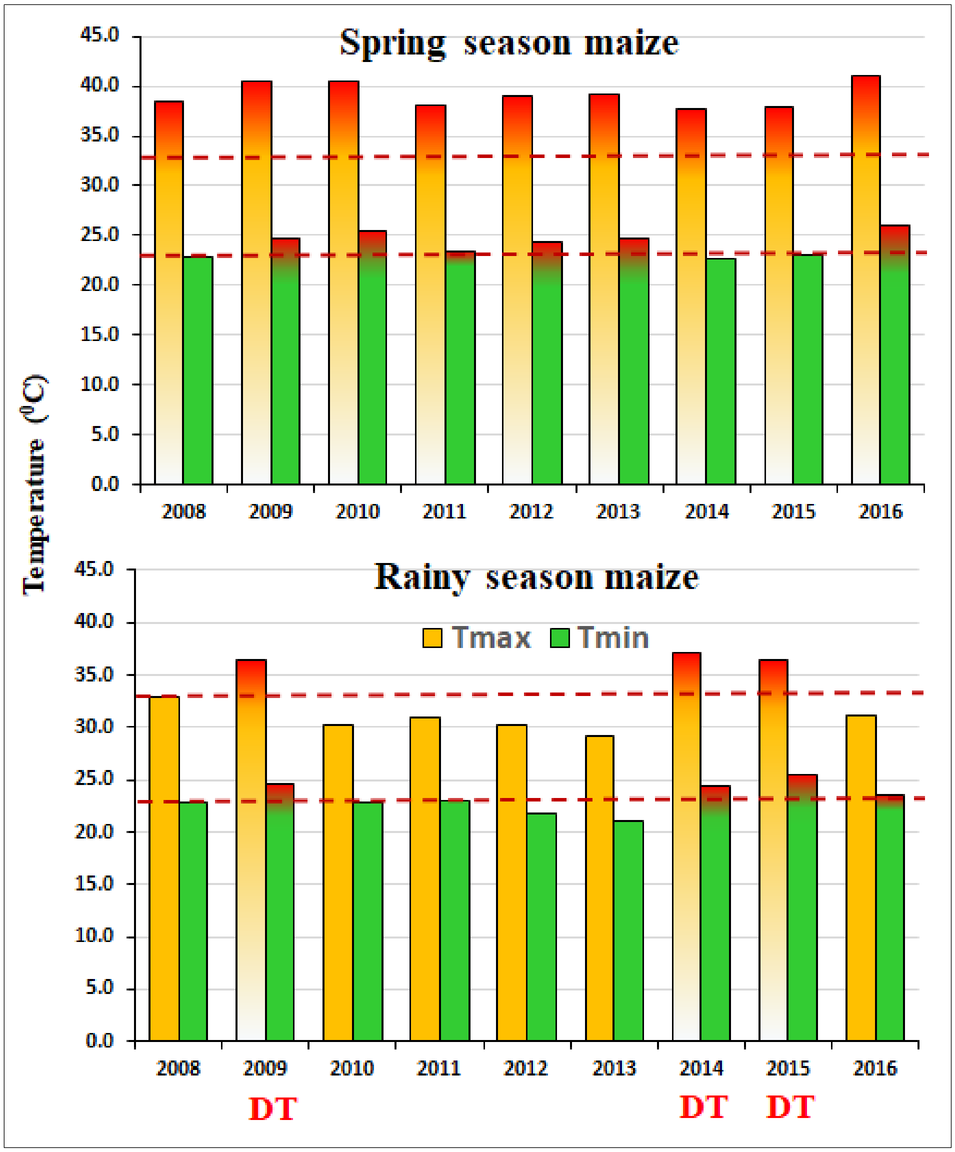


Figure 3. Temperature regime during flowering/grain-filling stage of maize crop in South Asia.

Lowland tropics, especially wet-lowland, are most congenial for biotic stresses, including diseases and insect-pests of economic importance. Turcicum leaf blight (*Exserohilum turcicum)*, Maydis leaf blight *Helminthosporium maydis*, Rust (*Puccinia polysora)* and Downy mildew (*Pernosclerospora spp.)* are the most common foliar diseases in Asian maize. Though reasonable sources of resistance to these diseases exist in Asian maize germplasm, new introductions and the evolution of more virulent strains are posing a major challenge to the longevity of such resistance. Therefore, host-plant resistance breeding programs require close monitoring of virulence changes in the pathogen and identification of new resistance sources to new virulent strains. Banded leaf and sheath blight (BLSB) is emerging as a major threat in most parts of Asian tropics, especially in the area where rice-maize rotation is followed. The main concern lies mainly in the lack of good sources of resistance to BLSB. Maize in Asian tropics is prone to several stalk rots, caused by range of causal organism. *Diplodia* ear rots are the most common, but *Fusarium* and *Aspergillus* ear and kernel rots are also found, especially after a dry spell or insect attack, and often lead to dangerous levels of mycotoxin in grain. Stem borers, including *Ostrinia furnicalis, Sesamia inferens* and *Chilo partellus,* are widely distributed in

Asia. Some partial resistance to these pests has been identified, which is largely dependent on inoculum load and intensity of infestation.

Climate-change effects – dealing with uncertainties

Rainfed systems, which represent a major part of maize mega-environments in Asian tropics, are more dependent on prevailing weather conditions, and therefore extremely vulnerable to climate change effects. Studies suggest that Asia will experience an increasing frequency of extreme weather conditions with high variability beyond the current capacity to cope up with (ADB, 2009; Cairns *et al*., 2012). Several climate modelling studies suggest sharper increases in both day and night temperatures in future, which could adversely impact maize production in the tropical regions (Lobell et al., 2011; Cairns et al., 2012). Such impacts are already being experienced in the region in several real and recognizable ways, such as shifting seasons and higher frequency of extreme weather events, such as drought, waterlogging and heat stress coupled with emergence of new/complex diseases. One of the major and well-realized effects of climate change has been the reduction in the number of rainy days (although there has been no significant change in total rainfall) in South (Kashyapi *et al*., 2012) and Southeast Asia (Manton *et al*., 2001). This has resulted in heavy rainfall events within a reduced number of days, thus extending the dry periods within same cropping season. The erratic distribution pattern in monsoon rains results in extremes of water regimes within the cropping season, causing contingent/intermittent waterlogging at some crop stage(s) and drought periods at other stages. Most of the Asian tropics is identified as a hotspot for climate change effects, and associated negative effects due to climate variability, including weather extremes (ADB, 2009). Climate change effects is a fact, well experienced in terms of weather extremes with increased frequency in recent years. One of the biggest challenges with climate change is the uncertainty in weather pattern, especially year-to-year variability and extremes with space and time. During most critical two months period, rainy season maize crop may be exposed to variable moisture regimes in the same area in different years (Figure 4).

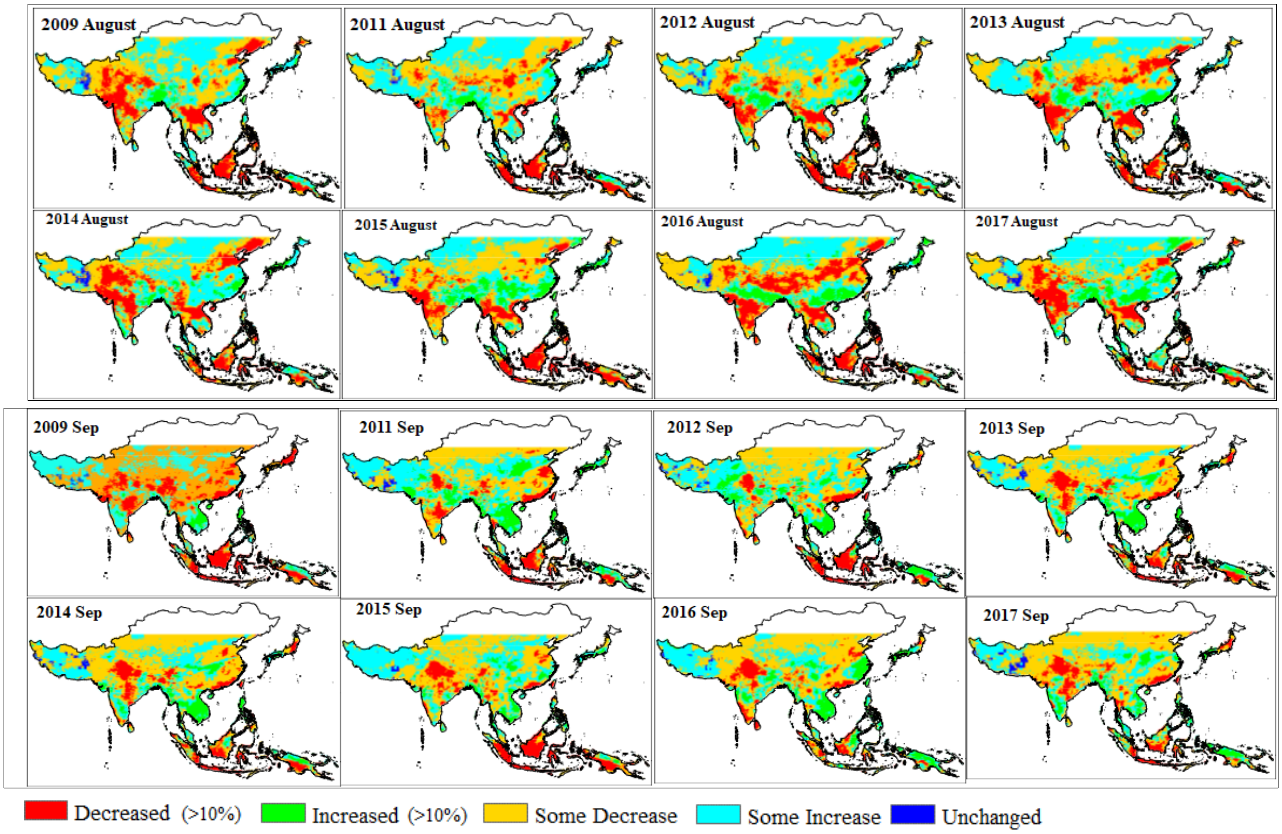


Figure 4. Variation in monsoon rains in Asian tropics during 2009-2017 in relation to 2010 (close to normal year.

With the increasing climate variability and uncertainties, current agricultural research - including development of crop varieties - needs to pay major attention to resilience towards variable weather conditions rather than tolerance to individual stress in a specific situation or crop stage. Plant breeders need to identify and deploy new genes and physiological mechanisms that contribute to climate-resilient varieties. Recent advancements in plant breeding and biotechnology are contributing efforts to engineer plants with tolerance to abiotic stresses; however, future plant breeding efforts must focus on integrating multiple climate adaptation traits in new cultivars to provide tolerance to a broad spectrum of adverse conditions. Drought and heat stresses often occur at the same time due to the integrated nature of these stresses in drought-prone environments. Drought is defined as a deficit of rainfall while heat stress is defined as an increase in temperature beyond a threshold level for a period enough to cause irreversible damage to plant growth and development. Plant breeding for heat stress tolerance in crop plants has lagged behind other abiotic stress tolerance research. Investigations into the genetic mechanisms influencing heat stress responses are underway. An extensive screen of lines developed for drought-prone environments in tropics indicated that very few of these lines combine heat and drought tolerance traits. Heat and drought stress tolerance were poorly correlated suggesting that heat and drought tolerance are controlled by different genetic mechanisms. As genetic sources of high-temperature tolerance are identified, it is hoped that inheritance studies will reveal a genetic architecture that can be manipulated to enhance crop productivity in a range of stressful environments.

Stress-resilient maize – an option for current and future climate

Challenged with growing problems of food security and climate change, Asian agriculture must become more productive, more resilient and more climate-friendly. Varieties with increased resilience to abiotic and biotic stresses will play an important role in autonomous adaptation to climate change (Fedoroff *et al*., 2010). Efforts to develop field crops with enhanced stress tolerance are of vital concern. Millions of smallholders in Asia grow maize under rainfed conditions for their subsistence. The future of maize production, and consequently, the livelihoods of several million smallholder farmers in such climate vulnerable regions are based on access to climate resilient cultivars.

C4 crops are known for their wider adaptability. However, recent trends in climatic conditions and associated variabilities seem to be challenging the threshold limit of even C4 crop, like maize. Maize production can be increased by the availability of invaluable genetic diversity which harbors favorable alleles for higher yield, biotic and abiotic stress tolerance (Prasanna *et al*., 2012). Maize varieties with increased resilience to abiotic and biotic stresses will play an important role in adaptation of climate change vulnerable farming communities in tropical Asia. Targeted crop improvement, aided by precision phenotyping, molecular markers and doubled haploid (DH) technology, offers a powerful strategy to develop climate change adapted germplasm. However, given the time lag between the development of improved germplasm and the adoption of the same by farmers in the targeted region(s), it is of utmost importance that necessary actions are initiated early in selected tropical Asian countries that are likely to be most affected by the changing climate (Cairns et al., 2012).

Using a crop growth simulation model for maize (CERES-Maize) Tesfaye *et al*. (2018) quantified the impact of climate change on maize and the potential benefits of incorporating drought and heat tolerance into the commonly grown (benchmark) maize varieties at six sites in Eastern and Southern Africa, and one site in South Asia. Simulation results indicate that climate change will have a negative impact on maize yield at all the sites studied but the degree of the impact varies with location, level of warming and rainfall changes. Combined hotter and drier climate change scenarios (involving increases in warming with a reduction in rainfall) resulted in greater average simulated maize yield reduction than hotter only climate change scenarios. Incorporating drought, heat and combined drought & heat tolerance into benchmark varieties increased simulated maize yield under both the baseline and future climates. While further evidence is still required to document the risk-reduction benefits of the climate-resilient maize on the numbers of chronically poor farmers, there is an increasing body of evidence confirming the benefits of climate-resilient maize to increase yields, reduce yield variability and, ultimately, increase food security (Cairns and Prasanna, 2018).

There is a myth that breeding for stress tolerance/resilience causes yield drag under optimal growing/high yield conditions. There are seldom optimal conditions in stress-prone ecologies in Asian tropics. Even if breeding and selection processes are planned using top-down approach, i.e. product design first followed by designing breeding and selection strategy accordingly, it is not impossible to develop hybrids with improved stable performance across un-stressed and stressed environments. In collaboration with national maize programs and private sector partners, CIMMYT-Asia maize program has initiated several projects largely focusing on saving achievable yields across environment by incorporating reasonable level of tolerance/resistance to key stresses, without compromising on yields under optimal conditions. Integrating the power of genomics with precision phenotyping, and focusing on reducing genotype x environment interaction effects, new generation of maize germplasm were developed with multiple stress tolerance that can grow well across variable weather conditions within season. These new generations of maize cultivars are being targeted to those stress-prone marginal environments where maize crop is invariably exposed to a wide range of challenging growing conditions, such as drought, heat, waterlogging and various virulent biotic stresses. The goal is to develop and deploy suitable maize germplasm for current climatic conditions and maintain a rich germplasm/product pipeline to effectively feed the requirement of emerging challenges due to future climatic situations in Asian tropics.

In CIMMYT-Asia maize program, we focused on enhancing resilience in maize germplasm for an array of climatic conditions. The overarching goal of the stress-resilience maize program has been to improve upside yield potential with downside risk reduction. This is achieved by focusing on and integration of the following key components:

- **Precision phenotyping** for key traits at several representative sites as well as under-managed stress screens.
- **Integration of novel breeding tools**, including genome-wide association studies (GWAS), genomic selection (GS), and double haploid (DH) technology to fast-track stress-resilience breeding pipeline.
- **Research collaboration** with committed NARS partners in the region for sustainable deployment and delivery of stress-resilient cultivars.

Phenotyping with precision

Irrespective of breeding approach, whether conventional or molecular breeding, high quality phenotyping is the key to success for genetic improvement for targeted traits. To realize true success of breeding program (or power of novel molecular breeding approaches), it is essential to appreciate the principles of phenotyping and apply them in practice (Zaidi *et al*. 2016b; Zaidi *et al*., 2016c; Zaman-Allah *et al.*, 2016).

Managed stress screen

Precision phenotyping involves a detailed characterization of phenotype of test entries under well-defined conditions (for example - managed drought stress). The intent is to precisely study the overall phenology of the test entries, which is the foundation for establishing genotype-phenotype associations in a molecular breeding approach. Quality of phenotypic data is defined by the precision in phenotyping environment. Understanding the target population of environment and simulating similar but more precise and uniform conditions (managed stress) is a pre-requisite for generating useful phenotypic data. Phenotyping sites need to be carefully developed based on key information about the site, including:

- A minimum set of medium-term (past 10 years) weather data (daily maximum and minimum temperature, humidity, rainfall, and sunshine hours).
- Soil type - physical and chemical properties.
- Cropping history of the site.
- Field levelling, irrigation & drainage facility.

The overall purpose of these managed stress trials is to simulate the targeted stress with desired level of stress intensity and uniformity at critical stages of crop growth, in a way that the available genotypic variability is clearly expressed and could be recorded.

Trait-based selection along with yield under stress

In general, the major trait of interest is always grain yield. However, under abiotic stresses heritability of grain yield is usually low, whereas heritability of some secondary traits remains reasonably high, while the genetic correlation between those traits and grain yield increases significantly (Banziger et al., 2000). At times, selection only based on high grain yield under stress is misleading; for example, selecting a high yielding test entry with prolonged anthesis-silking interval (ASI; >5.0 days). Such an entry can produce high yield as it is fed by the synchronous availability of pollen from other test entries in the trial, a luxury that is not available in farmer fields where a single hybrid is grown in a large area.

In case of molecular breeding projects, detailed phenotyping is essentially required to support the huge volume of genotypic information generated and unearth the power of that valuable information. It is essential to dissect complex traits into components that can enhance understanding of the cascade of events involved in conferring tolerance and add value in genomic region discovery efforts. However, for a secondary trait to be considered in phenotyping portfolio, it must comply with some basic requirements (Edmeades *et al.*, 1998), such as:

- Significant genetic variability exists for the trait.
- Significant genetic correlation with grain yield in the target environment, i.e. relationship is causal, not casual.
- Heritability of the trait is higher than grain yield itself, i.e. less affected by genotype x environment interaction.
- Trait should not be associated with poor yields under optimal conditions, i.e. it must confer tolerance rather than avoidance.
- Rapid and reliable measurement, which is less expensive than measuring yield itself.

Recently, initiatives are being taken to establish field-based, high throughput phenotyping platform (HTPP) to increase the throughput, more detailed measurements with better precision (Makanza *et al.*, 2018). The target is to develop field-based HTTP using low cost and easy-to-handle tools, so that it becomes an integral and key component in the breeding pipeline of stress-resilient maize.

Developing stress-resilient maize

High yields under optimal conditions (yield potential) and reasonably good yields under stress conditions (adaptation to stress conditions) are not mutually exclusive. Therefore, we focus more on improved stable yields across stressed and non-stressed environments (i.e. resilience, rather than just tolerance to a particular stress). This is achieved by defining the phenotyping and selection strategy across a range of environments and select the progenies that have high-stable performance across stressed and non-stressed environments. To increase the efficiency of breeding pipelines, CIMMYT-Asia maize program uses a combination of approaches including index selection for stress-adaptive secondary traits along with grain yield, and modern molecular breeding approaches, e.g. genome-wide association studies (GWAS), rapid-cycle genomic selection (RC-GS) and double haploid (DH) technology. The strategies that helped in developing new Asia-adapted maize germplasm pipeline with enhanced stress tolerance for individual or across stresses, without compromising optimal condition performance, are described below.

Constitution of base germplasm

The constitution of base germplasm is a key factor in a stress resilience breeding program targeting products that perform across non-stressed and a set of stresses with varied intensity. In CIMMYT Asia and Africa maize programs, association mapping panels were constituted involving 300-500 maize inbred lines representing genetic diversity of tropical maize. These include, drought tolerant maize for Africa (DTMA) panel, CIMMYT Asia association mapping panel (CAAM) and heat tolerant association mapping (HTAM) panel. These panels were genotyped using various marker systems, including 1536 (Illumina-Golden Gate), 55K (Illumina-Infinium) and GBS (Genotyping by Sequencing - around 900K SNPs). Across-site phenotyping data was generated and through genome-wide association analysis (GWAS), major genomic regions associated with key biotic (Gowda et al., 2015; Zerka *et al.*, 2018; Gowda et al., 2018) and abiotic stresses - including heat or drought (Babu *et al*., 2014; Cerrudo *et al*., 2018), waterlogging (Zaidi *et al.,* 2015) and root traits (Zaidi *et al*., 2016d) - were identified. The study resulted in following major outputs:

- Identification of major genomic regions associated with drought, water-logging or heat tolerance.
- Introgression of those regions in elite but stress-susceptible, Asia-adapted maize inbred lines with established commercial value through accelerated back cross approach using molecular markers and doubled haploid technology.

New generation of stress-resilient maize hybrids

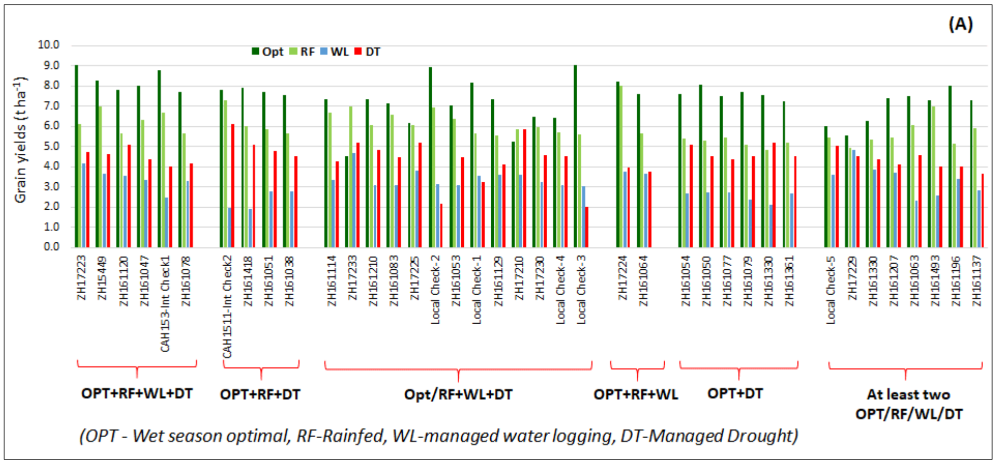
While introgression of major genomic regions identified is being executed, the large-scale robust phenotyping data helped in identification of highly promising donor lines for various complex traits (abiotic & biotic stresses). These promising trait donor lines for one or multiple stresses were used in various ways in breeding stress-resilient maize hybrids.

First generation hybrids

The first-generation maize hybrids were identified in two ways:

1. Promising test crosses from across site results of association mapping panel, as ready hybrid combination for individual stresses, and few hybrids, with stable performance across stresses and unstressed environments.
2. Elite donor lines identified after across site phenotyping of association mapping panel testcrosses with known heterotic pattern were crossed using north-Carolina design-II.

Hybrids from the above two sources were evaluated across range of stresses, including both biotic and abiotic stresses, as well as under optimal growing conditions. The best hybrids with combination of traits (and respectable yields under optimal trial) were identified based on across location trials results (Figure 5). These hybrids were licensed to partners (on semi-exclusive basis) and taken forward for deployment and scale-out in collaboration with public sector and seed company partners in the region.



(OPT - Wet season optimal, RF-Rainfed, WL-managed water logging, DT-Managed Drought)

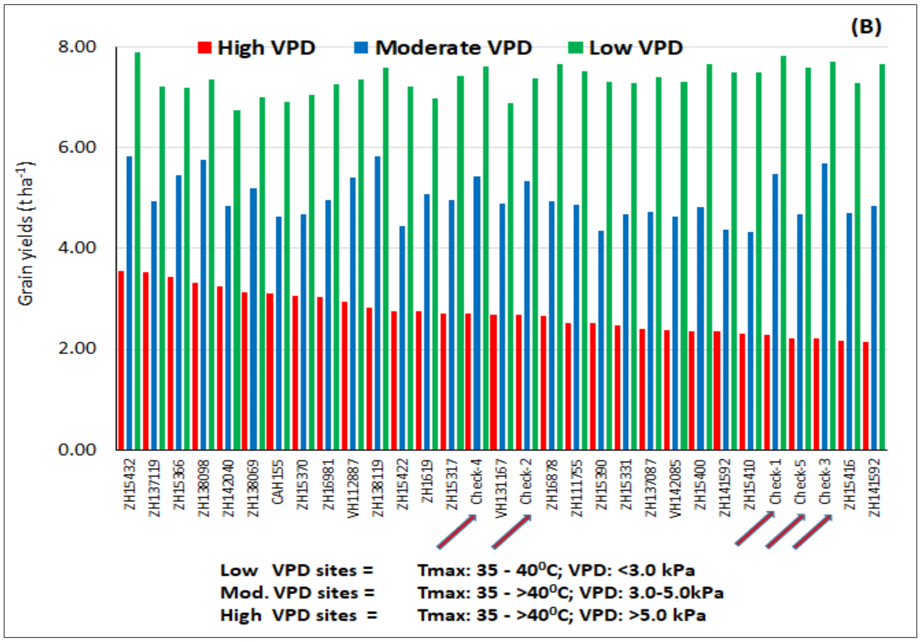


Figure 5. Stress-resilient maize hybrids –choice for various stress-prone ecologies (A) during rainy season prone variable moisture regimes and (B) spring season prone to heat stress.

Second generation hybrids

The inbred lines with promising performance in one or multiple stresses were used as trait donors in developing multi-parent synthetic populations (8-10 lines), which were used as base populations (Cycle-0 or C0) in stress-resilient breeding program. These populations were advanced through rapid-cycle genomic selection approach, C1 was constituted by inter-mating top 10% $F_{2:3}$ progenies based on their test-cross performance across several locations under stressed and non-stressed environments. Marker/haplotype/QTL effects were estimated by analyzing genotype of $F_{2:3}$ families and phenotype datasets from $F_{2:3}$ test-crosses. The C1 was subjected to next two cycles (C2 and C3) through rapid-cycle genomic selection (RC-GS) using genomic estimated breeding values (GEBVs) for grain yield (GY) across stressed and non-stressed environments. The advanced cycles were subjected to double-haploid (DH) induction, and these DH lines were used in developing new hybrid combinations for identification of new generations of stress-resilient hybrids for stress-prone target environments of South and Southeast Asia. These hybrids have gone through stage-1 testing across various stresses and optimal moisture conditions, along with promising 1st generation hybrids and popular commercial hybrids as check entries in the trials. Selected hybrids were advanced to stage-II, and are being tested to at least two more stages, i.e. stage-III and MLT (multilocation testing in larger plots), before finalizing best-bet hybrids for licensing to partners for deployment and scaling out.

Efforts have also started to follow genomic selection in the breeding pipeline which will help to dynamically create training populations and recalibrate GS models based on the breeding program; to effectively predict the breeding values bringing down time and cost, leading to enhancing genetic gains.

Productive partnership for efficient delivery of products and scale-out

In recent years, CIMMYT-Asia maize program has focused on developing strong partnerships and collaborations with a range of stakeholders, including public sector institutions, state agricultural universities, private sector and NGOs with required technical expertise and complementary strengths. Partnerships between public institutions actively engaged in maize R&D and private seed sector with good market share in the target countries are critical. Private sector partners play a key role in bringing products to a logical end through extensive multi-location testing of elite stress-resilient hybrids in target agro-ecologies/markets, multiplication of certified or quality declared seed, and marketing and delivery of the hybrids to the maize-based farming communities in Asian tropics.

The different types of partnership arrangements explored and developed, included:

i. *Partnership through bi-laterals projects*

CIMMYT-Asia program is implementing several bilateral projects in partnership with NARS and seed companies in the region; key among them Heat Tolerant Maize for Asia (HTMA) funded by USAID, Climate Resilient Maize for Asia (CRMA) funded by GIZ, Germany, Improved Maize for Asian Tropics (IMTA), and so on. Partnership in these projects is based on in-kind contribution by committed partners who are involved in all aspects of the project implementation - starting from research, development as well as product deployment and scale out.

ii. *International Maize Improvement Consortium (IMIC)-Asia*

IMIC-Asia is implemented in consortium mode, where willing private sector partners join the consortium on annual fee payment basis (public sector are honorary members). Consortium members jointly decide the R&D plan and product portfolio of IMIC then CIMMYT-Asia implements the breeding activities targeting the development of agreed type of germplasm, including early and advanced generation lines. These are then shared with partners through biannual IMIC-field days. Some ready hybrid combinations were also demonstrated in IMIC-field day, which were selected or largely preferred by SMEs with weak R&D capacity. IMIC also offers a platform for experimental hybrid testing, where partners can submit their pipeline hybrids, hybrid trials are constituted by CIMMYT, and evaluations done across locations on the sites shared by IMIC partners.

iii. *Partnership with on-going developmental project in the region*

There are different on-going developmental programs and projects in the region - supported by international donors – such as Nepal Seed and Fertilizer (NSAF), Nepal, Agriculture Innovation Project (AIP) funded by USAID, Pakistan, Cereal System Initiative for South Asia (CSISA) funded by USAID, and so on. Partnership with these projects helps in deployment of suitable products in targeted agro-ecologies. Partnership with developmental projects implemented by state governments (such as Stress Resilient Maize for Odisha (SRMO) supported by *Rashtriya Krishi Vikas Yojna* (RKVY), Government of Odisha, India, and *Rythukosham* project of Government of Andhra Pradesh, India), help in reaching remote areas where private seed companies may not have interest and/or reach.

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Identification and promotion of heat stress resilient maize hybrids for *Terai* region of Nepal

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Introduction

Maize occupies second position among food crops in terms of area planted (891583 ha) and production (2231517 t) with an average yield of 2503 kg ha^{-1} in Nepal. The productivity of improved maize is 45.21 percent (2547 kg ha^{-1}) higher compared to farmers' popular variety (1754 kg ha^{-1}) (MoAD, 2017). Raise in air temperature beyond a threshold level for a period enough to cause permanent damage to plant growth and development is called heat stress (Irmak, 2016). Spring and early summer maize are mainly affected by heat stress resulting yield losses up to 75 percent in Nepal (Koirala et al., 2013) because of leaf firing, silk damage and tassel blast.

Heat and drought stress during pollination and fertilization can cause more yield loss than almost any other period in the maize crop's development (Nielsen). In heat stress environment, the period between pollination and fertilization is critical to determine grain yield (Cicchino et al., 2011). Yield losses due to heat and drought stress depend on stage of the crop, severity and duration of the stress (Monsanto, 2012). When temperature rises above 38^0C, silks do not emerge at all and pollen grains burst causing very poor pollination or no pollination at all producing barren ears. It happens because of poor pollen viability and poor pollen shed (Cairns et al., Herrero and Johnson, 1980; Schoper et al., 1987; Mitchell and Petolino, 1988). Grain yield and grain starch content is also reduced in maize grown under heat stress environment (Khodarahmpour, 2011). When leaf temperature is above 38^0C, net photosynthesis is inhibited (Crafts-Brandner and Salvussi, 2002). During grain filling period, if temperature rises from 22^0C-28^0C, yield losses reach to 10% in the US Corn Belt (Thomson, 1966). Reduction in maize yield is higher with 2^0C rise in temperature than the reduction produced by precipitation dropped more than 20% (Lobell and Burke, 2010). Similarly, 13% yield reduction due to increase in temperature by 2^0C was observed (Rowhani et al., 2011).

The impact of climate change is of global concern. In the west and central part of Jilin province of China, maize yield is estimated to decrease by 15% or more by 2050 (Wang et al., 2011). Due to more than 5^0C temperature increase in Iowa by the end of the 21st century, maize yield is estimated to decrease by 18% (Ummenhofer et al., 2015). Likewise, 15% to 50% maize yield reduction is predicted from the late 20th century to middle and late 21st century in Iowa (Xu et al., 2016). Crops' yields could increase up to 20% in east and south-east Asia by the mid-21st century (IPCC, 2007); however, in central and south Asia, yields might decrease up to 30% within the same period. In case of Africa and Latin America, 10% yield reduction have been predicted (Seed Map) to 2055 (Jones and Thornton, 2003). It is estimated that average temperature in Nepal increased at an annual rate of 0.06^0C from 1977 to 2000. The increment was 0.04^0C in Terai and 0.08^0C in the Himalayas (Patricia, 2012). Therefore, this project was initiated in 2013 with aim to identify and deploy heat stress resilient maize hybrids in Nepal.

Materials and Methods

Project entitled "Heat stress resilient maize for Asia" supported by USAID was implemented at NMRP Rampur, RARS Nepalgunj and ARS Surkhet in Nepal from 2013 - 2016. Under this project, genotypes were received from CIMMYT Hyderabad. Total 57 trials consisting of 7764 single cross hybrids were evaluated using multinational company hybrids and NMRP developed hybrids as checks. Based on heat stress resilience during reproductive stage, grain yield and other agronomic attributes, 24 hybrids were selected and demonstrated/evaluated in farmers' fields of hybrid growing areas (Dumarwana, Nijgadh, Keureni and Rampur) having plot size of 30 m^2 in 2014/015. Seven hybrids out of 24 were selected and redemonstrated in 2015/016 with one additional site Anandapur (data not included) in Chitwan including two NMRP developed hybrids as checks. One location was used as a replication. Row-to-row and plant-to-plant distance was maintained at 60 cm and 25 cm, respectively. Fertilizers were applied @180:60:40 NPK kg ha^{-1}. Intercultural operations were carried out as per recommended. Grain yield was calculated to 80% shelling recovery and adjusted to 15% moisture level. Data were analyzed using GenStat.

Results and Discussion

In 2014/015, varietal differences among the evaluated genotypes for all the traits under observation were recorded non-significant except for ear height. Grain yield ranged from 6.79 (CAH1521) to 9.67 (RML-95/RML-96) t ha^{-1} (Table 1). In 2015/016, highly significant results were recorded for plant and ear heights whereas non-significant for rest of the traits. It shows similar performance of the tested genotypes for grain yield, plant aspect and *E. turcicum* scoring. Plant and ear heights were in between 158 and 196, and 69 and 129 cm, respectively (Table 2). Non-significant results were observed for grain yield and *E. turcicum* scoring when combined over years and locations indicating similar performance of the evaluated hybrids. Mean grain yield of the hybrids was 7.88 t ha^{-1} which is more than three times higher compared to national average productivity of 2.50 t ha^{-1}. Plant and ear heights ranged from 157-194 and 67-118 cm, respectively. Most of the genotypes had ear height below the middle portion of the plant, thus were resistant to lodging.

Effect of environment alone, and genotype by environment on various traits was observed to be non-significant, showing broad adaptability of the demonstrated genotypes. However, year wise significant variations were evident for all the traits except for *E. turcicum* scoring (Table 3). Based on stakeholders' preferences and a two-year multilocations' data, hybrids namely CAH151 and CAH153 were registered for general cultivation in Terai and inner Terai regions of Nepal for commercial cultivation in 2017 as Rapur Hybrid-8 and Rapur Hybrid-10, respectively. While analyzing maximun temperature data of Chitwan, Nepal from 1981 to 2012, Bhandari and his colleagues (2014) found that average temperature rise in this period was in between 0.03^{0}C and 0.13^{0}C per annum. Increased maize yields are expected in the hills and mountains but decreased in the Terai with 4^{0}C rise in temperature (Gautam, 2008). Thus, it is anticipated that these heat stress resilient hybrids will greatly contribute to food and feed security in Terai and inner Terai regions of Nepal.

Table 1. Grain yield and other quantative traits of promosing heat stress resilient maize hybrids combined over locations during winter 2014/015.

EN	Genotype	Grain yield, t ha^{-1}	Plant height, cm	Ear height, cm	Plant aspect, 1-5 scale	*E. turcicum,* 1-5 scale
8	RML-95/RML-96	9.67	168	84	1.5	1.8
1	CAH151	9.16	168	71	1.5	1.5
9	RML-86/RML-96	8.94	170	84	1.9	1.9
2	CAH153	8.93	171	74	1.5	1.5
5	CAH1511	8.55	174	60	1.9	1.6
7	CAH1515	8.52	160	70	2.1	1.5
3	CAH158	8.1	191	106	1.9	1.4
6	CAH1513	7.78	156	56	2	1.8
4	CAH1521	6.79	175	81	1.5	1.6
Mean		8.49	170	76	1.8	1.6
Minimum		6.79	156	56	1.5	1.4
Maximum		9.67	191	106	2.1	1.9
CV (%)		19.91	9.5	21.3	23.5	13.9
SD		1.69	16.1	16.2	0.41	0.22
ISD		2.47	23.5	23.7	0.60	0.33
F-test		ns	ns	*	ns	Ns

* = significant at P<0.05, ** = significant at P<0.01 and *** = significant at P<0.001

Table 2. Grain yield and other quantative traits of heat stress resilient maize hybrids combined over locations during winter 2015/016

EN	Genotype	Grain yield, t ha^{-1}	Plant height, cm	Ear height, cm	Plant aspect, 1-5 scale	*E. turcicum*, 1-5 scale
2	CAH153	8.53	179	71	1.6	1.9
5	CAH1511	8.33	178	73	2.0	2.3
7	CAH1515	7.70	164	69	2.1	1.6
6	CAH1513	7.30	158	84	1.8	2.3
1	CAH151	7.23	186	85	1.9	1.6
3	CAH158	6.90	196	129	2.3	1.8
4	CAH1521	6.65	194	99	1.8	1.8
8	RML-95/RML-96	6.45	171	93	2.0	2.1
9	RML-86/RML-96	6.38	161	92	2.5	1.9
Mean		7.27	176	88	2.0	1.9
Minimum		6.38	158	69	1.6	1.6
Maximum		8.53	196	129	2.5	2.3
CV (%)		16.35	5.7	16.7	18.3	28.1
SD		1.19	10.06	14.8	0.36	0.54
ISD		1.74	14.68	21.6	0.53	0.78
F-test		ns	***	***	ns	ns

* = significant at P<0.05, ** = significant at P<0.01 and *** = significant at P<0.001

Table 3. Grain yield and other quantative traits of heat stress resilient maize hybrids combined over locations and years (2014/015-2015/016)

EN	Genotype	Grain yield, t ha^{-1}	Plant height, cm	Ear height, cm	Plant aspect, 1-5 scale	*E. turcicum*, 1-5 scale
2	CAH153	8.73	175	72	1.6	1.7
5	CAH1511	8.44	176	67	1.9	1.9
1	CAH151	8.19	177	78	1.7	1.6
7	CAH1515	8.11	162	69	2.1	1.6
8	RML-95/RML-96	8.06	169	88	1.8	1.9
9	RML-86/RML-96	7.66	165	88	2.2	1.9
6	CAH1513	7.54	157	70	1.9	2.0
3	CAH158	7.50	194	118	2.1	1.6
4	CAH1521	6.72	185	90	1.6	1.7
Grand mean		7.88	173	82	1.9	1.8
Minimum		6.72	157	67	1.6	1.6
Maximum		8.73	194	118	2.2	2.0
Genotype (G)		ns	***	***	*	Ns
Environment (E)		ns	ns	ns	ns	Ns
G × E		ns	ns	ns	ns	Ns
Year × E		***	***	***	*	Ns

* = significant at P<0.05, ** = significant at P<0.01 and *** = significant at P<0.001

Conclusion

Maize hybrids CAH151 and CAH153 were identified heat stress resilient during reproductive phase. Grain yield and other agronomic traits of these hybrids were also preferred by stakeholders. Thus, these two hybrids were registered in 2017 for commercial cultivation in Terai and inner Terai regions of Nepal as Rapur Hybrid-8 and Rapur Hybrid-10, respectively.

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Genomic Regions Associated with Heat Stress Tolerance in Tropical Maize

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Introduction

Maize (*Zea mays* L.) is one of the most versatile crops in terms of wide adaptability and potential productivity. The last decade recorded impressive growth in maize, as most Asian countries showed significant increase in maize production (FAOSTAT, 2018). With progressive climate change, global mean temperature and variance are expected to increase in the future (Lobell et al. 2011). Fluctuations in temperature occur naturally during plant growth and reproduction. Although maize crop can survive brief exposure to temperature extremes (<0ºC and >35ºC), exposure to temperatures above 35ºC for a long period is considered unfavorable for crop growth. Temperatures beyond 40ºC, particularly during grain filling stage, can have dramatic negative effects on grain yields (Lobell and Field, 2007, Alam et al., 2017). Cereal production in South Asia and South Africa is most likely to be affected by climate change if new strategies for improvement are not found (Fischer and Edmeades, 2010; Cairns and Prasanna, 2018). Breeding heat tolerant cultivars is essential for future food security. Considering the complexity of heat stress, the is to identify superior alleles through genome-wide association studies (GWAS) for use in forward breeding through marker-assisted introgression of desirable genomic regions in elite genetic background. With this rationale the study was aimed to identify the genomic regions associated for grain yield under heat stress.

Materials and Methods

Germplasm and experiment design

A collection of 543 maize inbred lines constituted an association mapping panel named as heat tolerant association mapping panel (HTAM panel). The panel was constituted by involving select advanced stage maize inbred lines derived from CIMMYT's tropical and sub-tropical pools and populations from Latin America, Africa, Asian maize program and a few lines from MMRI, Pakistan, Purdue university, USA and Kaveri seed company, India. The lines with reasonably good adaptation in Asian tropics were selected for constituting the HTAM panel, avoiding sister lines or over-representation of lines derived from any specific pools or populations. The inbred lines were crossed to a CIMMYT tester line, CML451, which has a high general combining ability and is widely used in Asian region maize breeding programs.

The test crosses were evaluated in nine environments in South Asia (Table 1) during spring season under natural heat stress condition during flowering and early grain filling period. The sowing period of spring maize was chosen as mid-March so that flowering and early grain filling period was naturally exposed to the heat stress (>35ºC). The layout of test crosses was taken in Alpha lattice design with two replications in a 4m row length with row to row spacing of 0.75m and plant to plant spacing of 0.2m. Grain yield was recorded plot wise and grain weights were adjusted to 12.5% moisture content.

Table 1. Descriptive statistics for Grain yield, of HTAM panel test crosses evaluated under heat stress conditions.

Location	Latitude & Longitude	No of Entries Tested	H^2	Mean	Min	Max	LSD	σ^2g	σ^2e
BG-1	16.73N; 6.79E	290	0.62	2.77	1.26	4.36	1.86	0.74	0.90
LU	30.99N; 5.74E	290	0.50	3.61	1.99	5.26	2.38	0.75	1.48
NG	28.05N; 1.61E	290	0.36	2.25	1.54	3.22	1.60	0.19	0.67
RA	16.22N; 7.38E	335	0.34	2.48	1.71	3.60	2.41	0.39	1.51
BJ	18.25N; 9.02E	420	0.53	2.56	1.15	4.87	2.32	0.80	1.40
BG-2	16.73N; 6.79E	285	0.69	3.19	1.27	5.49	2.23	1.43	1.30
HY	17.51N; 8.27E	479	0.61	3.65	2.20	5.24	1.72	0.61	0.77
JA-1	31.32N; 5.57E	471	0.39	4.63	3.23	5.51	2.03	0.35	1.08
JA-2	31.32N; 5.57E	435	0.41	6.81	5.42	8.06	2.53	0.59	1.67

BG-1-Bhemarayangudi, Karnataka, India; LU-Ludhiana, Punjab, India; NG-Nepalgunj, Nepal; RA-Raichur, Karnataka, India; BJ-Bejanki, Telangana, India; BG-2-Bhemarayangudi, Karnataka, India; HY-Hyderabad, Telangana, India; JA-1, JA-2-Jalandhar-1, Punjab, India; H^2-Broad sense heritability; σ2g-Genotypic variance; σ2e-Error variance.

Genotyping and association mapping

A total of 955,690 SNPs were generated through GBS v2.7 using Illumina Hi-seq 2000/2500 at Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA. The physical coordinates of GBS SNPs was derived from AGPv2. The criteria for filtering SNPs for GWAS, PCA and LD analysis was done based on Suwarno et al. 2015 with slight modifications. SNPs were filtered based on criteria of call rate (CR) >0.7 and with minor allele frequency (MAF) > 0.05 for association analysis, and with call rate of 0.9 and minor allele frequency of 0.1% for PCA. BLUPS for grain yield was done separately for each location. The GWAS analysis based on mixed linear model (MLM) for each location and across location was done in SNP and Variation Suits v8.x (GoldenHelix, Inc., Bozeman, MT, www.goldenhelix.com).

Results and Discussion

Analysis of variance for grain yield under heat stress was significant for all the environments. The magnitude of genotypic variance for grain yield ranged from 0.19 in Nepalgunj (NG) to 1.43 in Bhemarayangudi (BG-2), with the heritability >0.34 (heritability ranging from 0.34 to 0.69). The wide range of genotypic variance is due to their polygenic inheritance and environmental influence (Bassi et al. 2016; Desta and Rodomiro, 2014).

Mean grain yield for the environments under heat stress ranged between 2.25 t ha^{-1} in Nepalgunj to 6.81 t ha^{-1} in Jalandhar (Table 1). During the cropping period, particularly at flowering to early grain filling stage, crop was continuously exposed to temperature above 35°C along with moderate to high vapor pressure deficit (VPD) conditions. Heat stress significantly affected various plant functions, crop growth and development, reproductive success and eventually grain yields. There are many factors responsible for these effects as reported earlier in various studies on maize (Cicchino et al., 2010; Alam et al., 2017) and other cereals such as pearl millet (Gupta et al., 2015) and rice (Bheemanahalli et al., 2016). The wide variability of grain yield under heat stress over nine environments makes the panel a valid source for performing the genome wide association analysis (GWAS) to identify the genomics region associated with grain yield under heat stress conditions.

The total number of SNPs generated for the HTAM panel was 955,690 SNPs. The SNPs for principal component analysis were filtered based on CR >0.9 and MAF > 0.1. Principal component analysis using genome-wide markers revealed moderate population structure with the first two principal components (Figure 1). The germplasm from MMRI, Pakistan, Purdue University and Kaveri Seed Company clearly separated in different axes from the rest of the CIMMYT tropical and sub-tropical lines. In general, larger LD block and slower rate of LD decay results in low mapping resolution. In diverse maize germplasm the LD decay occurs rapidly within few kilo-base pairs due to high rate of recombination (Tenaillon et al., 2001). The average LD decay of the present panel was 13.7kb at $r^2=0.1$ and 4.76 kb at $r^2=0.2$. Several studies using GBS for large number of tropical and temperate maize germplasm indicated that higher mapping resolution can be obtained from tropical germplasm because LD decay is faster in tropical germplasm than in temperate germplasm (Suwarno et al., 2014).

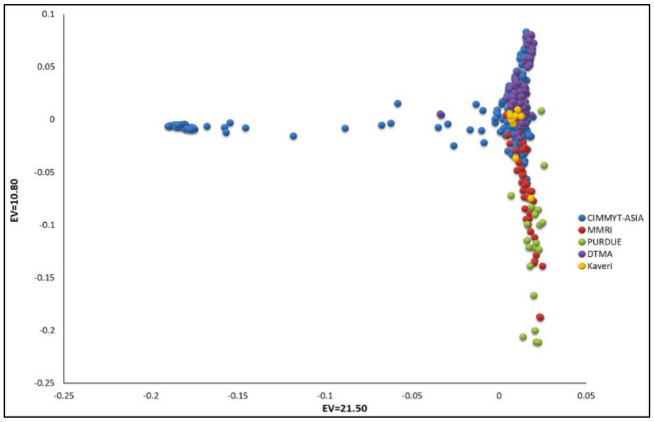

Figure 1. Grouping of accessions of HTAM panel based in first two Principal components.

SNPs for GWAS for individual location were filtered from total number of SNPs based on CR >0.7 and MAF > 0.05. The SPNs used for GWAS ranged from 281,145 (Across) to 289,060 (BG-1, LU and NG) SNPs (Table 2). A total of 680 SNPs were found to be significantly associated with grain under heat stress for across environment at P value $\geq 10^{-3}$ (Table 3, Figure 2). Similarly, GWAS for individual location identified significant SNPs ranged from 345 SNPs (BG-1) to 622 SNPs (JA-1) at P value $\geq 10^{-3}$. In each environment, number of SNPs ranging from 21 to 44 were found to be highly significant SNP (P value $\geq 10^{-5}$) associated with trait under study. Except for one SNP at BG-1, none of the other SNPs were common between the individual and across environment. Among the highly significant SNPs (P value $\geq 10^{-5}$) 98 SNPs were found in different gene model, and 76 among them were unique for various biological pathways. Genes found to have significant associations with target traits could be re-sequenced in a diverse panel of germplasm to identify causal mutations and the most favorable alleles for trait improvement, and to develop simple PCR-based markers for MAS (Yan et al., 2010).

Table 2. Number of SNPs used for GWAS and PCA analysis in individual and across location.

Location	Total No. of SNPs used for GWAS	Total No. of SNPs used for PCA	No of Entries Tested
Across	281,145	113,280	499
BG-1	289,060	124,496	290
LU	289,060	124,496	290
NG	289,060	124,496	290
RA	288,826	124,832	335
BJ	281,901	115,936	420
BG-2	289,061	124,496	285
HY	281,268	113,263	479
JA-1	282,186	114,654	471
JA-2	286,786	121,597	435

BG-1-Bhemarayangudi, Karnataka, India; LU-Ludhiana, Punjab, India; NG-Nepalgunj, Nepal; RA-Raichur, Karnataka, India; BJ-Bejanki, Telangana, India; BG-2-Bhemarayangudi, Karnataka, India; HY-Hyderabad, Telangana, India; JA-1, JA-2-Jalandhar-1, Punjab, India; H2-Broad sense heritability; σ^2g-Genotypic variance; σ^2e-Error variance.

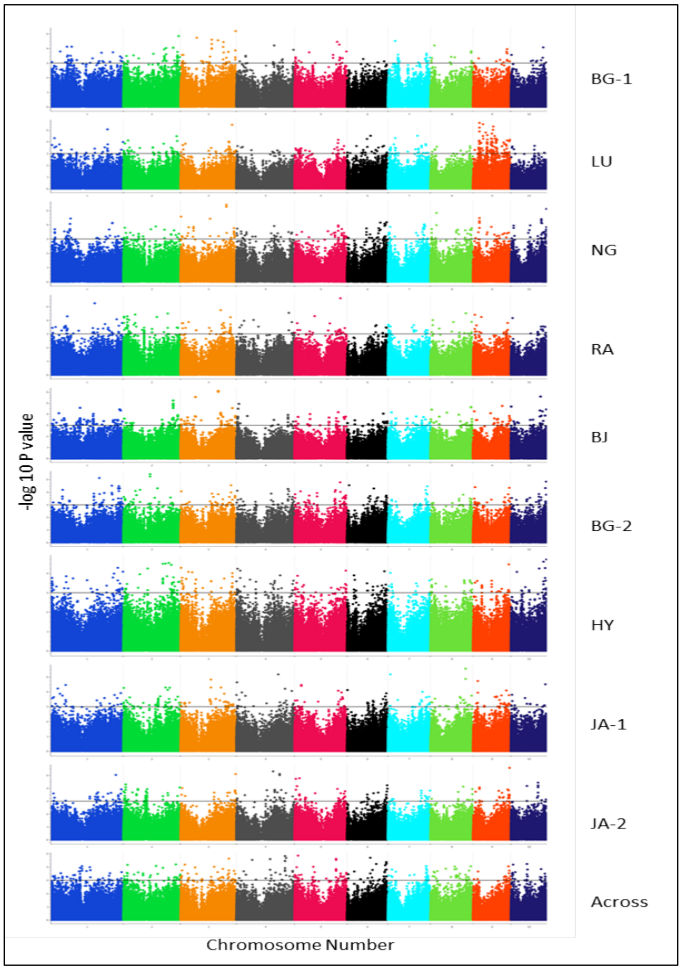

Figure 2. Manhattan plot from the Q+K (MLM) model for grain yield under heat stress.

BG-1-Bhemarayangudi, Karnataka, India; LU-Ludhiana, Punjab, India; NG-Nepalgunj, Nepal; RA-Raichur, Karnataka, India; BJ-Bejanki, Telangana, India; BG-2-Bhemarayangudi, Karnataka, India; HY-Hyderabad, Telangana, India; JA-1, JA-2-Jalandhar-1, Punjab, India; H2-Broad sense heritability; σ2g-Genotypic variance; σ2e-Error variance.

The significant SNPs that were in the range of P value 10^{-4} to 10^{-3}, a few SNPs ranging from 1 to 20 SNPs were common among the individual and across environments (Table 3). Similarly, all significant SNPs compared among individual locations showed that SNPs ranging from one SNP to 21 SNPs were common (Table 4). A total of 69 SNPs were found to be common for more than three environments (Table 5). SNPs that were common in more than three environments varied from one (three environments) to six (six environments) SNPs. These combinations of SNPs may be useful for environment specific breeding. Though many significant SNPs were identified for individual and perfect across location, very few were found to be common. This could be because the grain yield in each location is the composite of an observable expression of a genome interacting with given environment (Awada et al., 2018).

Table 3. Total numbers of SNPs identified at various level of P-Values in individual and across locations.

Location	**Total No. Of Significant SNPs ($P \geq 10^{-3}$)**	**No. of SNP significant at $P \geq 10^{-5}$**	**No of SNPs common with Across**	**No. of SNP significant at $P10^{-4}$ to 10^{-3}**	**No of SNPs common with Across**

Across	680	34	-	646	-
BG-1	345	44	1	301	0
LU	494	29	0	465	1
NG	590	29	0	561	5
RA	409	21	0	388	2
BJ	501	23	0	478	3
BG-2	535	25	0	510	20
HY	562	38	0	524	14
JA-1	622	40	0	582	2
JA-2	590	23	0	567	0

Table 4. Numbers of significant SNPs ($P \geq 10^{-3}$) common between individual environments.

Location	BG-1	LU	NG	RA	BJ	BG-2	HY	JA-1
BG-1(345)	-							
LU (494)	0	-						
NG (590)	21	3	-					
RA (409)	6	1	6	-				
BJ (501)	1	2	0	0	-			
BG-2 (535)	0	0	0	1	3	-		
HY (562)	0	0	0	0	2	4	-	-
JA-1 (622)	1	0	0	3	0	0	0	
JA-2 (590)	0	0	0	0	0	0	1	0

Value in parenthesis is the total number of significant SNPS identified in individual location for Grain yield under heat stress condition

BG-1-Bhemarayangudi, Karnataka, India; LU-Ludhiana, Punjab, India; NG-Nepalgunj, Nepal; RA-Raichur, Karnataka, India; BJ-Bejanki, Telangana, India; BG-2-Bhemarayangudi, Karnataka, India; HY-Hyderabad, Telangana, India; JA-1& JA-2-Jalandhar-1, Punjab, India.

Table 5. Number of significantly associated SNPs common in three and more than three environments

		Environment Code

No. of common SNPs	No of Environments	BG-1	LU	NG	RA	BJ	BG-2	HY	JA-1	JA-2
6	6									
3	5	✓		✓		✓	✓	✓		
1	5	✓	✓	✓		✓		✓		
5	4	✓			✓	✓		✓		
4	4	✓				✓	✓			✓
4	4				✓			✓	✓	✓
3	4	✓		✓			✓	✓		
3	4	✓	✓	✓	✓					
2	4	✓		✓		✓	✓			
1	4	✓	✓				✓	✓		
1	4	✓			✓		✓	✓		
1	4	✓			✓		✓	✓		
1	4	✓	✓					✓	✓	
1	4					✓	✓	✓		✓
1	4	✓		✓				✓		✓
1	4	✓	✓	✓				✓		
4	3					✓	✓			✓
3	3	✓	✓	✓						
2	3	✓		✓		✓				
2	3	✓		✓				✓		
2	3		✓			✓	✓			
2	3			✓			✓			✓
2	3		✓		✓			✓		
1	3	✓				✓	✓			
1	3	✓			✓	✓				
1	3	✓		✓						✓
1	3	✓			✓					✓
1	3	✓	✓		✓					
1	3					✓	✓	✓		
1	3		✓				✓	✓		
1	3			✓	✓		✓			
1	3					✓		✓		✓
1	3		✓			✓		✓		
1	3			✓				✓		✓
1	3		✓	✓				✓		
1	3		✓						✓	✓
1	3		✓		✓					✓
Total =69		21	14	15	11	14	15	20	3	12

BG-1-Bhemarayangudi, Karnataka, India; LU-Ludhiana, Punjab, India; NG-Nepalgunj, Nepal; RA-Raichur, Karnataka, India; BJ-Bejanki, Telangana, India; BG-2-Bhemarayangudi, Karnataka, India; HY-Hyderabad, Telangana, India; JA-1& JA-2-Jalandhar-1, Punjab, India.

Conclusion

For effective use of the GWAS approach for further breeding processes, the SNPs identified based on across location alone may not be effective as very few SNPs were common with individual location SNPs. The better approach would be to perform individual location GWAS and the significant SNPs that are common between locations may greatly help to increase our understanding of the genetic architecture of complex traits under heat stress conditions. Major genomic regions with favorable alleles for grain yield under heat stress can be introgressed into elite and locally adapted genetic background through step-wise marker-assisted validation-cum-introgression strategy.

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Outbreak of Fusarium Ear Rot on Maize in Thailand

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Introduction

Ear rots in maize occur worldwide wherever maize is grown (Kommedahl and Windels, 1981). Many fungal species infect maize grains including *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp, and *Stenocarpella* spp. (Payne, 1999 a). *Fusarium* is one of the major fungal genera associated with maize in Thailand (Darnetty and Salleh, 2013). This pathogen causes losses in grain yield and quality, due to contamination of grains by mycotoxins, primarily fumonisin (Parsons and Munkvold, 2012). Mycotoxins are persistent, thermostable metabolites that reproduce in food and feeds, possibly causing health problems to humans and animals (Sydenham *et al.,* 1991). Different species of fungi in the genus *Fusarium* can cause maize ear rot including *F. verticillioides*, *F. proliferatum*, *F. nygamai*, and *F. graminearum* (Mukanga *et al,* 2010). The most common species are *F. verticillioides* syn. *F. moniliforme* (Teleopmorph: *Gibberella fujikuroi*). Mycelium in infected crop debris produces macroconidia and microconidia, which are disseminated by wind and rain splash, infecting ears through silks and colonizing kernels. *F. verticillioides* can also infect maize plants systemically in which case ears may be infected through the ear shank. Insects such as the European corn borer have also been reported to act as vectors, and transfer *F. verticillioides* spores between plants or cause plant injury that enables the fungi to infect the plant.

Fusarium ear rot is characterized by cottony mycelium growth that typically occurs on a few kernels, or is limited to certain parts of the ear, unlike Gibberella ear rot. Mycelium is generally white, pale pink or pale lavender, and infected kernels typically display white streaking (also known as 'starburst' symptoms) on the pericarp and germinate on the cob. Infection occurs close to ear tips and can easily be confused for damage and injury caused by ear borers. Under severe infestation, the entire ear appears withered and is characterized by mycelium growth between kernels (Payne, 1999a).

Management of ear rot of maize is very challenging. Foliar sprays with fungicides are neither effective nor economically feasible for maize growers in Thailand. Integrating cultural practices, seed treatment and cultivation of resistant maize varieties is possibly the most effective way to manage the disease and reduce mycotoxin contamination in maize grains. Research conducted on this disease in Thailand determined disease incidence and severity, affected locations, and the causal fungus. Evaluation of host resistance for pathogen infection of maize inbred lines and pre-commercial hybrid varieties, and identification of mycotoxin chemotypes of the causal *Fusarium* sp. from affected grains was also carried out in this study. Data obtained may highlight the possibility of improving maize plant response to ear rot disease.

Materials and Methods

Sample collection

The occurrence of ear rot of maize was determined over two-year (2016 and 2017) growing seasons (September to October) from fields in six locations/ districts that mainly cultivate maize and are affected by this disease. Each sample was collected from one randomly selected cob of each hybrid variety, and 23 cobs were obtained from each location. In total, 138 samples were collected from experimental fields in six districts. Sampling date and location (geographic coordinates) were recorded from a global positioning system (GPS). Rainfall, relative humidity and temperature data were collected during growing periods

(August to October), obtained from the Department of Meteorology website. Symptomatic cobs sampled were placed in plastic bags and delivered to the National Corn and Sorghum Research Center (NCSRC), Nakhon Ratchasima for further analysis.

Identification and isolation

The infected kernels of each sample cob were surface-disinfected for one minute in 3.5% NaOCl, then rinsed twice in sterile distilled water. Thirteen maize kernels were plated on moist, sterile filter paper in plastic Petri plates, then incubated at room temperature (25±2ºC) for seven days. For identification, some of the cultures were transferred to a 1/4-strength Potato Dextrose Agar (PDA) medium for single spore isolation and then incubated for seven days at room temperature. The morphological and cultural characters, i.e. the pigmentation and the extent of mycelial growth, shape and size of macroconidia, microconidia, nature of conidiogenous cells, the presence or absence of macroconidia, chlamydospores and perithecia, were used to identify the species. These characteristics were compared with those described previously (Barnett and Hunter, 1972; Mathur and Kongsdal, 2003).

Pathogenicity assay

Three corn varieties (WS4452, CP888 and NS3) were used in pathogenicity test. Conidial inoculum of six Fusarium isolates, identified as *F. verticillioldes*, was prepared following the procedure of Zainudin *et al.* (2016) with minor modification. The concentration was modified to 2×10^6 conidia/mL using a hemacytometer. After that, one mL of conidial suspension was injected into the ear region of 70-day old plants. Two controls were set up, one inoculated with sterile distilled water and one not inoculated. Twenty-one days after inoculation, the cobs were manually dehusked and scored for discoloration of kernels. Evaluation was done based on a disease scale from 1 to 7 as described by Reid and Hamiton (1996). To ascertain the pathogenicity phenotypes of *F. verticillioides*, all inoculated ears showing Fusarium ear rot symptoms were re-isolated for single-spore, and reidentified based on their cultural and morphological characteristics.

Maize inbred screening trial

A set of 60 inbred lines belonging to NCSRC, Kasetsart University, Nakhon Ratchasima province, were evaluated during rainy season (2017) under field trial at NCSRC research station with the aim of finding maize resistance to Fusarium ear rot. Plots were arranged as randomized complete block designs with three replications to evaluate plant resistance under artificial infection. The silk channel inoculation method (SILK) described by Reid et al. (1993) where two ml of a macroconidial suspension of *F. verticilliodes* at concentration of 10^5 conidia ml^{-1} was injected with a blunt needle into the silk channels of individual ears. Individual plots were 5 m long, 3 m wide and consisted of four rows planted with 25 seeds per row. At maturity when kernel moisture was less than 20%, ears were manually harvested and after hand de-husking, the severity of *F.verticilliodes* infection was measured using the disease rating of Reid and Hamiton (1996) as follows: 1=0% infection, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50%, 6=51-75%, and 7=76-100%.

Maize hybrid screening trial

The screening of hybrids was conducted under field trials during both rainy and dry seasons in 2017. Maize hybrid of 20-precommercial and 3-commercial varieties belonging to NCSRC, Kasetsart University were screened for natural disease infection at six locations in four provinces where fungal target isolates were typical. The field set up for all experiments was arranged in randomized complete block designs with three replications. Individual plots were 5 m long, 3 m wide and consisted of four rows planted to 25 seeds per row as previously described. When plant reached physiological maturity, all cobs were harvested per varietyand maize ear rot infections evaluated on site based on the symptoms and nature of damage. Disease severity of cob rot in each ear in the sample was assessed using the disease rating of Reid and Hamiton (1996) as above mentioned. The incidence of infected cobs per farmstead was calculated using the following formula:

Cob rot incidence = $100(x/N)$ where, x the number of infection cobs with a rating of 2 or more and N total number of cobs in maize sample.

On-farm visual assessment

Eight commercial maize varieties were evaluated in four districts in four provinces during rainy season, 2017, under natural infection. The selection of districts was based on maize production levels and accessibility. Maize ear rot infections were evaluated on site based on the symptoms and nature of damage. Disease severity of cob rot in each ear in the sample was assessed using the disease rating of Reid and Hamiton (1996).

ELISA assay for fumonisin production

The maize grain samples of eight commercial varieties from on-farm visual assessment were used for fumonisin production upon natural infection by local endemic isolates**.** Extraction procedures and ELISA kit quantified for fumonisin analysis were carried out based on method described by Berardo *et al.* (2011) and the manufacturer's instruction (Romer Labs Singapore Pte. Ltd.). To obtain each sample solution for fumonisin detection, 100 mL of methanol/water (70:30, v/v) was added to 20 g ground kernel sample of field-test-infected ears. The mixture was then shaken vigorously for three minutes and the extract filtered through a Whatman No.1 filter. The samples were tested with the AgraQuant® Total Fumonisin Assay (Romer Labs Singapore Pte. Ltd.), which detected total fumonisins (FB_1, FB_2 and FB_3) at concentrations as low as 0.25 ppm. Data of fumonisin content was averaged across two replicates.

Data analysis

Maize ear rot incidence and severity, and mycotoxin concentration were analyzed separately using a Duncan's Multiple Range Test (DMRT) (P=0.05). Responses from the collection were analyzed by the SX Statistic Program version 8.

Results and Discussion

Occurrence and identification of ear rot

Ear rot was observed at all locations surveyed with 100% disease prevalence. The highest incidence was 100% in Chuntuk, Pak Chong, Nong Bun Mak, and Phop Phra, and the lowest at 25% was recorded at plot sites at Tambon Pak Chong in Pak Chong district. Over the two years of study, the most severity of ear rot was 38% at Chuntuk, Pak Chong district followed by Phop Phra, and Nong Bun Mak, with 35 and 28% respectively (Table 1). Data obtained provides documentation of ear rot incidence and its geographic distribution in six crop districts in Thailand. This indicates that fungal disease associated with maize ears is quite high during the growing seasons studied.

Table 1. Distribution of 138 isolates of *Fusarium verticilloides*, disease incidence, disease severity, and rainfall of each location in 2016.

Geographic origin	**Number of isolates**	**Disease incidence**	**Disease severity**	**Rainfall (mm)**
Chuntuk, Pak Chong, Nakhon Ratchasima[1/]	23	100%	38%	116.5
Pak Chong, Pak Chong, Nakhon Ratchasima[1/]	23	25%	9%	105.9
Nong Bun Mak, Nakhon Ratchasima	23	100%	28%	105.6
Phop Phra, Tark	23	100%	35%	217.3
Muak Lek, Saraburi	23	27%	8%	148.9
Khok Charoen, Lop Buri	23	26%	5%	130.7

[1] Chuntuk and Pak Chong are different Tambons in Chuntuk, Pak Chong district, Nakhon Ratchasima province. Other plot sites showed only district and province.

One hundred and thirty-eight isolates of *Fusarium* spp. from infected maize grains from six districts were identified for their species level. The results showed that cultural and morphological identification of pathogenic fungus preliminary revealed *F. verticillioides* was the causal agent of all isolates obtained from infected samples. *F. verticillioides* is a saprophyte (not responsible for maize ear rot) and a parasite of maize; that can be found as a systemic endophyte in a symptomless biotrophic state, or as a hemibiotrophic pathogen depending on environmental conditions (Bacon *et al*, 2008). The symptoms were mostly present on the husk and/ or kernels in the form of a white to pink or salmon-colored, cottony mold that occurs on single or multiple kernels scattered or clustered on the ears. Infected kernels are frequently tan or brown or have white streaks. Regardless of the occurrence of symptoms, the presence of this fungus in maize constitutes an imminent risk due to its ability to produce fumonisins, a mycotoxin recognized to be a possible carcinogen to humans (Voss *et al*, 2002).

After four days on potato dextrose incubation at room temperature, pure colonies of *F. verticillioides* (25±2°C) were white, cottony, and tinged with purple. After a 7-day incubation the conidiation showed abundant microconidia of single-celled and oval formed false heads on monophialides. Macroconidia present with sickle-shaped to straight with 3-5 septate.

A pathogenicity test was conducted on varieties WS4452 (susceptible), NS3 (moderately resistant) and CP888 (resistant). All six isolates of *F. verticillioides* identified as pathogenic caused ear rot symptoms with a significantly different disease severity from the control ($P \leq 0.05$) and were used as inoculum for pathogenicity assays. On the susceptible WS4452, isolates F14, F23, and F40 induced the highest disease severity scores of 6.7, 6.0 and 6.3 respectively. The left isolates showed disease severity scores of 1.6-5.6 (data not shown). All treatments were found to have significant difference ($P \leq 0.05$) from the control. After 21 days of artificial inoculation, no symptom was detected on control ears of nontreated controls. In contrast, the ears inoculated with all isolates of *F. verticillioides* showed typical symptoms of ear rot disease. All tested isolates caused different degrees of ear rot symptoms on cobs. These isolates confirmed pathogenicity procedures (Zainudin *et al.* 2016). These results also confirm that the identified causal fungus is *F. verticillioides* and the inoculation method used to produce ear rot symptoms in this study is effective.

Responses to F. verticillioides infection in inbred lines

Evaluation under artificial inoculation using the silk channel inoculation method was conducted at NCRSC and 60 Fusarium-inoculated inbred lines were determined at maturity during rainy season in 2017. In Table 2, all data reported represented averages across replication. Fusarium ear rot severity for all 60 inbred lines ranged from two (1 to 3%) to seven (i.e., 76 to 100% ear rot symptoms), and almost all the entries had severity levels of more than 10%. Only one out of 60 inbred lines (Ki30) showed low disease severity (rating 2.6). The results showed that most of Ki1 to Ki60 lines had high disease severity, therefore were ranked as susceptible and highly susceptible to Fusarium ear rot respectively. Because the inbreds were tested during the rainy season, highly favorable environment contributed to their disease severity induction. This was consistent with Sutton (1982), who reported environmental factors such as rainfall and temperature affect the severity of *Fusarium* spp. infection. The difference in disease severity was thus attributed to differences in environmental conditions affecting disease development and infection. Fusarium ear rot is most severe under hot, dry weather conditions that occurs after flowering (Gxasheka *et al*., 2015). Moreover, Sweets and Wright (2008) pointed out that most of the fungi are more prevalent when the rainfall is above normal during silking to harvest.

Another factor that might contribute to differences in the reaction of inbred lines to ear rot is pathogen diversity. The most virulent isolate (F14), when used as inoculum with an effective silk channel inoculation method (with minor modification in this study), showed adequate disease assessment critical for evaluation of maize resistance to ear rot. Resistant inbred line Ki30 generated enough disease pressure and overcame specific source of resistance. Validations of Fusarium ear rot phenotypes of Ki30 can therefore facilitate development of breeding program to further improve *F. verticilloides* resistance in maize.

Maize hybrid evaluation experiments

Twenty pre-commercial hybrids and three commercial cultivars were subjected to natural infection in field trials at six locations in four provinces where outbreak of Fusarium ear rot has previously been observed. Rainy season experiments resulted in higher mold severity scores than dry season of the years (data not shown) according to natural infestation of ear rot. Three out of six locations at Park Chong (at Tambon Chuntuk), Nong Bun Mark, and Phop-Phra districts showed 100% ear rot incidence. Most pre-commercial hybrids bred by NCSRC showed higher severity than commercial cultivars. However, the effect of two pre-commercial cultivars, KSX5720 and KSX5911 on ear rot severity was not significantly different when compared with those commercial cultivars. These two pre-commercial cultivars revealed moderately resistant tendency with less severity level present under extreme disease pressure of 100% ear rot incidence in the three locations tested. The commercial hybrids also showed moderate resistance similar to that occurring in KSX5720 and KSX5911, at 3.6 and 4.0 respectively. Most of the hybrids were ranked either susceptible or highly susceptible, and the other cultivars tested exhibited high severity of ear rot infection (Table 2). There was a trend of declining disease incidence and severity in these moderately resistant cultivars. This indicated that the ear rot resistance expressed in these genotypes reduces the extent of *F. verticilloides* colonization under high disease pressure of infection by different fungal isolates naturally epidemic in the location tested sites.

Table 2. Disease incidence and disease severity of Fusarium ear rot on pre-commercial field maize under natural infection in six locations determined.

Location / Maize cultivar[1/]	Chuntuk, Pak Chong Nakhon Ratchasima		Pak Chong, Pak Chong Nakhon Ratchasima		Nong Bun Mak Nakhon Ratchasima		Phop Phra Tark		Muak Lek Saraburi		Khok Charoen Lop Buri	
	DI[2/]	DS[3/]	DI	DS	DI	DS	DI	DS	DI	DS	DI	DS
1. KSX5402	100	6.3	20.0	2.6	100	5.3	100	4.6	22.3	3.0	30.0	2.0
2. KSX5603	100	5.0	30.3	4.3	100	6.0	100	5.0	40.7	3.6	20.0	2.0
3. KSX5614	100	4.3	24.3	3.0	100	4.0	100	3.3	30.3	3.3	31.0	2.0
4. KSX5720	100	3.6	30.0	3.0	100	3.6	100	3.3	20.0	2.0	20.0	2.0
5. KSX5805	100	5.6	44.3	4.3	100	6.0	100	6.0	30.6	3.3	21.0	2.0
6. KSX5819	100	4.6	20.0	2.6	100	5.0	100	4.3	23.3	2.3	35.0	2.3
7. KSX5901	100	6.0	28.0	3.6	100	5.0	100	5.3	65.0	4.6	21.6	2.0
8. KSX5902	100	5.0	33.0	4.0	100	6.0	100	3.3	25.0	2.0	20.0	2.0
9. KSX5903	100	4.6	16.0	2.6	100	4.6	100	4.6	34.3	3.3	39.3	2.3
10. KSX5904	100	5.0	N	N	100	4.3	100	5.3	26.6	2.6	21	2.0
11. KSX5906	100	5.0	12.3	2.6	100	5.6	100	5.0	31.6	3.0	26.6	2.0
12. KSX5908	100	4.3	17.6	3.0	100	5.3	100	4.3	23.3	2.3	31.6	2.3
13. KSX5909	100	4.3	20.0	2.6	100	4.6	100	3.0	33.3	3.3	41.0	3.6
14. KSX5911	100	4.0	23.6	3.0	100	4.0	100	4.0	13.0	1.6	20.0	1.6
15. KSX5912	100	4.6	20.0	3.0	100	5.0	100	3.6	18.6	2.0	19.7	1.6
16. KSX5919	100	4.6	18.0	2.3	100	4.6	100	4.3	25.0	3.0	20.0	2.0
17. KSX5924	100	4.6	30.0	3.3	100	5.0	100	4.3	31.0	3.0	50.0	3.6
18. KSX5927	100	5.3	43.3	4.0	100	6.0	100	5.0	28.3	3.3	20.3	3.0
19. KSX5934	100	5.3	22.6	3.3	100	6.0	100	5.3	24.3	3.0	23.3	3.0
20. KSX5937	100	5.0	53.3	4.0	100	4.6	100	4.0	18.3	3.0	24.7	2.3
21. NS3	100	4.0	15.6	2.6	100	2.6	94.3	3.3	13.3	2.6	20.3	2.0
22. CP888	100	3.6	10.0	2.0	100	3.6	91.7	2.6	22.3	2.6	22.7	1.6
23. SW4452	100	5.0	10.0	2.6	100	4.3	96.7	3.6	20.0	3.3	26.7	2.3
% CV	0.00	10.40	12.23	13.78	0.00	10.59	1.34	12.29	15.42	15.86	12.07	17.18

[1/] Numbers 1 to 20 are precommercial and 21to 23 = commercial cultivars.

[2/] Mean of disease incidence (%).

[3/] Mean of disease severity score (1=0% infection, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50%, 6=51-75%, and 7=76-100%).

On-farm visual assessment and fumonisin analysis

The present work addressed the effects of Fusarium ear rot on commercial hybrids of field-grown maize and fumonisin content of affected grains at harvest from five growing locations in four provinces. The average of Fusarium ear rot severity of eight commercial hybrids analyzed at maturity after harvest is shown in Table 3. The affected husks on grains were randomly selected from the sites that showed low disease severity from those five locations. Of eight commercial hybrids tested, SW4452 showed high level of disease severity (ranked=5) at Wang Thong district. Investigation of fumonisin accumulation in infected grains using ELISA analysis revealed that all commercial maize tested had low levels of fumonisin concentration. In some location samples however, fumonisin was detected with over 5 ppm concentration levels. A maximum of 20.61 ppm of fumonisin was detected in PAC139 grains and mean of disease severity ranked with four level at Wang Thong district. However, cultivars PAC559, SW4452, and DK6818 had high levels of disease severity, but low fumonisin accumulation was observed. These results seem to indicate that there is no correlation between Fusarium ear rot severity (visualization) and concentration of fumonisin accumulated in grains (ELISA). Similar results were obtained by Clement *et al.* (2004) when testing the correlation between fumonisin production and the severity of ear rot symptoms. These results suggest that fumonisin production by *F. verticilloides* under natural (uncontrolled) conditions is not a consequence of the severity of ear rot symptoms in maize grains. Thus, a toxigenic potential present in this parasitic fungus from Thailand is complex and has a chance to vary in the range of fumonisin contaminated in food and feeds. Further study should be conducted to determine if (i) the virulent effect of *F. verticilloides* isolates in fumonisin production, (ii) the tendency quantified in number of isolate populations compared to the visible symptoms and mycotoxin accumulation, (iii) fumonisin production under both in vitro and in vivo culture conditions, and (iv) fumonisin accumulation in grains of resistant and susceptible maize plants, were responsible for development of ear rot severity and fumomisin production. Data obtained could address the stability of reduced mycotoxin accumulation in maize grains.

We observed that high humidity, increased precipitation, and high temperature contributed to development of ear rot epidemics in different locations, resulting in variation in the rank among moderately susceptible and susceptible phenotypes. Current study revealed that the severity of ear rot in rainy season (August-October) was greater than dry season (December-February). This matches results obtained by Czembor *et al.* (2015) that temperature and rainfall are the main factors affecting the development of *Fusarium* species causing important diseases of maize and other small grain cereals.

This study is the first attempt at identifying a causal fungus of ear rot epidemics in Thailand and evaluating a subset of maize phenotypes classified as moderately resistant and susceptible to *F. verticilloides* infection. The capacity of this parasitic fungus to induce ear rot symptoms under field grown maize in correlation to its fumonisin production was also described. More work is needed to further explain the results. The results of the current study are validated in breeding program and growers' fields as a few sources of ear rot resistance have been identified to date.

Table 3. Fusarium ear rot severity and fumonisin concentration of eight commercial maize hybrids evaluated after harvest in 2017 growing season.

District	Si Satchanalai		Pak Chong		Tak Fa1		Tak Fa2		Wang Thong	
Commercial maize hybrid	Fusarium ear rot	Fumonicin (ppm)	Fusarium ear rot	Fumonicin (ppm)	Fusarium ear rot	Fumonicin (ppm)	Fusarium ear rot	Fumonicin (ppm)	Fusarium ear rot	Fumonicin (ppm)
PAC139	2[1/]	11.83	2	0.17	3	0.93	2	4.47	4	20.61

PAC129	2	1.42	2	4.07	3	1.41	2	2.05	2	0.56
CP888	2	1.03	2	0.21	2	0.06	2	5.91	2	3.37
DK9898	2	0.50	3	1.0	3	0.85	3	7.58	3	8.74
NS3	2	3.00	2	1.99	2	0.25	2	1.14	3	0.58
PAC559	2	8.25	3	2.29	3	1.89	3	5.11	4	5.70
SW4452	2	3.64	2	1.65	3	1.47	4	1.48	5	1.48
DK6818	2	0.71	3	2.25	3	5.73	3	2.63	4	1.69

[1/] Mean of disease severity score (1=0% infection, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50%, 6=51-75%, and 7=76-100%).

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Defense Response Boost through Cu-Chitosan Nanoparticles and Plant Growth Enhanced Coupled with Multiple Disease Control

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Introduction

Environmental contamination has become a challenging issue because of uncontrolled and rampant use of synthetic agrochemicals for plant growth and protection (Tilman et al. 2002). Perpetual use of agrochemicals causes several adverse affects including, increased resistance in plant pathogenic microbes, negative impact on non-target organisms and deterioration of soil health (Kashyap et al. 2015; Zhan et al. 2015). Globally, crops are severely affected by diseases which lead to qualitative and quantitative losses in agriculture (Savary et al. 2012). Emphasis on development of biomaterial based biodegradable agrochemicals for effective and safe application in crops is needed. Chitosan, a versatile biomaterial that is non-toxic, biocompatible and biodegradable in nature, is being touted as a viable alternative (Katiyar et al. 2015; Xing et al. 2015). Chitosan is well recognized as antimicrobial (Kong et al. 2010; Goy et al. 2016), immunomodulatory (Amborabe et al. 2008; Falcon-Rodriguez et al. 2011; Popova et al. 2016; Sathiyabama et al. 2016) and an agent promoting plant growth (Kananont et al. 2010; Sathiyabama et al. 2016). Higher physiological and biochemical responses of chitosan-based NPs as compared to bulk chitosan (Van et al. 2013; Saharan et al. 2015 and 2016) is due to smaller size, high surface to volume ratio and surface charge. Hence, chitosan-based NPs have various applications in agriculture including plant growth (Van et al 2013; Saharan et al. 2015; Sathiyabama and Parthasarathy, 2016; Abdel-Aziz et al. 2016; Saharan et al. 2016) and disease protection (Saharan et al. 2015; Kheiri et al. 2016; Manikandan and Sathiyabama, 2016; Sathiyabama and Manikandan, 2016; Saharan and Pal, 2016).

Recently, chitosan-based NPs have been evaluated as potent inducers of antioxidant and defense enzymes (Chandra et al. 2015; Sathiyabama and Manikandan, 2016). In our previous studies, we have reported Cu-chitosan NPs as effective antifungal and plant growth promoting agents (Saharan et al. 2013; Saharan et al. 2015). Further studies revealed that application of Cu-chitosan NPs enhanced maize seedling growth by mobilizing reserve food through the enhanced activities of alpha amylase and protease (Saharan et al. 2016). While maize is an important food crop worldwide, it is prone to various fungal diseases like Curvularia leaf spot (CLS) disease which can cause yield losses of up to 60% (Bisht et al. 2016). Many strategies have been applied to control CLS disease using chemicals and other bio-agent but there is no report on evaluation of Cu-chitosan NPs against CLS disease in maize. This is the first report on the efficacy of Cu-chitosan NPs to induce the defense responses against CLS disease in maize and sustainable plant growth. Our results convincingly establish Cu-chitosan NPs as a potent inducer of systemic acquired resistance for effective control of CLS disease of maize and as an agent of plant growth.

Materials and Methods

The experiments performed in the study are summarized in Table 1 and details are as below.

Table 1. Experimental outline

Experiment	**Analysis/method**	**Remarks**
Synthesis of Cu-chitosan NPs	Ionic gelation method (Saharan et al. 2015)	Cu-chitosan NPs were synthesized
In-vitro Cu release	Using AAS (Saharan et al. 2015)	Cu release from Cu-chitosan NPs was evaluated with respect to pH and time
Pot experiment		
Antioxidant and defense enzymes assay	Methods described as (Giannopolitis and Ries, 1977; Chance and Maehly, 1955; Moerschbacher et al. 1988; Taneja and Sachar, 1974)	Activities of SOD, POD, PAL and PPO were estimated
Chlorophyll content (a, b)	As described by Stangarlin et al. (2010)	Chlorophyll content (a, b) was quantified
Disease assessment	Using 1 to 9 standard disease rating scale (Sobowale, 2011)	DS and PEDC were calculated
Copper content in leaves	AAS method described by Adrian, 1973	Cu content in leaves was determined
Statistical analysis	JMP version-12 using Tukey−Kramer HSD test	Significant difference between treatment *(p* = 0.05) were calculated

Materials

Chitosan (low molecular weight and 80% N-deacetylation) and sodium tri-polyphosphate (TPP) were procured from Sigma-Aldrich, St. Louis, MO, USA. Chemicals for enzyme assay and other experiments were procured from HiMedia and SRL, Mumbai, India. The seeds of cultivar Suryal local were obtained from the Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, India. Inoculum of *Curvularia lunata* was received from Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, India.

Synthesis of Cu-Chitosan NPs

Cu-chitosan NPs were prepared based on the ionic gelation method as earlier described by us (Saharan et al. 2015). Synthesized NPs were characterized for various physico-chemical characteristics by dynamic light scattering (DLS), fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The synthesized NPs had the same characteristics as reported (Saharan et al. 2015 and 2016).

In-vitro Cu release profile

In-vitro experiments were conducted to study the effect of pH and time on the release of Cu from Cu-chitosan NPs. In brief, freeze dried Cu-chitosan NPs were dispersed in deionized water with pH adjusted in the range of 1 to 7. The contents were centrifuged at 10,000×g for 10 minutes and supernatants were collected for further analysis. Similarly, in separate experiments, the NPs were dispersed at 4.5 pH for 0, 24, 48, 72, 96, 120 and 144 hours followed by centrifugation at 10,000×g for 10 minutes. The supernatants, thus obtained, from both the experiments were analyzed for Cu contents using atomic absorption spectrophotometer (AAS 4141 model, Electronics Corp. of India Ltd., India).

Pot experiment for disease assessment and plant growth

Seeds of disease susceptible maize cultivar Suryal local were surface sterilized with 10% sodium hypochlorite for 10 minutes and further treated for four hours with different concentrations of Cu-chitosan

NPs (0.01, 0.04, 0.08, 0.12 and 0.16%, w/v), bulk chitosan (0.01%, w/v), $CuSO_4$ (0.01%,w/v) and commercially available fungicide (0.01% Bavistin, w/v). The treated seeds were dried and sown in earthen pots filled with standard clay type soil in net house condition. The plants were subjected to foliar spray of the same treatments as the seeds, until runoff at 35 days of sowing. After 10 days of foliar treatments, cultures of *C. lunata,* prepared on sorghum seed (Hou et al. 2013), were inoculated on plants. Disease assessment was performed after appearance of symptoms on leaves at 15 days of inoculation. Disease Severity (DS) was recorded on 1 to 9 standard disease rating scale (Sobowale, 2011). Further, the DS and percent efficacy of disease control (PEDC) were calculated by using the formula given by Chester (1959) and Wheeler (1969).

DS = Sum of all individual disease rating × 100 / total number of plants assessed x maximum rating
PEDC = Disease severity in control - disease severity in treatment × 100 / disease severity in control.

The plants were harvested at maturity (95 days) to determine plant height, stem diameter, root length and root number. Chlorophyll a and b were quantified in 3rd leaf after 24 hours of foliar spray (Stangarlin et al. 2010). Cu content was also measured in 3rd leaf of treated plant after harvest using AAS (Adrian, 1973).

Measurement of enzyme activity

Activities of antioxidant [superoxide dismutase (SOD) and peroxidase (POD)], defense enzymes [phenylalanine ammonia lyase (PAL) and polyphenol peroxidase (PPO)] were estimated in third leaf after 24 hours of foliar spray of various treatments. For enzyme extraction, 0.2g samples were homogenized in 5 ml of extraction buffer (phosphate buffer for SOD and PPO at pH 7.4 and 6.8, respectively; tris-HCl buffer at pH 7.5 for POD and borate buffer at pH 8.8 for PAL). The homogenates were centrifuged at 10,000 x g for 20 minutes at 4ºC and supernatants were taken for enzymes assay. SOD (EC 1.15.1.1) activity was determined at 560 nm, reduction of nitroblue tetrazolium (NBT) as an indicator of superoxide anion production (Giannopolitis and Ries, 1977). POD (EC 1.11.1.7) activity was measured spectrophotometrically as described by Chance and Maehly (1955) by oxidation of guaiacol in the presence of hydrogen peroxide. Increase in absorbance at 470 nm was recorded due to formation of tetra guaiacol. PPO (EC 1.10.3.1) was assayed according to Taneja and Sachar (1974) and activity was expressed as change in absorbance at 490 nm. PAL (EC 4.3.1.5) was estimated as described by Moerschbacher et al. (1988) where the deamination of L-phenylalanine to trans-cinnamic acid and ammonia was measured at 290 nm. Activities of all the enzymes were expressed in µmol/min/g tissue.

Statistical analysis

Each experiment was repeated twice, and each treatment replicated thrice. Statistical analysis of the data was performed with JMP software version 12. The significant differences among treatment groups were determined using the Turkey Kramer HSD at $p = 0.05$.

Results and Discussion

Cu-chitosan NPs

Laboratory synthesized, stable and biologically active Cu-chitosan NPs (physical and mean hydrodynamic diameters 150±12.4 nm, and 374.3±8.2 nm, respectively with zeta-potential +22.6 mV), reported in our previous papers (Saharan et al. 2015 and 2016), were used to evaluate their effect on antioxidant and defense system, disease control and plant growth promotion in maize.

Cu release profile

Release of Cu from Cu-chitosan NPs was studied in the pH range 1 to 7 (Figure 1). With decrease in pH from 3 to 1, release of Cu increased rapidly from 21.5% to 44.11% due to protonation of amino group of chitosan. At pH above 6, release of Cu drastically decreased (4.94%) due to deprotonation of amino group of chitosan (Figure 1a). Release study was further continued at 4.5 pH with respect to time. Cu release increased slowly and steadily with time and at 96 h ~85% of Cu released from Cu-chitosan NPs (Figure 1b). The release profile indicated that acidic pH expedited the Cu release and over time (at pH 4.5), a slow and sustained release of Cu from Cu-chitosan NPs was evident.

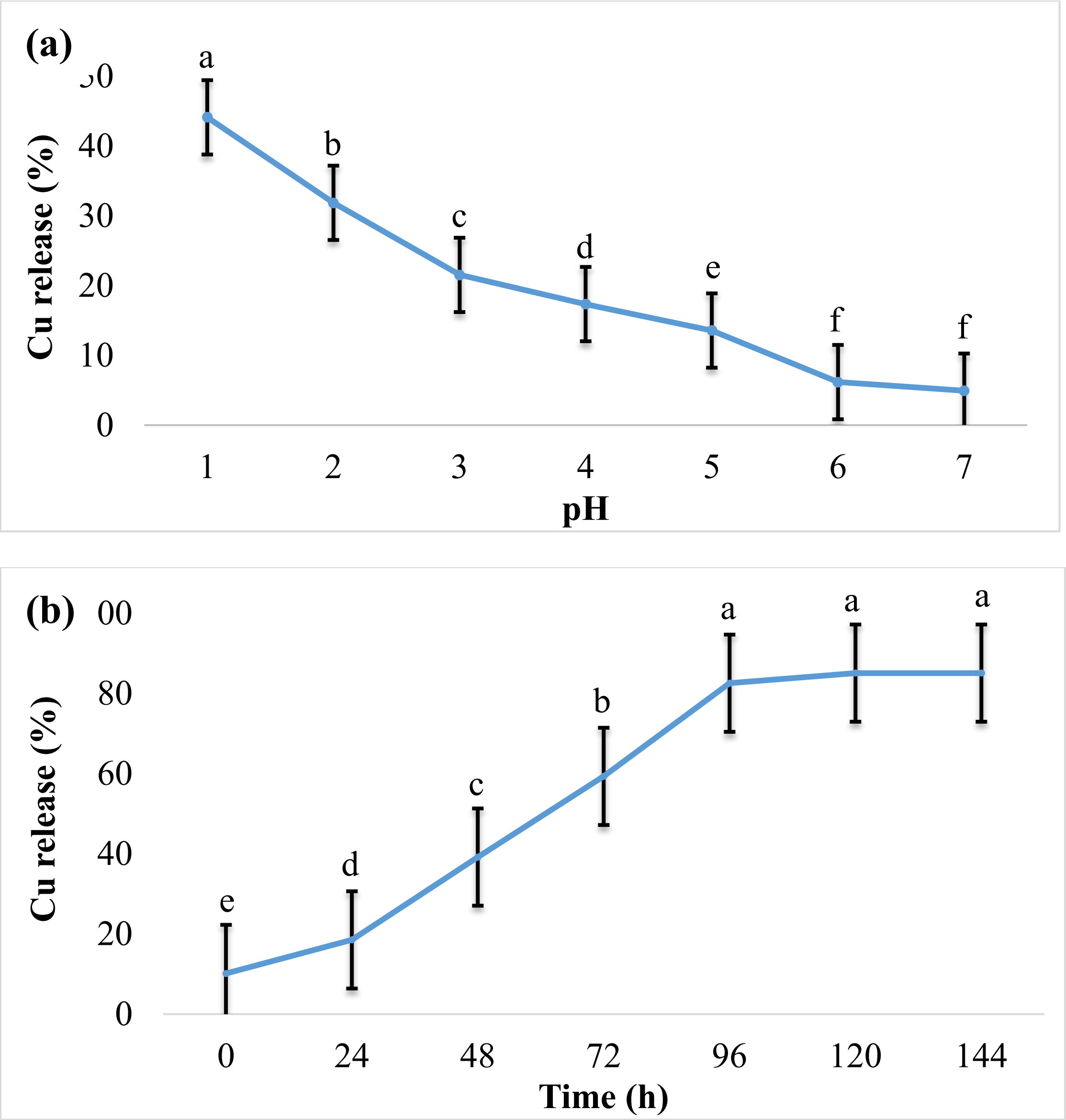
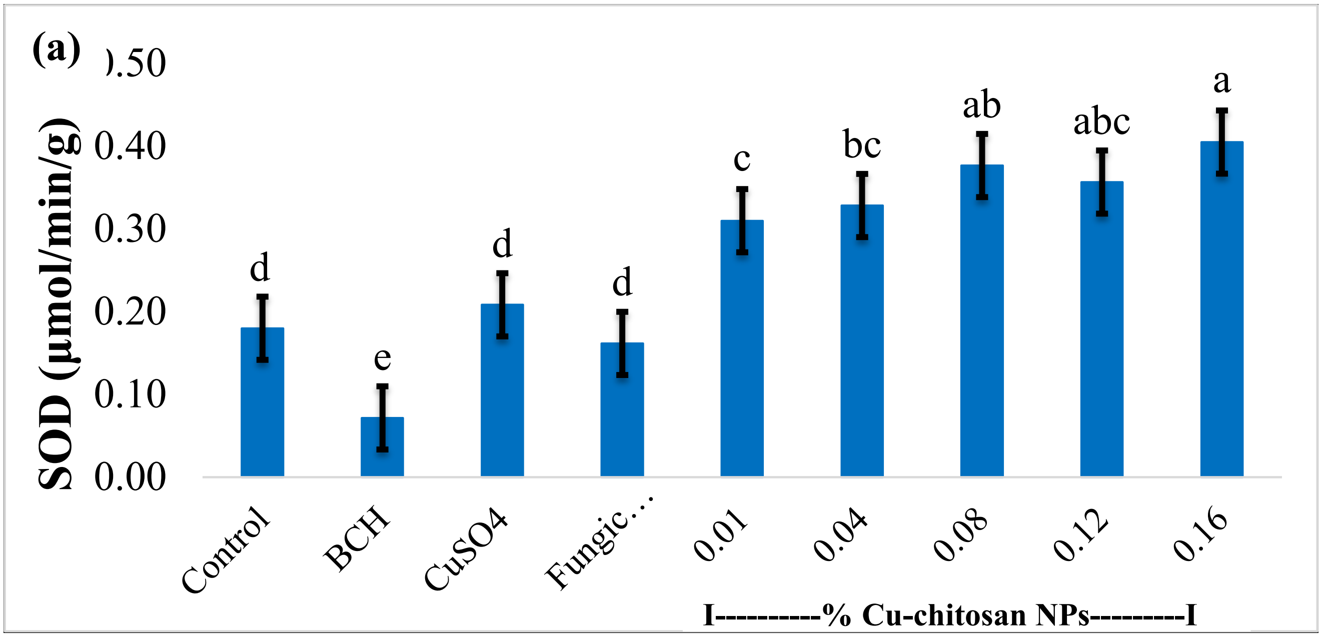
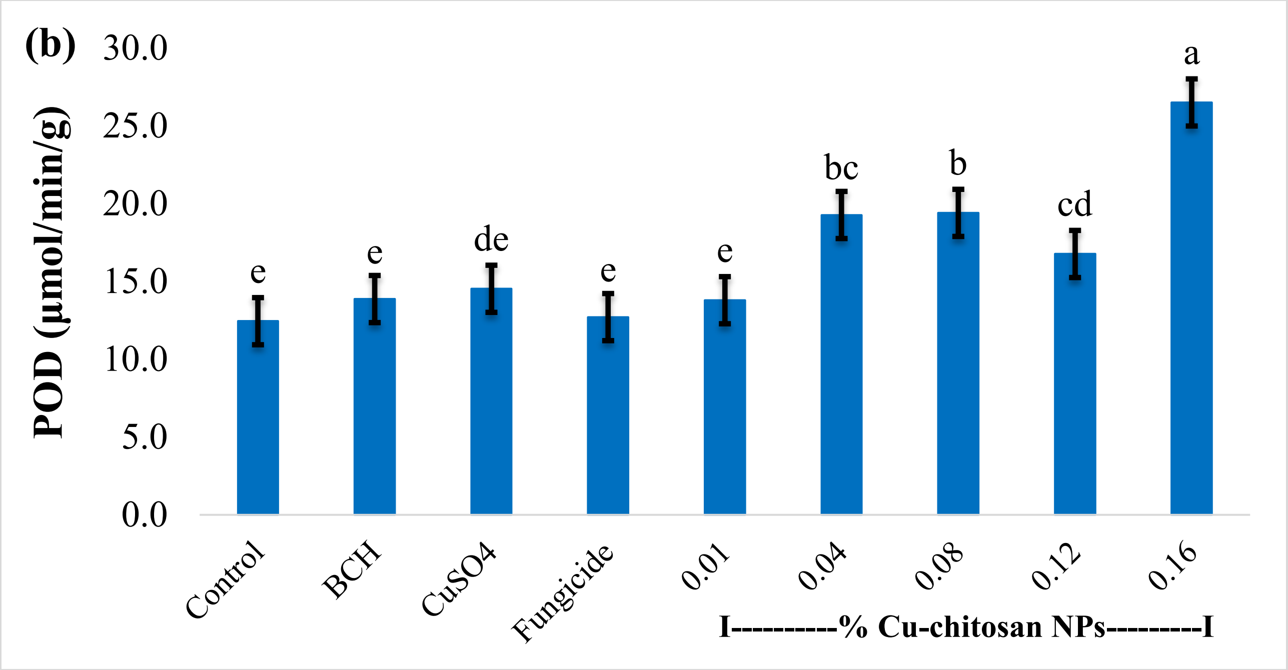


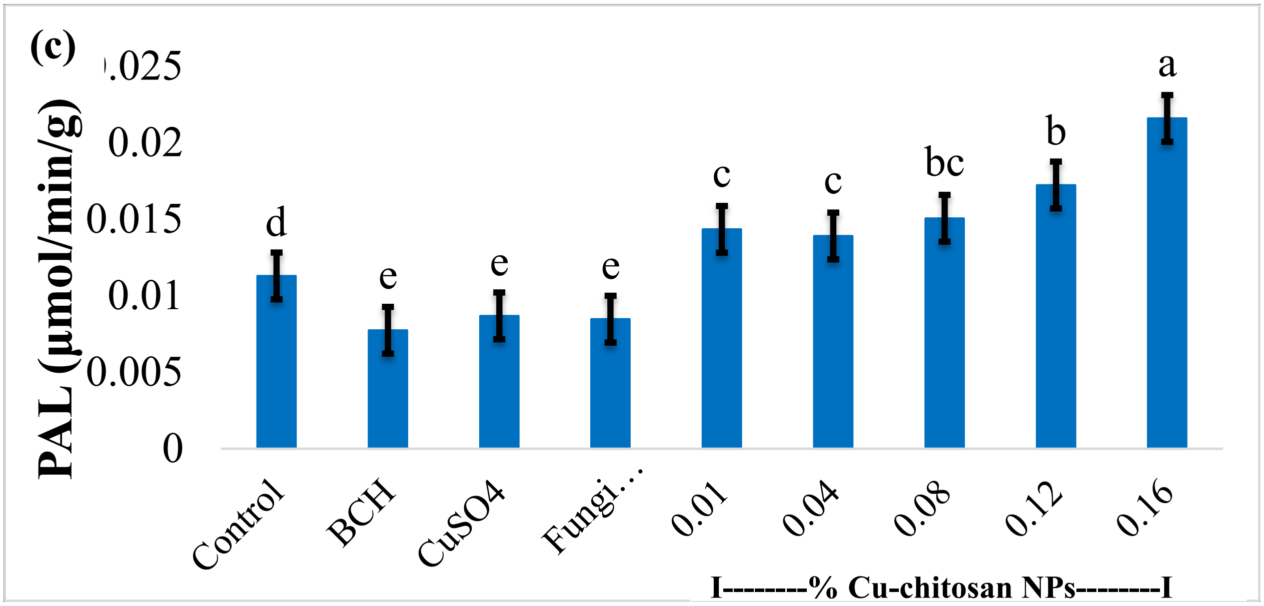
Figure 1. *In-vitro* Cu release from Cu-chitosan NPs (a) at different pH (b) time. Each value is the mean of triplicates. Same letter in graph is not significantly different at $p = 0.05$ as determined by Tukey−Kramer HSD.

Effect of Cu-chitosan NPs on activities of antioxidant and defense enzymes

To estimate the activities of antioxidant and defense enzymes, leaf samples were collected after 24 hours of foliar treatments. Application of NPs substantially induced the enzyme activities in leaves. SOD activity was significantly higher in all the treatments of NPs (Figure 2a). Similarly, 1.5- to two-fold higher POD activity was recorded in 0.04 to 0.16% NPs treated plant leaves compared to control and bulk chitosan treated plants (Figure 2b). Likewise, Cu-chitosan NPs treated plants leaves showed two- to three-fold increased PAL activity compared to bulk chitosan treatment (Figure 2c). The activity of PPO was also enhanced by NPs treatment compared to control, bulk chitosan and $CuSO_4$ treatments (Figure 2d).







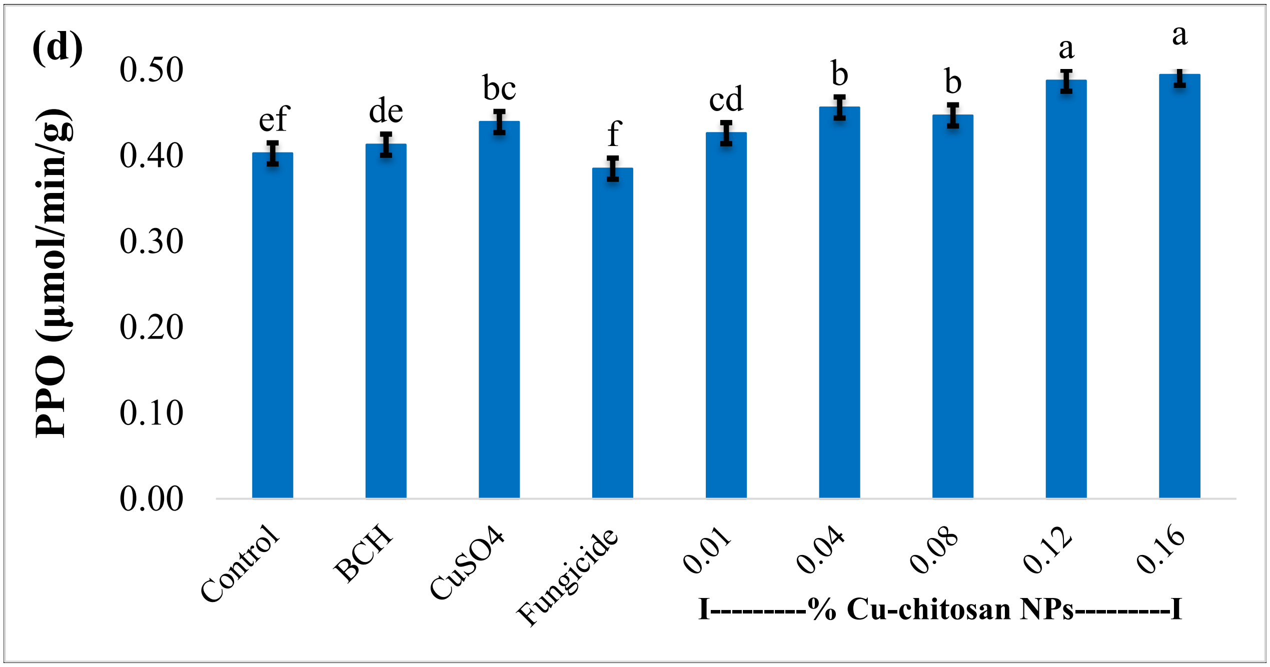


Figure 2. Effect of Cu-chitosan NPs on (a) SOD (b) POD (c) PAL (d) PPO enzymes in maize plant leaves after 24 h of foliar spray. Each value is the mean of triplicates. The same letter in the graph of each treatment is not significantly different at $p = 0.05$ as determined by Tukey−Kramer HSD, control with water. BCH (bulk chitosan, 0.01%) dissolved in 0.1% acetic acid. $CuSO_4$ (0.01%) and fungicide (0.01% of Bavistin)

Effect of Cu-chitosan NPs on CLS disease

In pot experiment, symptoms of CLS disease began 3-4 days after fungal inoculation in control plants. The early appeared small chlorotic spot gradually extended into a large eye shaped lesion, leading to the formation of leaf necrosis (Figure 3a). On the contrary, in NPs treated plants, the disease symptoms in the form of small lesions without chlorosis visualized 7-8 days after fungal inoculation (Figure 3b). The spread and severity of disease was also slow in subsequent days. After 15 days of inoculation, data for DS and PEDC were recorded. DS decreased with increasing concentrations of NPs compared to other treatments (Table 2). Commercially available fungicide (0.01% bavastine), used as positive control, showed 29.3% DS. All the plants treated with 0.04 to 0.16% Cu-chitosan NPs showed significantly lower DS to an extent of 24.6-22.6%. The Cu-chitosan NPs at 0.04-0.16% significantly controlled CLS disease as depicted by higher value of PEDC (Table 2).

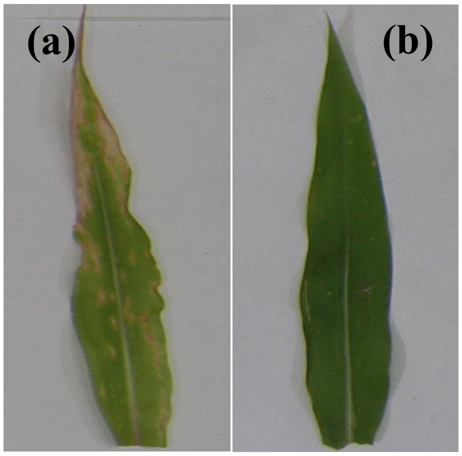


Figure 3. Symptoms of CLS disease on maize plant leaf in pot experiments (a) large necrotic lesion in control (b) micro lesions on Cu-chitosan NPs (0.16%) treated leaf.

Table 2. Effect of Cu-chitosan NPs on CLS disease

Treatment (%)	**DS (%)**^{A}	**PEDC (%)**^{A}
Control (water)	44.00±1.15^{a}	00.00±0.00^{e}
BCH (0.01)	32.67±0.66^{b}	25.72±1.01^{d}
$CuSO_4$ (0.01)	32.00±1.15^{bc}	27.24±2.31^{cd}
Fungicide (0.01)	29.33±0.66^{bc}	33.31±0.85^{bc}
Cu-chitosan NPs		
0.01	28.67±0.66^{c}	34.83±0.87^{b}
0.04	24.67±0.66^{d}	43.86±2.06^{a}
0.08	24.67±0.66^{d}	43.93±0.78^{a}
0.12	23.33±0.66^{d}	46.97±0.76^{a}
0.16	22.67±0.66^{d}	48.48±0.76^{a}

Disease data was recorded after 15 days of inoculation using 1 to 9 standard disease rating scale. ^{A}Each value is the mean of triplicates. The same letter in the table of each treatment is not significantly different at $p = 0.05$ as determined by Tukey−Kramer HSD. BCH (bulk chitosan) dissolved in 0.1% acetic acid and Fungicide (0.01% of Bavistin)

Effect of Cu-chitosan NPs on plant growth

To evaluate the effect of NPs on plant growth, various growth characteristics (plant height, stem diameter, root length, root number and chlorophyll content) were recorded. Statistical analyses showed that Cu-chitosan NPs significantly enhanced the growth of maize plants in pot experiments compared to control, bulk chitosan, $CuSO_4$ and fungicide treatments (Figure 4). Higher values of plant height, stem diameter, root length and root number were recorded in 0.01% to 0.12% NPs treated plants (Figure 5a-d). A significant increase in chlorophyll a and b content (10.58 to 16.22 mg/g and 0.58 to 1.03 mg/g) was recorded in 0.01% to 0.12% of NPs treatments. In $CuSO_4$ treatment, chlorophyll a and b content were least (4.53 and 0.20 mg/g) followed by 0.16% NPs (6.81 and 0.35 mg/g) treatment (Figure 5e-f). To illustrate the possible association between plant growth and Cu, Cu content was estimated in treated plant leaves by AAS. Increasing concentrations of Cu-chitosan NPs (0.01-0.16%) showed increased Cu content (8.6-28.5 mg/kg dry weight) in treated leaves (Table 3). $CuSO_4$ (0.01%) treated plant leaves had 24.1 mg/kg dry weight of Cu, whereas in water and bulk chitosan treatment, same content was observed (Table 3).

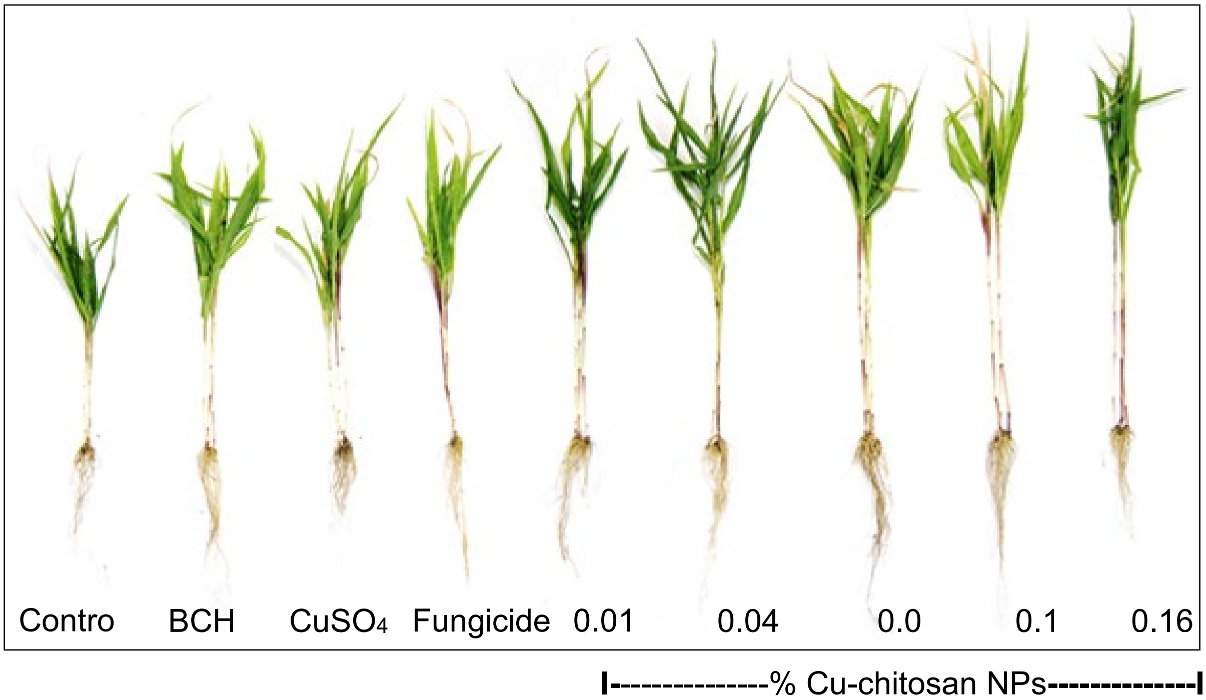
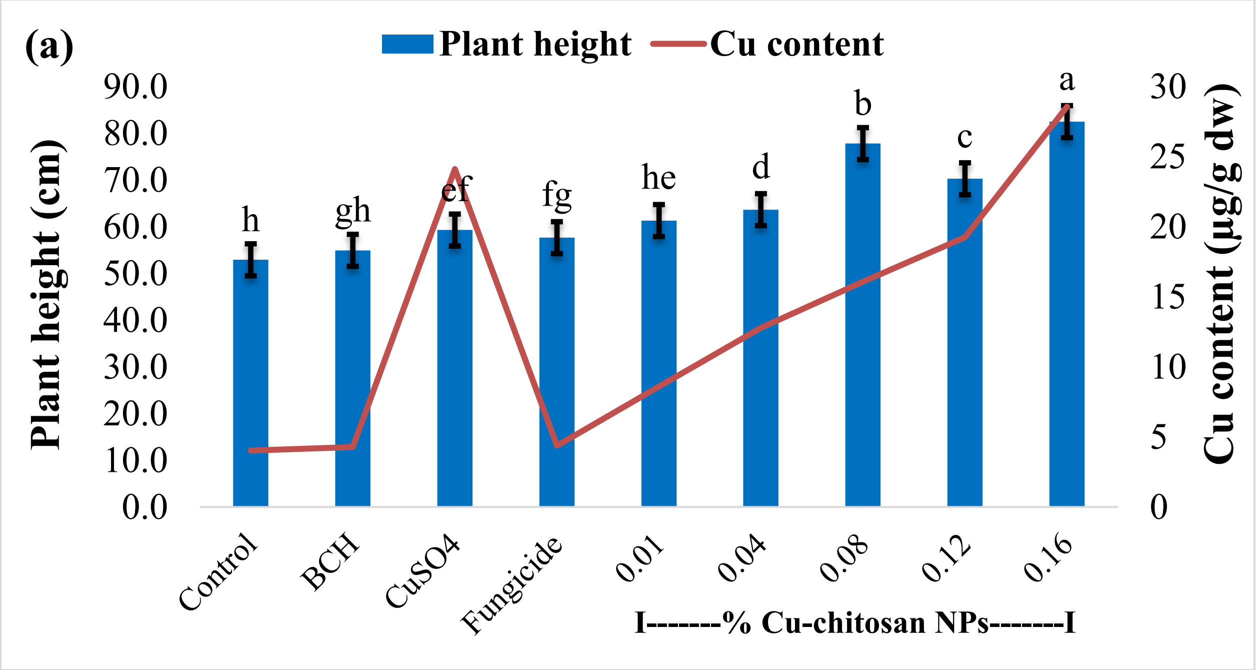
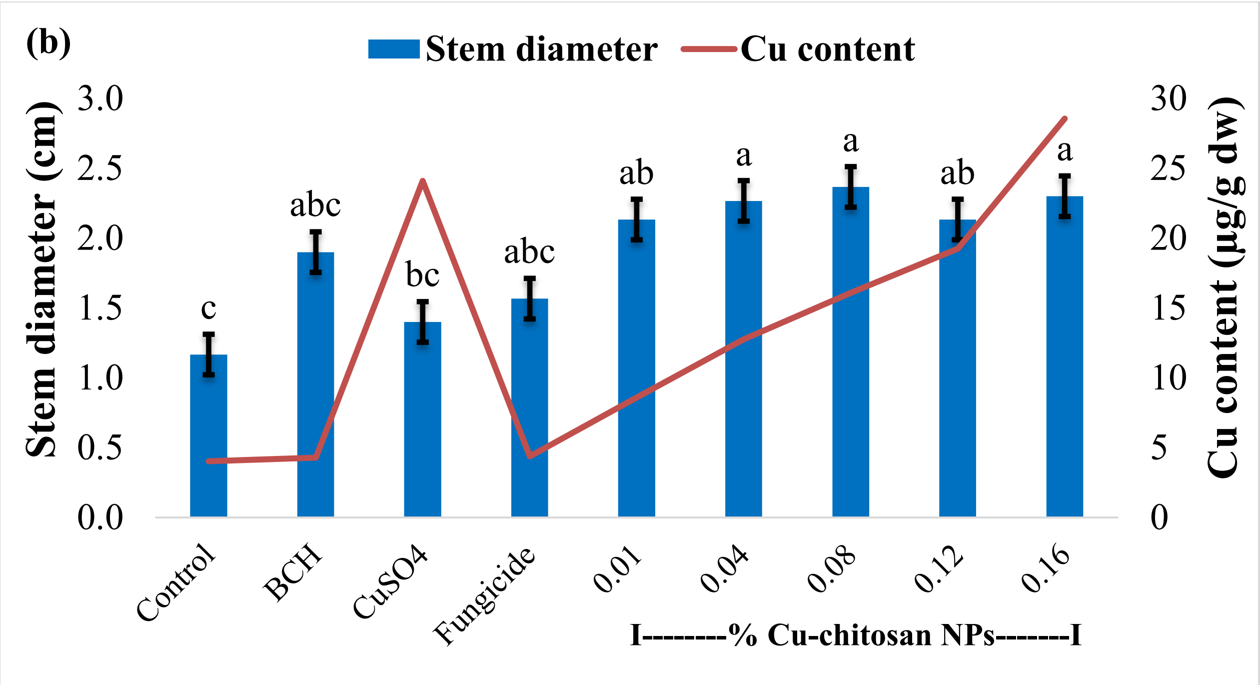
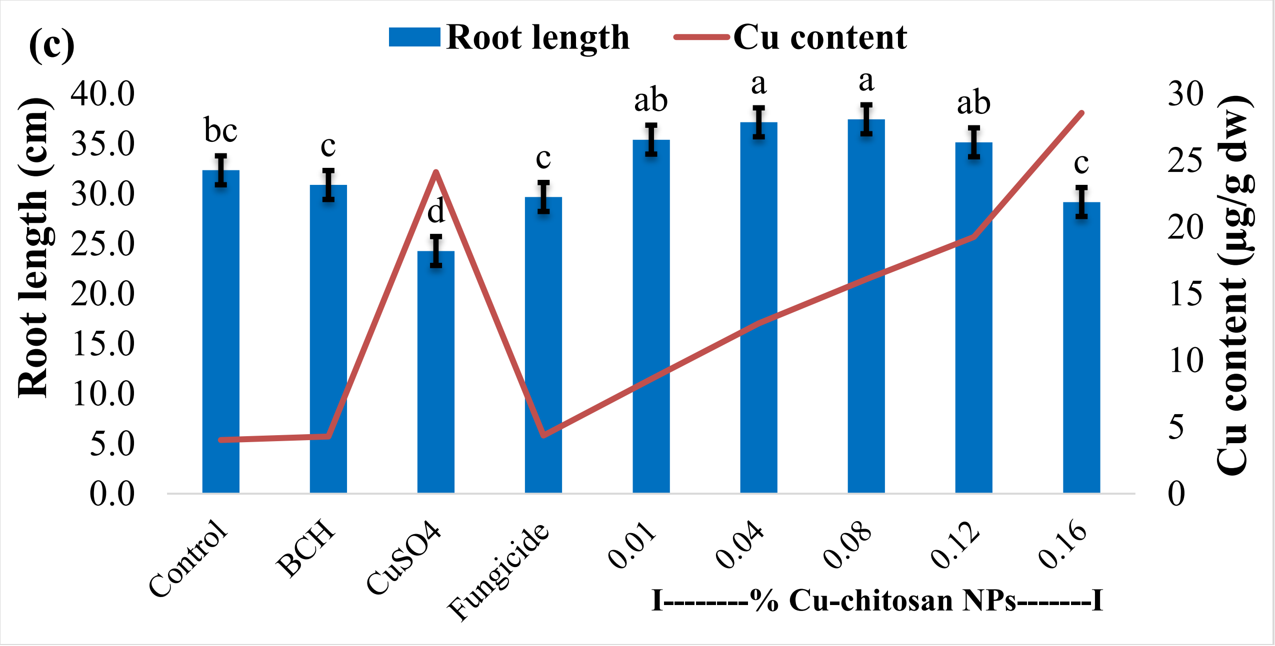
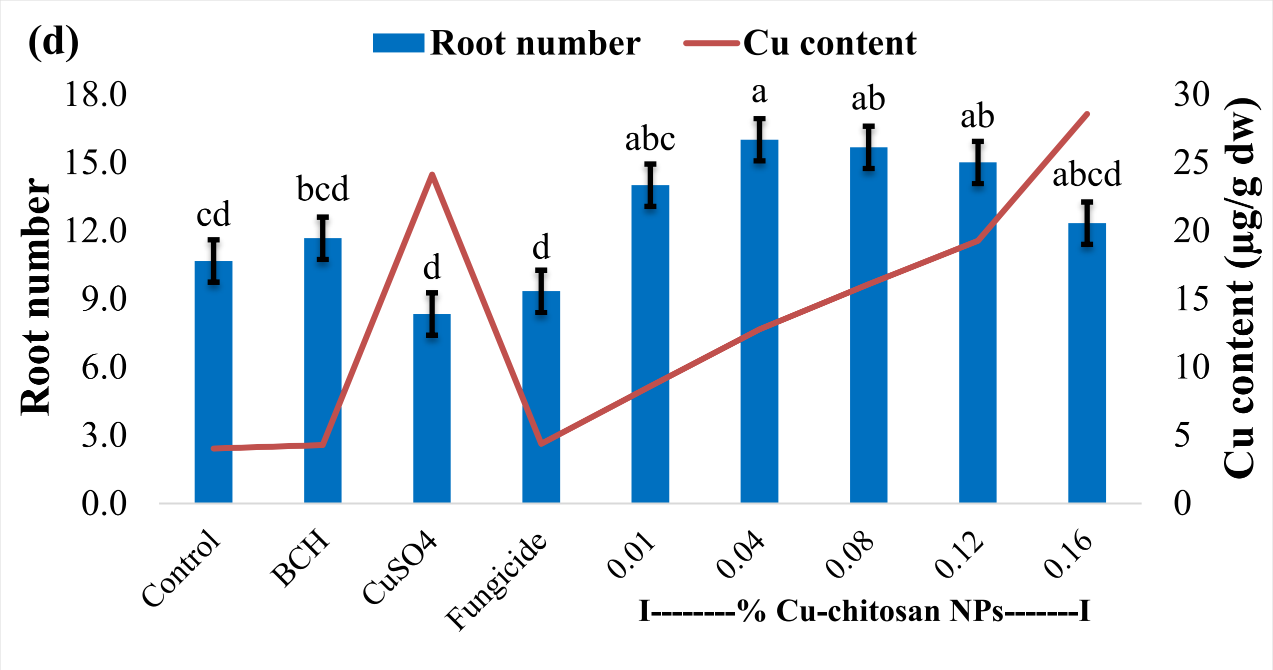


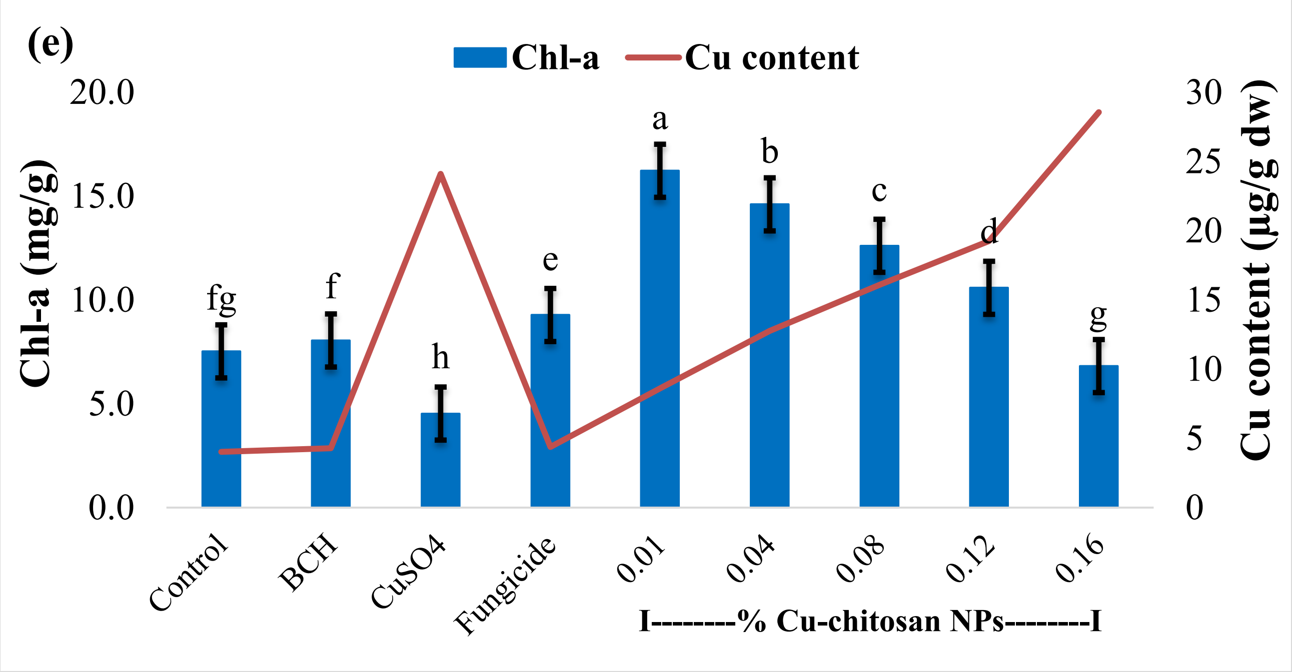
Figure 4. Effect of Cu-chitosan NPs on plant growth of maize in pot condition




(b)
Stem diameter
Cu content
Stem diameter (cm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Cu content (µg/g dw)
30
25
20
15
10
5
0
c
abc
bc
abc
ab
a
a
ab
a
Control
BCH
CuSO4
Fungicide
0.01
0.04
0.08
0.12
0.16
I--------% Cu-chitosan NPs-------I


(c)
Root length
Cu content
Root length (cm)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Cu content (µg/g dw)
30
25
20
15
10
5
0
bc
c
d
c
ab
a
a
ab
c
Control
BCH
CuSO4
Fungicide
0.01
0.04
0.08
0.12
0.16
I--------% Cu-chitosan NPs-------I


(d)
Root number
Cu content
18.0
15.0
12.0
9.0
6.0
3.0
0.0
Root number
30
25
20
15
10
5
0
Cu content (µg/g dw)
cd
bcd
d
d
abc
a
ab
ab
abcd
Control
BCH
CuSO4
Fungicide
0.01
0.04
0.08
0.12
0.16
I--------% Cu-chitosan NPs-------I


(e)
Chl-a
Cu content
20.0
15.0
10.0
5.0
0.0
Chl-a (mg/g)
30
25
20
15
10
5
0
Cu content (µg/g dw)
fg
f
h
e
a
b
c
d
g
Control
BCH
CuSO4
Fungicide
0.01
0.04
0.08
0.12
0.16
I--------% Cu-chitosan NPs--------I

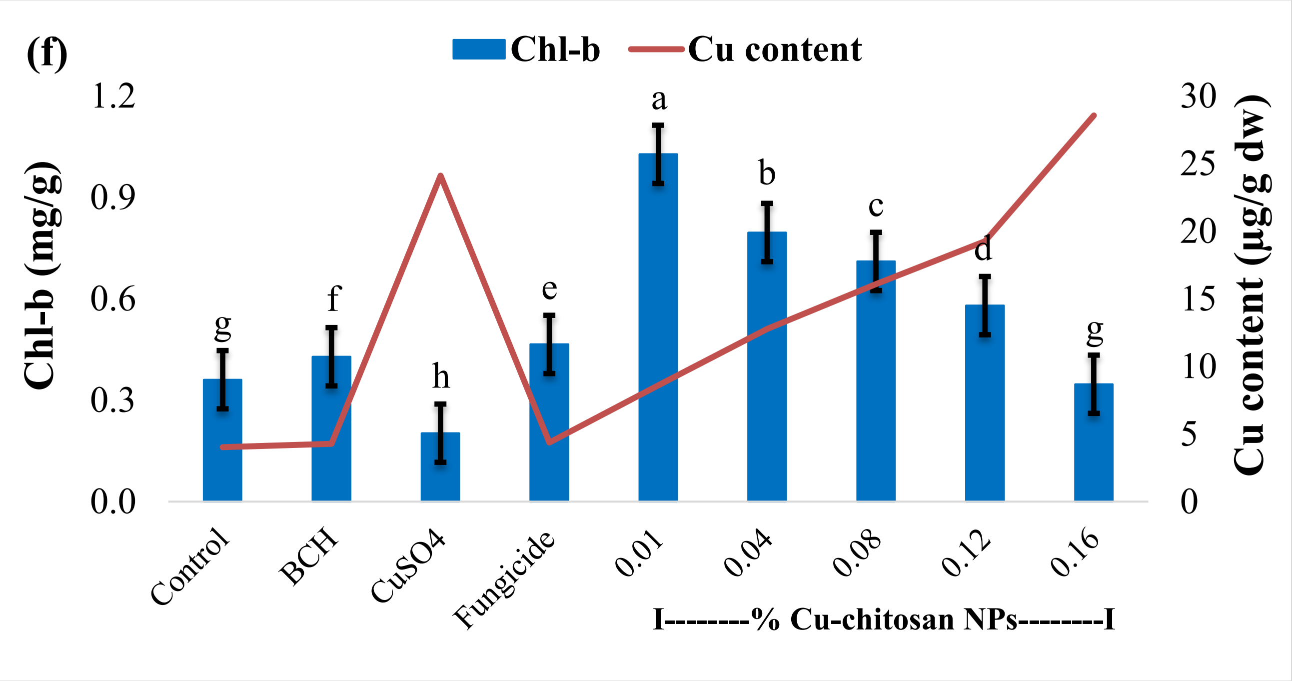


Figure 5. Effect of Cu-chitosan NPs on (a) plant height (b) stem diameter (c) root length (d) root number (e) chlorophyll a content (f) chlorophyll b content. Each value is the mean of triplicates. The same letter in the graph of each treatment is not significantly different at *p* = 0.05 as determined by Tukey−Kramer HSD, control with water. BCH (bulk chitosan, 0.01%) dissolved in 0.1% acetic acid. $CuSO_4$ (0.01%) and fungicide (0.01% of Bavistin)

Table 3: Cu content in maize leaves in various treatments

Treatment (%)	**Cu content^A (µg/g dw)**
Control (water)	4.02±0.37^g
BCH (0.01)	4.28±0.24^g
$CuSO_4$ (0.01)	24.10±0.67^b
Fungicide (0.01)	4.37±0.37^g
Cu-chitosan NPs	
0.01	8.60±0.47^f
0.04	12.77±0.33^e
0.08	16.07±0.13^d
0.12	19.25±0.61^c
0.16	28.55±0.50^a

Data was recorded in 3rd leaf after harvest. ^{A}Each value is the mean of triplicates. The same letter in a column of each treatment is not significantly different at *p* = 0.05 as determined by Tukey−Kramer HSD. BCH (bulk chitosan, 0.01%) dissolved in 0.1% acetic acid and fungicide (0.01% of Bavistin).

Chitosan NPs have previously been reported as immune modulators through induction of antioxidant/ defense enzyme activity in tea and finger millet plants (Chandra et al. 2015; Sathiyabama and Manikandan, 2016). In the present study, foliar application of Cu-chitosan NPs in pot experiments substantially induced antioxidant/ defense enzyme activity in maize leaves. NPs treated plant leaves showed four- to six-fold higher activity of SOD compared to bulk chitosan (Figure 2a). The higher activity of SOD effectively converts highly toxic superoxide radicles into less toxic H_2O_2 species (Bowler et al. 1992; Del Rio et al. 2002). A significantly higher activity of POD, a key enzyme to scavenge H_2O_2 into H_2O and O_2, was also recorded in NPs treated leaves (Figure 2b) (Almagro et al. 2009). The elevated activities of SOD and POD after NPs treatments might be responsible for balancing, degeneration and scavenging of reactive oxygen species (ROS) to protect plants from oxidative stress during pathogen invasion. In Cu-chitosan NPs treated plant leaves, PAL activity also increased from 46.15% to 66.66% and PPO activity increased from 3.05% to 16.39% compared to bulk chitosan treatment (Figure 2c-d). The increased activity of POD, PAL and PPO might be associated with production of suberin, melanin and lignin (Goamez-Vasquez et al. 2004; Fugate et al. 2016) for cell wall strengthening, which further acts as a mechanical barrier to invading plant pathogen (Bruce and West, 1989; Kuzniak and Urbanek, 2000; Goamez-Vasquez et al. 2004; Fugate et al. 2016). In pot experiment, DS and PEDC were recorded to determine the efficacy of Cu-chitosan NPs against CLS disease. A significant control of CLS disease was recorded on Cu-chitosan NPs treatments (0.04-0.16%) compared to others (Table 2). These Cu-chitosan NPs (0.08-0.12%) have previously been reported as very effective against early blight and *Fusarium* wilt of tomato (Saharan et al. 2015). The defense response of Cu-chitosan NPs might be due to direct activity like (a) through membrane destruction by electrostatic interaction of chitosan with microbial cell surface **(**Ing et al. 2012; Xing et al. 2015) and (b) positively charged NPs could bind to DNA/RNA which affects transcription and translation processes and inhibits fungal proliferation (Ing et al. 2012). Alternatively, indirect activity might be exerted through aroused plant immune response by enhanced activities of antioxidant and defense enzymes. Furthermore, we foresee that Cu-chitosan NPs releases Cu rapidly in acidic pH (Figure 1a) which is created upon fungal infection, and the released Cu may act weighty on the fungus (Saharan et al. 2015). Altogether Cu-chitosan NPs lead to abate *C. lunata* spreading and contributed resistance in maize plants against CLS disease. These NPs significantly enhanced seedling growth of tomato (Saharan et al. 2015) and maize by mobilizing reserved food through higher activities of α-amylase and protease (Saharan et al. 2016).

To take advantage of the growth promotory effect of Cu-chitosan NPs (as reported in our previous study), maize seeds were treated with Cu-chitosan NPs followed by foliar spray in pot experiment. Statistical analyses showed that Cu-chitosan NPs notably increased plant height, stem diameter, root length, root number and chlorophyll content (Figure 5). However, at higher concentrations of Cu-chitosan NPs (0.16%) and $CuSO_4$ (0.01%) treatment, chlorophyll content significantly decreased (Figure 5e-f). It has previously been proposed that accumulation of Cu interferes with chlorophyll biosynthesis and cause deficiency of Mg and Fe (Lidon and Henriques, 1991; Patsikka et al. 2002; Kupper et al. 2003). Concurrently, root length and root number were affected at higher concentrations of NPs (0.16%) and $CuSO_4$ (0.01%) (Figure 5c-d). In AAS analyses, we quantified Cu content in treated plant leaves and allied it with plant growth characters (Table 3; Figure 5). We disentangled that the trend of plant growth was virtually related to Cu content, and this is in line with our previous study (Saharan et al. 2016). The toxicity was envisaged only on chlorophyll content, root length and root number at $CuSO_4$ (0.01%) and 0.16% NPs treatments which could be endowed by elevated accumulation of Cu (28.5 and 24.1 µg/g, Table 3). The accumulated level of Cu in present study is more than toxic level of Cu in maize leaves, which is reported to be 20 µg/g (Borkert et al. 1998). Therefore, we expect that for conceivable plant growth, Cu uptake must be controlled to avoid its sudden exposure to plant cells (Saharan et al. 2016) which can be achieved by slow release of Cu from Cu-chitosan NPs (Figure 1b). Results in present study categorically suggest that enhanced activities of antioxidant and defense enzymes in Cu-chitosan NPs treatments correlate with plant growth and systemic acquired resistance in treated plants.

Conclusions

Demand for food and feed crop that is free from synthetic components has exponentially increased in recent times, mostly to avert toxic effects of synthetic components and evade development of resistance in pathogens. It is imperative that a new approach to strengthening innate plant immunity be adopted, to cope with mutating plant pathogens, reduce chemical use and promote sustained plant growth. Cu-chitosan NPs have been proved as promising plant protection and growth agents in past and recent studies. Their unique ability to sustain plant growth under disease conditions makes them very effective and usable agents. These bio-based nanomaterials could be pivotal to sustainable agriculture without harming the ecosystem. The synthesized NPs have immense potential to be commercially explored for agricultural use. Further research under field conditions is underway.

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Comparative Analysis of Biochemical and Physiological Responses of Maize Genotypes under Waterlogging Stress

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Introduction

Almost every plant requires water and oxygen for its growth, development and survival, but excess water is counterproductive. If a plant's root is submerged in water (waterlogging), oxygen uptake is hindered, which results in death of the plant. Waterlogging - a serious abiotic stress - causes destruction of plants in various physiological, biochemical, anatomical and metabolic changes such as; reduction of chlorophyll content, reduced uptake of nutrients from the soil, nitrogen deficiency, reduction in CO_2 assimilation rate, chlorosis and, most importantly, oxidative stress. Oxidative stress produces reactive oxygen species (ROS) causing the damage of protein, lipid, nucleic acid, even DNA, of the plant cell (Hasanuzzaman et al., 2017; Setter et al., 2009). The waterlogging stress conditions may be caused by excess precipitation, faulty irrigation, poor drainage, unpredicted rainfall and so on. Under waterlogging stress conditions, plants produce reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) etc. that cause damage, ultimately leading to cell death. Plants possess different self-defense mechanisms to cope with waterlogging (viz. formation of adventitious roots, increased production of ethylene etc.). The most effective adaptive mechanism is the production of enzymatic (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase etc.) and non-enzymatic antioxidants (tocopherols, carotenoids etc.) that scavenge ROS species (Phukan et al., 2015; Ashraf, 2012). In this study, the effect of waterlogging stress on maize was studied, highlighting the physiological damage and ROS scavenging enzymatic effects. The aim of the study was to screen six maize genotypes under waterlogging stress and demonstrate physiological changes and anti-oxidative defense in selected genotypes.

Materials and Methods

Six maize genotypes (CML 54 × CML 487, P18, CML 54, CML 486 × CML 487, CML 486 and CML 487) collected from the Plant Breeding Division of Bangladesh Agricultural Research Institute (BARI) were selected for the study. The seeds of selected maize genotypes were sown in the glass house during the rainy season (*Kharif*) and the experiment conducted in the Molecular Breeding Laboratory of BARI. Eight day old seedlings were transferred to hydroponic conditions using Hoagland solution (Hoagland and Arnon, 1950). During this period, the seedlings were maintained in the glass house at 22°C, pH 6.2 and 12 hours of light. The waterlogging stress-imposed leaf samples were collected at the 0, 2nd, 4th and 6th day of waterlogging stress treatment for further biochemical tests in the laboratory. All the chemicals used for the experiment were of analytical grade and obtained from Sigma Aldrich Chemical (St. Louis, Missouri, USA).

Determination of chlorophyll content and membrane damage

The canopy cover was measured with the aid of Green Seeker Handheld Crop Sensor, Trimble (Hunt, 2013). Chlorophyll content was measured by non-destructive method using chlorophyll meter SPAD-502 plus, Konica Minolta (Putra and Soni, 2017) and by destructive method in the laboratory using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The total chlorophyll content (chl a and chl b) was determined by measuring the absorbance of the homogenized leaf samples with the spectrophotometer at different nm (Lichtenthaler, 1987) and calculated using the equations proposed by Arnon, 1949. Lipid peroxidation in the roots was determined by a modified histochemical staining using Schiff's reagent

(Srivastava et al., 2014). The loss of plasma membrane integrity in the roots was measured by histochemical staining using Evan's blue solution with a slight modification (Schützendübel et al., 2001).

Protein extraction and quantification

The supernatant of leaf extract was collected and used for PAGE and other enzymatic assays. Protein in the crude extract was determined according to the Coomasie Brilliant Blue G-250 dye binding method (Bradford, 1976). The absorbance was recorded at 595 nm. Protein concentration was calculated with the reference of standard curve using BSA (Bovine Serum Albumin).

Determination of Enzymatic Activity

Catalase (CAT, EC: 1.11.1.6) activity was measured according to the Csiszár et al., 2007, method. Peroxidase (POD, EC: 1.11.1.7) activity was estimated according to Hemeda and Klein, 1990. Ascorbate peroxidase (APX, EC: 1.11.1.11) activity was assayed following the method of Nakano and Asada, 1981. Glutathione peroxidase (GPX, EC: 1.11.1.9) activity was measured as described by Elia et al., 2003. Changes in proteins having iso-enzymatic activity of the ROS scavenging enzymes (CAT, POD, APX, GPX) were studied using SDS-PAGE under non-reduced, non-denatured conditions at 4°C according to Laemmli, 1970. The reference methods were used with modifications where needed.

Statistical Analysis

Data generated from this study was analyzed by STATISTIX 10 software where needed, and following completely randomized design (CRD) with three replications. Means were separated by Duncan's Multiple Range Test (DMRT) and $P \leq 0.05$ was considered the significance level. The graphs were prepared in MS Excel, 2010. Mean values ± standard error (SE) was presented in graphs from at least three independent experiment.

Results

Membrane damage and photosynthetic pigments

Maize roots are the first to be damaged during waterlogging stress condition. Overproduction of ROS under stress causes lipid peroxidation, protein oxidation, enzyme inhibition and eventually leads to cell death (Gill and Tuteja, 2010). Root staining with Schiff's reagent showed intense pink/ red color in CML 54 genotype compared to other genotypes, suggesting lipid membrane damage due to production of ROS in O_2 deprived conditions. Root staining with Evan's blue showed intense blue color in CML 54 and CML 486, suggesting more cell membrane damage compared to other genotypes in waterlogging stress (Picture not shown). The extent of damage is negatively correlated with the synthesis of anti-oxidative enzymes and positively correlated with the synthesis of ROS. These studies are supported by previous studies (Hasanuzzaman et al., 2017; Jaiswal and Srivasta, 2016; Tang et al., 2010).

In CML54 × CML487, the canopy cover was decreased by 8%, 10% and 11.36% respectively with increased treatment of 2nd, 4th and 6th day of waterlogging, indicating the decrease of chlorophyll pigment due to deficiency of nitrogen caused by oxygen deprivation under waterlogging. In P18, the decrease of canopy cover was 7.5%, 11% and 9.37%; in CML 54, 16%, 13%, 30%; in CML486 × CML487, 5%, 11%, 18%; in CML 486, 26%, 9%, 23% and in CML 487, 8%, 6% and 3% respectively with the increase of waterlogging stress at 2nd, 4th and 6th day (Table 1).

Table 1. Canopy cover reading of six maize genotypes under waterlogging stress at 0, 2nd, 4th and 6th day.

Canopy Cover				
	Day 0	**Day 2**	**Day 4**	**Day 6**
CML 54 × CML487	0.53±0.00^{a}	0.49±0.01^{ab}	0.44±0.00^{a-d}	0.39±0.03^{b-e}
P18	0.40±0.06^{b-e}	0.37±0.04^{c-f}	0.33±0.02^{e-h}	0.29±0.01^{gh}
CML54	0.37±0.02^{c-f}	0.31±0.02^{e-h}	0.27±0.01^{e-h}	0.19±0.01^{h}
CML486 ×CML487	0.39±0.01^{b-e}	0.37±0.02^{c-f}	0.33±0.01^{d-h}	0.27±0.01^{f-h}
CML486	0.46±0.02^{a-c}	0.34±0.02^{d-g}	0.31±0.0$_{2}^{e-h}$	0.24±0.01^{gh}
CML487	0.39±0.01^{ab}	0.36±0.03^{c-f}	0.34±0.03^{e-h}	0.33±0.00^{gh}

Table 2. SPAD reading of six maize genotypes at 0, 2nd, 4th and 6th day of waterlogging stress condition.

Table 3. Effect of waterlogging stress on total chlorophyll content at 0, 2nd, 4th and 6th day of treatment.

SPAD				
	Day 0	**Day 2**	**Day 4**	**Day 6**
CML54 × CML487	36.55±0.58^{b-e}	34.15±0.31^{d-g}	29.98±0.20^{f-i}	26.08±0.98^{hi}
P18	34.18±0.71^{a}	33.53±0.59^{ab}	31.33±0.37^{bc}	27.53±0.25^{e-h}
CML54	32.60±0.46^{ab}	26.69± 0.20^{b-d}	23.85±0.25^{c-g}	18.30±0.11^{f-i}
CML486 × CML487	36.47±0.07^{ab}	27.45±0.78^{e-h}	25.30±0.20^{g-i}	21.88±0.91^{i}
CML486	32.10±0.12^{b-e}	27.75±0.43^{e-h}	22.43±0.36^{e-h}	18.03±0.95^{hi}
CML487	31.10±0.84^{b-f}	29.43±0.51^{e-h}	26.98±0.13^{g-i}	24.83±0.72^{j}

Total Chlorophyll				
	Day 0	**Day 2**	**Day 4**	**Day 6**
CML54 ×CML487	0.49±0.07^{a-c}	0.43±0.06^{bc}	0.39±0.04^{b-d}	0.36±0.04^{c}
P18	0.67±0.08^{a-d}	0.62±0.02^{a-e}	0.59±0.07^{cd}	0.57±0.009^{b-d}
CML 54	0.46±0.06^{a-c}	0.32±0.04^{a-d}	0.28±0.07^{c}	0.16±0.02^{d}
CML486×CML487	0.85±0.08^{ab}	0.70±0.07^{a-c}	0.56±0.02^{bc}	0.48±0.03^{a-d}
CML486	0.63±0.04^{a-d}	0.51±0.01^{ab}	0.44±0.05^{a-c}	0.32±0.06^{cd}
CML487	0.76±0.07^{a}	0.67±0.06^{d}	0.60±0.06^{b-d}	0.52±0.01^{a-d}

Decrease of SPAD value in CML 54 × CML 487 was 6.56%, 12%, 13%; in P18, 2%, 6% and 11%; in CML 54, 18%, 17% and 22%; in CML 486 × CML 487, 25%, 8%, 14%; in CML 486, 13.5%, 16% and 19% and in CML 487, the decreased value was 5.36%, 8.32% and 8% with increased waterlogging stress treatment of 2nd, 4th and 6th day respectively (Table 2).

In case of CML54 × CML487, the total chlorophyll content was decreased by 12%, 9% and 8% on the 2nd, 4th and 6th day compared to control respectively. In case of P18, the decrease rate was 8%, 5% and 3% with increased dose of waterlogging stress. In CML 54, the decrease rate was 30%, 12.5% and 43% respectively, and 18%, 20% and 14% in CML 486 × CML 487; 19%, 14% and 27.2% in CML 486 followed by 12%, 10% and 13% in CML 487 on the 2nd, 4th and 6th day of waterlogging stress condition (Table 3).

Activities of anti-oxidative enzymes

Maize seedlings exposed to waterlogging stress in different treatments had increased activity in some genotypes and decreased activity in others. CML 54 × CML 487 had increased POD activity of 31%, 1.21% and 5% on the 2nd, 4th and 6th day, respectively, compared to control (0 day). In P18, the activity increased

7% and 12% on the 2nd and 4th day, respectively, but the activity had decreased 10% on the 6th day. In CML 54, the POD activity increased 21% on day 2 and then gradually decreased at 12% and 4% on the 4th and 6th day respectively. In CML486 × CML487, the POD activity increased at 45% and 22% on the 2nd and 4th day, then decreased at 25% on the 6th day. In case of CML 486, the POD activity increased by 38% on the 2nd day then gradually decreased to 11% and 3% on the 4th and 6th day respectively. In CML 487, POD activity significantly increased at 19%, 7.35% and 14% on the 2nd, 4th and 6th day of treatment (Figure 1).

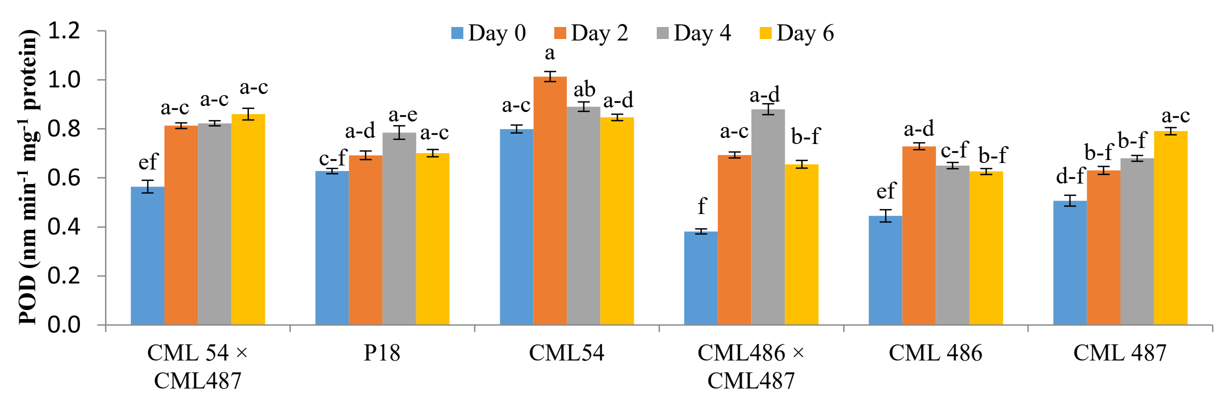

Figure 1. Activities of POD enzyme of six maize genotypes on 0, 2nd, 4th and 6th day of waterlogging stress. Values represent the mean ± SE from three independent experiments. Bars with the same letters are not significantly different at $P \leq 0.05$

The APX activity of CML54 × CML487 after waterlogging treatment significantly increased at 42%, 14% and 13% on the 2nd, 4th and 6th day respectively. In P18, the APX activity increased significantly at 9%, 15% and 3% on the 2nd, 4th and 6th day respectively. In case of CML 54, the APX activity increased 38% on the 2nd day then decreased significantly by 22% and 13% on the 4th and 6th day. In CML 486 × CML 487, activity increased by 47% and 39% on the 2nd and 4th day of treatment but decreased 22% with increased level of treatment on the 6th day. In CML 486, the value increased 30% on the 2nd day and decreased 24% and 6% on the 4th and 6th day of treatment. In case of CML 487, the value increased 35% on the 2nd day but decreased 16% and 9% on the 4th and 6th day respectively (Figure 2). The activities were calculated by comparing with the control (Day 0).

In CML54 × CML487, the GPX activity increased 19%, 1.6% and 1.28% on the 2nd, 4th and 6th day respectively. In P18, the activity increased at 6%, 5% and 6.45% in 2nd, 4th and 6th day of treatment respectively. In case of CML 54, the activity increased at 1% and 18% on the 2nd and 4th day of treatment but decreased 1.28% on the 6th day of treatment. In CML486 × CML487, the activity increased at 12%, 1.41% and 2.20% on the 2nd, 4th and 6th day. In case of CML 486, the activity increased on the 2nd and 6th day by 17% and 12% but decreased 19% at 4th day of treatment. In CML 487, the GPX activity significantly increased by 19%, 4% and 8% with the increasing dose of treatment, respectively (Figure 3).

The CAT activity of CML 54 × CML 487 after waterlogging stress treatment increased significantly by 6%, 10% and 4% on the 2nd, 4th and 6th day respectively, compared to control (0 day). In P18, the CAT activity increased significantly by 11%, 6% and 9% with increased treatment. In CML 54, the CAT activity increased by 2%, 23% and 10% with the increase of treatment. In CML486 × CML487, the activity increased 7% and 29% at 2nd and 6th day but decreased at 4th day by 22%. In CML 486, the activity increased by 13% and 14% on the 2nd and 4th day but decreased slightly to 4% on the 6th day of treatment. In case of CML 487, the activity decreased 4% and 3% on the 2nd and 6th day but increased 40% on the 4th day with increased treatment (Figure 4).

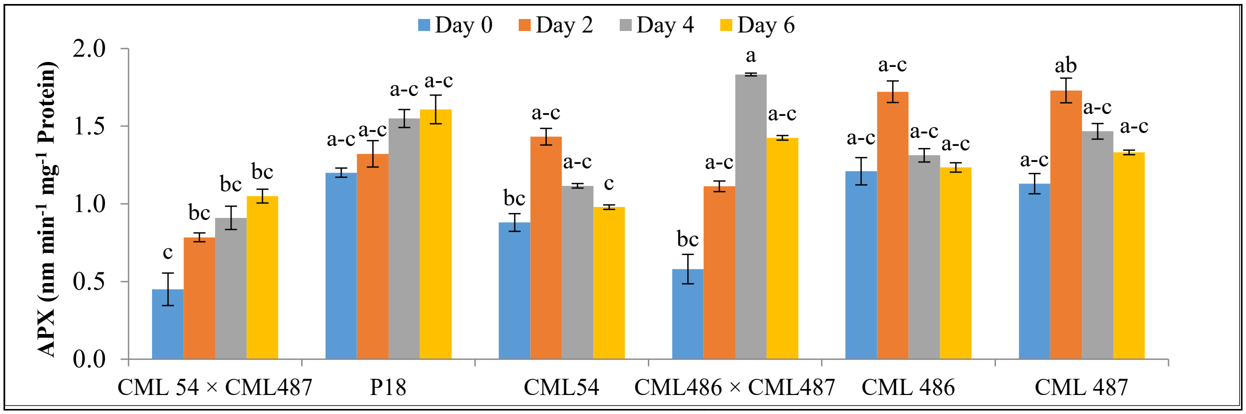

Figure 2. APX enzyme activity of six genotypes on 0, 2nd, 4th and 6th day of waterlogging stress conditions. Values represent the mean ± SE from three independent experiments. Bars with the same letters are not significantly different at P≤0.05.

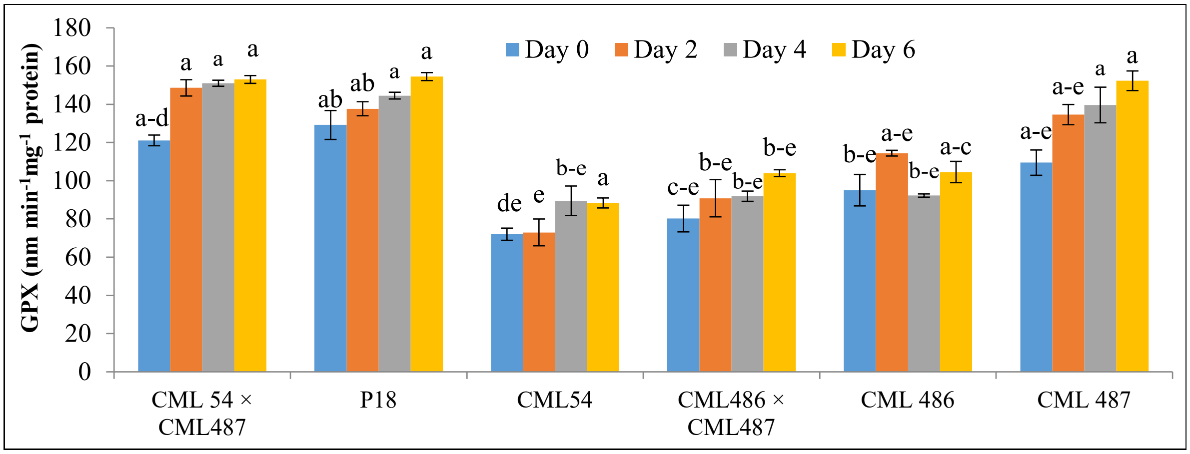

Figure 3. Activity of GPX enzyme in 0, 2nd, 4th and 6th day of waterlogging stress conditions. Values represent mean ± SE from three independent experiments. Bars with the same letters are not significantly different at P≤0.05.

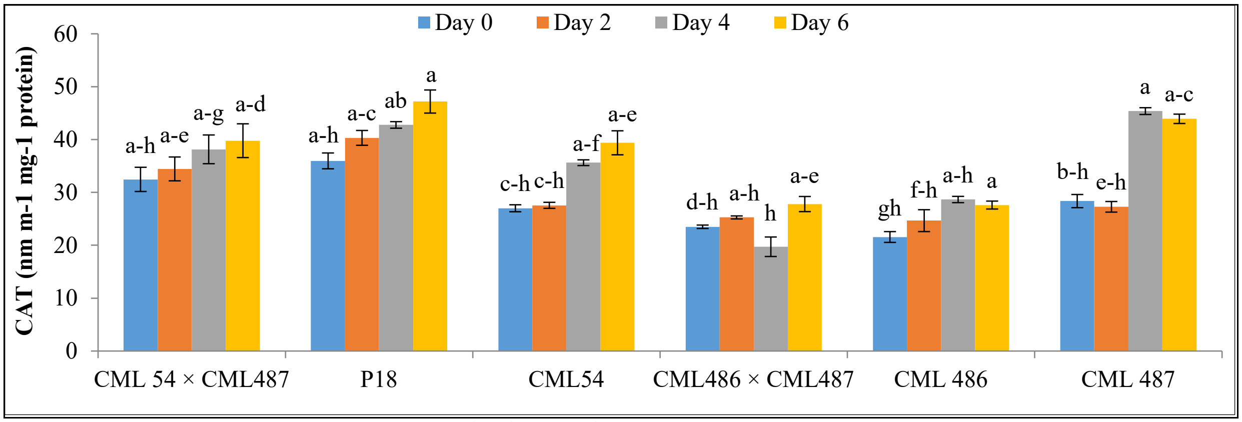

Figure 4. CAT enzyme activity on 0, 2nd, 4th and 6th day of waterlogging stress conditions. Values represent the mean ± SE from three independent experiments. Bars with the same letters are not significantly different at P≤0.05.

Discussion

In this study, N_2 and chlorophyll content decreased significantly with increase of waterlogging stress doses (2nd, 4th and 6th day of treatment), followed by N_2 deficiency. The reduction rate was more in waterlogging stress susceptible genotypes than in tolerant genotypes. CML 54 × CML 487, P18 and CML 487 showed less reduced canopy cover suggesting less reduced N_2 uptake followed by less reduced chlorophyll contents. These results are as reported in pigeon pea (Bansal and Srivastava, 2015) and maize (Wang et al., 2011).

The POD activity of the maize seedlings increased in CML 54 × CML 487 and CML 487. In case of P18 and CML 486 × CML 487, the activity of POD increased from 0 to 4th day, and then decreased. These results are consistent with previous studies (Hasanuzzaman et al., 2017; Tang et al., 2010). CAT activity was also found to be higher in genotypes that survived better under waterlogging stress. Immediately after waterlogging, increased CAT activity is linked with efficient detoxification of H_2O_2, conferring waterlogging resistance in maize. Three genotypes (viz. CML 54 × CML 487, P18 and CML 54) from our study showed increased CAT activity under waterlogging stress. In other three genotypes there was uneven regulation of CAT activity. These results are consistent with previous findings (Mubeen. et al., 2017; Phukan et al., 2016; Sairam et al., 2009; Zhang et al., 2007). CAT and APX are known to be the most important anti-oxidative enzymes performing in scavenging ROS species under waterlogging stress (Tang et al., 2010). The effect of APX activity has also been examined under waterlogging stress in maize seedlings. APX activity increased in CML 54 × CML 487 and P18. In rest of the genotypes the APX activity increased on the 2nd day but significantly decreased on the 4th and 6th day. The results are consistent with previous studies (Chugh et al., 2011; Sairam et al., 2009).

Finally, the activity of GPX in maize seedlings under waterlogging stress was determined for the first time. No previous studies investigated the GPX effect under waterlogging stress in maize. The binding of different xenobiotics and their electrophilic metabolites are known to produce less toxic and water-soluble conjugates to protect plants from oxidative stress, which is aided by the enzymes GPX and GST together (Gill and Tuteja, 2010). In this study, the increased activity of GPX was found in the genotypes CML 54 × CML 487, P18 and CML 487. In CML 54, CML 486 × CML 487 and CML 486, there was uneven regulation of GPX enzyme activity under waterlogging stress conditions.

The study, in total, suggests that among the six genotypes, CML 54 × CML 487, P18 and CML 487 showed the best performance against ROS species under waterlogging stress conditions. These genotypes possess better ROS scavenging activity during oxygen deprivation, and an increased survival rate. Other three genotypes (CML 54, CML 486 and CML 486 × CML 487) showed less effective results collectively (CAT, POD, APX and GPX) because in some cases there was uneven up- and down-regulation of the ROS scavenging enzymatic activities. SDS-PAGE gel electrophoresis was performed to confirm the result. In gel electrophoresis activity, two isoforms of CAT, three isoforms of POD, three isoforms of GPX and four isoforms of APX were found, which supported the result and was confirmed by the intesity of band in the gels. The more intense the band color, the more the anti-oxidative enzymatic expression. In this study, three isoforms of POD enzyme were found, a rare occurrence under waterlogging stress conditions. The genotypes (viz. CML 54 and CML 487) showing less anti-oxidative enzyme activity showed intense color in root staining, indicating excess lipid peroxidation and plasma membrane damage. In tolerant genotypes (viz. CML 54×CML 487, P18 and CML 487), higher anti-oxidative enzyme activity reduced H_2O_2 production, lipid peroxidation and membrane damage, resulting in their survival under waterlogging stress conditions.

Conclusion

From the study, it can be concluded that waterlogging tolerant maize seedlings possess higher ROS scavanging activities due to higher activities of anti-oxidative enzymes. The aforementioned enzymes are known to be the anti-oxidative enzymes which scavange ROS during oxidative stress. In this study, CML 54 × CML 487, P18 and CML 487 genotypes are noted to be the more effective genotypes while CML 54, CML 486 × CML 487 and CML 486 were less effective under stress. So, these ROS effective genotypes against waterlogging stress are going to be the target for Asian farmers in this era of climatic change and disaster. In this study, the GPX activity in waterlogging stress has been discussed for the first time in case of maize. So, further investigation is needed to prove the result. In POD enzyme gel electrophoresis, three POD isotypes were found whereas two isotypes are usually found. Further study is needed regarding POD enzyme activity under waterlogging stress conditions.

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Identification of Quantitative Trait Loci for Resistance to Shoot Fly in Maize

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Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the tropics and subtropics because of its variability and adaptation to varied environmental conditions. It ranks third in the world among cereals, after wheat and rice, in terms of area and production. In India, it is cultivated over an area of 9.63 million hectares with annual production of 25.90 million metric tons and average productivity of 2.69 metric tons per hectare (www.indiastat.com). Due to expansion of maize cultivation to newer areas and changing climate scenarios, ubiquitous prevalence of diseases and insect pests at the pre-harvest stage are prominent factors affecting productivity. Among 130 insect pests that affect maize crop, about two dozen (stem borers, shoot fly, armyworm, jassids, thrips, white ants, earworms, grasshoppers, leaf miners etc.) cause severe damage (Meihls et al. 2012; Siddiqui and Marwaha 1994). In North India, shoot fly (*Atherigona naqvii*) is the key pest that heavily damages spring maize crop at seedling stage and causes significant loss in yield, sometimes as high as 60% (Atwal 1976). The infestation of shoot fly in maize was first reported in 1925. Females are attracted both to the volatiles emitted by susceptible seedlings and to phototactic (optical) stimuli that facilitate orientation to the host for oviposition (Nwanze et al. 1998). It was reported that the female fly lays minute, rice grain shaped eggs - singly or in small groups - on the stem above the ground, in cracks and crevices around the plants in the soil and/or on the undersurface of the cotyledonary or first leaf of young seedlings (Sandhu and Kaushal 1976). After an incubation period of 1 to 3 days, the maggot - after hatching - slowly moves downwards, enters the central shoot by puncturing it from the lateral side and feeds on the growing point causing a typical damage named dead heart (Barry 1972; Kundu and Kishore 1970). Their infestation withers the central shoot of seedlings; hence, dead heart symptom appears. Cloudy weather favours the multiplication of this insect and it is believed that infestation is also higher in irrigated fields. Currently, the main preventive method for shoot fly is the use of pesticides such as seed treatment with Imidacloprid @ 6ml/kg of seeds. Early sowing during first fortnight of February also avoids build-up of shoot fly population. However, the intensive and indiscriminate use of chemicals leads to environmental pollution, kills natural enemies of the target pest, and may result in development of shoot fly populations that are resistant/tolerant to insecticides, ultimately leading to resurgence of shoot fly populations.

Identifying the relative resistance of maize against the shoot fly is one of the options to increase crop productivity. In India, about 2000 maize germplasm lines have so far been screened against *Atherigona* species and several resistant sources, from low to moderate levels of resistance, identified (Jindal et al. 2007; Kumar and Kanta 2012; Panwar 2005; Siddiqui et al. 1988). However, the genetics of shoot fly resistance in maize has not been investigated in detail, therefore no known source of cultivated maize accession is reported to confer absolute resistance to shoot fly. Host plant resistance can be broken down into three categories: antixenosis, antibiosis, and tolerance. The antixenosis for oviposition, i.e. non-preference of host plants, was not observed in maize genotypes to shoot fly and the resistance is mainly dependent on the degree of inherent tolerance, i.e. ability of plant to recover from injury (Jindal et al. 2007, Jindal 2013). Plant resistance to *Atherigona* spp. is a complex character and it depends on the interplay of several componential characters.

To develop crop cultivars with durable resistance to insect pests, it is important to identify germplasm with diverse combinations of factors associated with resistance to the target pests and then combine identified components of resistance in the same genetic background. Studies on shoot fly resistance in sorghum suggested that component traits for resistance are complex and quantitatively inherited (Hallali et al. 1983), with predominantly additive gene effects (Nimbalkar and Bapat 1992). Past studies established the polygenic nature of maize resistance to insect pests in general, and stem borer and storage pest resistance in particular, which were found to have low to moderate heritability values (Barros et al. 2011; Bergvinson 1999; Kim and Kossou 2003; Sandoya et al. 2010). Since the development of molecular markers (SSR, InDel, SNPs) and functional genomics, the genetic studies of shoot fly resistance in maize could be investigated. DNA marker-assisted breeding for a range of traits (particularly to overcome diseases and pests) has become one of the most important applications of biotechnology in recent times. A few molecular studies are reported for shoot fly resistance in sorghum that identified QTLs associated with resistance to shoot fly (Apotikar et al. 2011; Satish et al. 2009). Therefore, a detailed study of the underlying genetic basis of resistance to shoot fly in maize by using appropriate breeding material is the need of the hour, before formulating more effective breeding strategies. Thus, the present study aims to investigate the genetics and mapping of different component traits of shoot fly resistance in maize.

Materials and Methods

Plant material

The experimental material consisted of two parental inbred lines *viz.* CM143 and CM144 and 107 F_2 individuals & $F_{2:3}$ families. The CM143 (resistant) had good vigor and highly glossy and erect leaves, whereas CM144 (susceptible) had poor vigor, and dark green and drooping leaves. CM143 and CM144 are the parental lines of hybrid JH3459 which is widely grown in north western plains of India. The 107 $F_{2:3}$ families along with the two parents (CM143 and CM144) were raised in randomized block design in two replications at Punjab Agricultural University, Ludhiana. Each $F_{2:3}$ families were planted in one row of three meters (15 plants) in two replications. The plants were sown with a plant-to-plant and row-to-row distance of 20 cm and 60 cm, respectively. Standard agronomical practices were followed for raising the crop.

Evaluation of $F_{2:3}$ families against shoot fly attack

The fish-meal technique was used for increasing shoot fly abundance under field conditions. The moistened fish-meal was applied @50 g/m^2 one day after seedling emergence by broadcasting in the field to screen maize germplasms against *A. naqvii* (Jindal et al. 2007). The data on shoot fly infestation was recorded from ten plants of each $F_{2:3}$ family at different time intervals for different traits. Phenotypic data was recorded on various component traits, *viz.*, oviposition, leaf injury, dead heart, leaf surface glossiness, seedling vigor, plumule and leaf sheath pigmentation, leaf wetness, leaf length, leaf width, leaf area and stem girth. Ovipositional count was recorded by counting the total number of eggs laid on ten plants at random from each $F_{2:3}$ family. The mean number of eggs per plant was calculated on 5, 10 and 15 DAE (days after emergence). The mean value of leaf injury and dead heart percent was calculated as number of plants with leaf injury or dead hearts / total number of plants × 100 at 7, 14 and 21 DAE. The average values of leaf injury and dead heart percent recorded on 21 DAE were used for QTL identification. Leaf glossiness was visually scored on a scale of 1-5 at 5th leaf stage [1 = highly glossy (shining, light green, narrow and erect leaves) and 5 = non-glossy (dark green, dull, broad and drooping leaves)]. Seedling vigor was rated at 5th leaf stage on a 1-5 scale, where 1 = highly vigorous (plants showing maximum height, leaf expansion and robustness) and 5= poor seedling vigor (plants showing minimum growth, less leaf expansion). Pigmentation and leaf wetness were also recorded on 1-5 scale. The stem girth (cm) was recorded from seedling at 5th leaf stage. The mean diameter (d) of the seedling was recorded using vernier caliper.

Genotyping of F_2 individuals

Genomic DNA was isolated from young seedlings of 107 F_2 individuals along with both parents (CM143 and CM144) using the standard CTAB procedure (Murray and Thompson 1980). *In vitro* amplification using polymerase chain reaction (PCR) was performed in a 96-well microplate in an Eppendorf master cycler in 10 µl reaction volume. The PCR reaction mixture consisted of 50ng of DNA template, 0.5 µM of each of the relevant primers, 1.5mM $MgCl_2$, 0.2mM dNTPs, one unit of Homemade Taq polymerase, and 5X PCR buffer. PCR was performed following a profile of initial denaturation at 94^{0}C for five minutes, followed by 35 cycles of denaturation at 94^{0}C for one minute, annealing at 55-60^{0}C for two minutes, and extension at 72^{0}C for two minutes. The final extension was carried out at 72^{0}C for seven minutes. The 10 µl PCR products were resolved in 3% 0.5X TBE agarose gel and the bands were visualized under the UVP gel documentation system. For parental polymorphism survey, SSR (simple sequence repeats) markers were selected from maize database, covering all regions of 10 linkage groups spanning all bins. A total of 701 SSR markers were screened for parental polymorphism. Resulting 125 polymorphic SSR markers were then genotyped on F_2 population.

Data analysis

Critical difference (CD) and coefficient of variance (CV) were calculated using CPCS1 software (Cheema and Singh 1991). PROC CORR was used to estimate the Pearson correlation coefficients between different component traits. Square root transformation was applied to count data, i.e score for various traits, and arc sin square root transformation was applied to percentage data, *i.e* dead heart count and leaf injury count (Little and Hill 1978). Mapdisto version 1.7.7 (Lorieux 2007) was used to construct the linkage map that gave representation in the form of a graphic to mark the position of genes within a linkage group, with a threshold value of LOD score of 3.0 and recombination fraction of 0.3. The QTLs were identified by composite interval mapping (CIM) using Windows QTL cartographer version 2.5 (Basten et al. 2005). Interval mapping scanned QTL at every 2.0 cM and each point calculated a maximum likelihood QTL map. For better accuracy, forward and backward regression method was selected as the parameter for analysis. Threshold LOD score was calculated using permutation tests (1,000 permutations in each case) with 5% level of significance. Putative QTL were designated by the corresponding chromosome bin in which they were found.

Results and Discussion

Phenotypic data analysis

Plant resistance to *Atherigona* spp. is a complex character that depends on the interplay of number of componential characters (Jindal et al. 2015). It is therefore important to identify genotypes with different mechanisms of host plant resistance, increase the levels and diversify the bases of resistance to shoot fly. Plant morphology can have a strong impact on shoot fly population dynamics, especially in case of seedling characteristics that physically reduce feeding oviposition and shelter. The phenotypic trait means of the parents CM143 and CM144 and their $F_{2:3}$ families for various component traits are presented in Table 1. Differences between the parental lines were highly significant for all component traits. A wide range in each component trait expression among the $F_{2:3}$ families was observed. The frequency distribution of data revealed that the response of $F_{2:3}$ families for various component traits fitted into a normal curve. In the present investigation, traits like leaf length, leaf width, leaf area and stem girth were found to be negatively associated with resistance and showing positive additive effects, thus deciphering that the contribution of phenotypic variation among the lines is due to the susceptible parent i.e. CM144. Similarly, it was reported that the leaf length, width, area and stem girth were more in susceptible maize genotypes possibly related to susceptibility to shoot fly (Jindal 2013). It was also reported that the leaf surface wetness is associated with shoot fly resistance in sorghum as it reduces the movement of freshly hatched *A. soccata* larvae through the leaf sheath to the growing part (Nwanze et al. 1990).

Table 1. Mean values of parents and $F_{2:3}$ families for various morphological characters after shoot fly infestation

Parents/	Seedling	Leaf	Plumule and leaf	Leaf	Leaf	Leaf	Leaf	Stem

Population	vigor	glossiness	sheath pigmentation	wetness	length (cm)	width (cm)	area (cm^2)	girth (cm)
CM143	1.5	2	1	2	11.56	1.69	19.53	2.07
CM144	4.25	4.5	4	4.5	13.04	1.87	24.38	1.76
$F_{2:3}$ families	2.182	2.706	1.659	3.28	12.001	1.711	20.53	1.98

The egg count of $F_{2:3}$ families (10 plants of each family) after 5, 10 and 15 DAE fall within the range of 0-11.0, 10.0-25.0 and 17.0-43.0 respectively. Resistant parent CM143 had mean egg count of 6.0, 13.0 and 20.0, whereas susceptible parent CM144 had an average egg count of 9.5, 21.0 and 38.50 at 5, 10 and 15 DAE. It was observed that insect attack severity progresses with time as indicated from leaf injury and dead heart (Figure 1). The dead heart count at different time intervals revealed that the resistance present in CM143 is partial, *i.e* controlled by polygenes in additive manner along with modifier genes. This indicated that although there was complete expression of insect attack at 21 DAE, progress is slow up to certain period when favorable conditions are available. Additionally, inherent mechanisms of tolerance of plants drive the reaction either towards resistance or susceptibility. Dead heart accounted for the maximum CV of 31.97 with critical difference of 10.734 at 5% level of significance and leaf length showed least CV of 7.54, whereas non-significant differences between replications had been observed for all the component traits (Table 2). This indicated that leaf injury and dead heart are the major contributors to disease reaction and are directly associated with the oviposition of shoot fly attack.

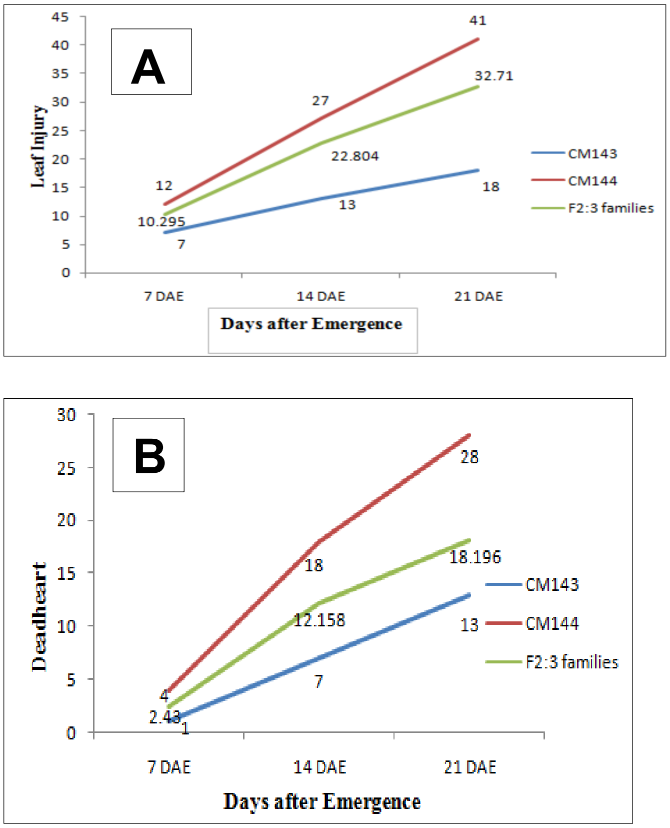

Figure 1. Leaf injury (A) and dead heart (B) progress curve for parental lines and of $F_{2:3}$ families at 7, 14 and 21 DAE.

Table 2. Critical difference and coefficient of variance among component traits.

Component Trait	C D	CV

Oviposition	5.186	9.3
Leaf injury	12.492	19.24
Dead heart	10.734	31.97
Seedling Vigor	1.333	30.78
Leaf Glossiness	1.028	19.15
Plumule & leaf sheath pigmentation	1.001	30.22
Leaf Wetness	1.094	16.82
Leaf length	2.098	7.54
Leaf width	0.379	10.56
Leaf area	8.157	15.64
Stem girth	4.76	11.54

Table 3. Correlation among various component traits.

Component Trait	% Dead heart	Seedling vigor	Leaf glossiness	Leaf wetness	Pigmentation	Leaf length	Leaf width	Leaf area	Stem girth	Oviposition
% Leaf injury	0.7924*	0.0183	0.2673	0.1777	0.0498	0.1646	0.2101	0.1963	00.2588	0.86279*
% Dead heart		0.0759	0.2889	0.2029	0.1008	0.1666	0.1718	0.1694	00.1832	0.94254*
Seedling vigor			0.2566	0.2524	0.0537	-0.19	-0.175	-0.205	-0.195	0.08948
Leaf glossiness				0.578*	0.1101	0.1101	0.0535	0.0581	0.0267	0.31321
Leaf wetness					-0.0644	0.1716	0.0249	0.0951	-0.03	0.17733
Pigmentation						-0.119	-0.236	-0.183	0.0183	0.11776
Leaf length							0.7303*	0.9224*	0.3589	0.10442
Leaf width								0.9252*	0.3756	0.14312
Leaf area									0.373	0.12609
Stem girth										0.20701

Construction of linkage map

A total of 701 SSR markers were surveyed between parents for polymorphism survey and 228 SSR markers were polymorphic, showing overall 32.52% polymorphism. A total of 125 polymorphic SSR markers were genotyped on mapping population. Five of the markers showed either a CM143 or CM144 type banding pattern in the whole population. This might be due to the presence of blocks of regions from either parent lacking recombination in that region, known as 'cold spot regions'. Similarly, distortion in the banding pattern in rice was observed while mapping *xa8* gene (Vikal et al. 2014). The genetic linkage map constructed had genomic coverage of 1211.16 cM. All the markers belonging to one chromosome grouped together as reported for maize chromosomes in maize database. Chromosome 8 had the smallest length of 62.56 cM because of few polymorphic markers. The longest map length was of chromosome 1 (262.10 cM). The average genetic distance between markers was 10.50 cM. In maize for different populations several linkage maps had been constructed with varied map length and marker intervals (Castro-Álvarez et al. 2015; Lennon et al. 2017).

QTL mapping

Putative QTL associated with various component traits with shoot fly resistance were detected on chromosome 1, 2, 4 and 9. A total of 18 QTLs were detected for shoot fly resistance component traits under study (Table 4). Four QTLs for leaf width were identified on chromosomes 1, 2 and 4, explaining 38.68% phenotypic variation among $F_{2:3}$ families. Two QTLs (present on chromosome 1 and 2) each for leaf length and leaf area overlapped, indicating both traits are highly correlated (Figure 2). The QTLs for leaf injury and leaf glossiness were detected in different genomic regions of chromosome 1, accounting for 11.96 and 12.98% phenotypic variation respectively. The QTL of seedling vigor was co-localized with leaf surface wetness on chromosome 2. The QTL for dead heart (*qDH9.1*) was flanked by marker interval of *bnlg127* and *umc1258,* explaining 15.03% phenotypic variance. This putative QTL was co-localized with oviposition accounting for 18.89% of phenotypic variance (Figure 3). Apotikar et al. (2011) also reported the co-localization of oviposition and dead heart QTL in sorghum. No epistatic interactions were found between any of the QTLs.

Table 4. Marker intervals showing putative QTLs for shoot fly resistance component traits in F_2 population from cross of CM143 x CM144

Component Trait	**QTL**	**Marker interval**	**Chromo-some**	**LOD score**	**Phenotypic variance (%)**	**Additive effect**
Leaf width	*qwidth1.1*	*bnlg1803-bnlg1083*	1	3.68	9.32	0.0526
	qwidth1.2	*umc1568-umc1073*	1	4.27	9.94	0.0595
	qwidth2.1	*bnlg1092-bnlg1338*	2	2.65	8.16	0.05
	qwidth4.1	*umc1940-umc2290*	4	3.18	11.26	-0.0457
Leaf length	*qlength1.1*	*bnlg1178-bnlg1614*	1	4.67	4.25	0.1162
	qlength2.1	*bnlg1887-umc1233*	2	2.94	10.85	-0.1465
Leaf area	*qarea1.1*	*bnlg1178-bnlg1803*	1	6.28	8.48	0.3731
	qarea2.1	*bnlg1887-umc1233*	2	2.55	9.82	-0.3392
Leaf injury	*qLI1.1*	*bnlg1083-umc1568*	1	3.14	11.96	0.1143
Leaf surface wetness	*qLW2.1*	*bnlg2277-bnlg2248*	2	3.22	7.30	-0.018
Leaf glossiness	*qgloss1.1*	*umc1431-bnlg100*	1	2.62	12.98	0.0501
Plumule and leaf sheath pigmentation	*qpigm4.1*	*bnlg1621b-bnlg1784*	4	3.08	7.58	0.1207
Seedling vigour	*qSV2.1*	*bnlg2277-bnlg2248*	2	2.73	11.13	-0.0483
Oviposition	*qEC9.1*	*bnlg127-umc1258*	9	4.09	18.49	-2.881
Dead heart	*qDH9.1*	*bnlg127-umc1258*	9	3.49	15.03	-0.0393
Stem girth	*qgirth9.1*	*bnlg1012-umc1078*	9	2.65	7.26	-0.1722
	qgirth9.2	*umc1657-umc1789*	9	4.27	1.05	-0.1118
	qgirth4.1	*bnlg1784-bnlg1189*	4	3.47	9.89	-0.067

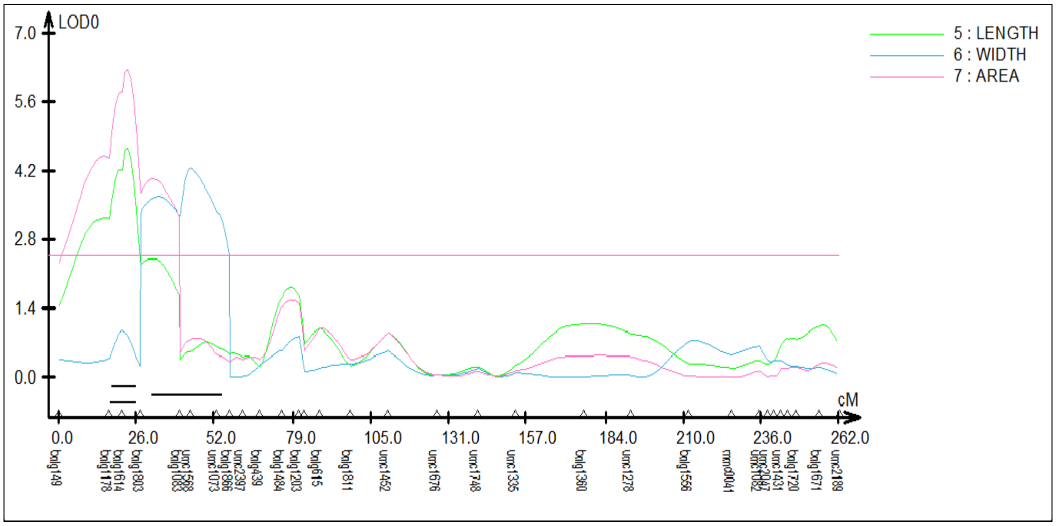

Figure 2. QTL Cartographer plot showing QTL peaks as obtained using composite interval mapping on chromosome 1 for leaf length, leaf width and leaf area.

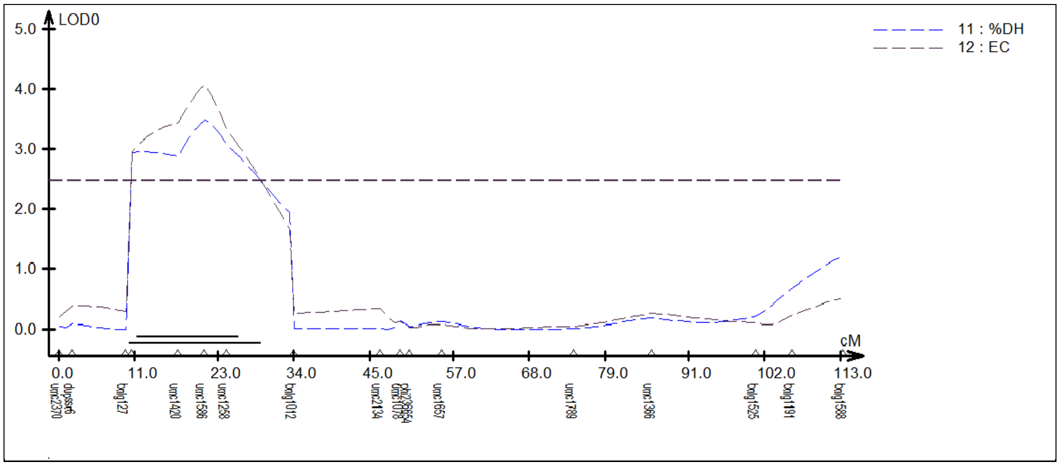

Figure 3. QTL Cartographer plot showing QTL peaks as obtained using composite interval mapping on chromosome 9 for oviposition and dead heart per cent at 21DAE.

In most of the studies, resistance alleles at a QTL are inherited from the resistant parent (Klump et al. 2011), except for a few QTLs harboring susceptible alleles from the susceptible parent (Balint-Kurti et al. 2008). This is consistent with the results reported here stating that eight of the QTLs possessed resistance alleles from CM143, whereas ten of the QTLs had susceptible alleles from CM144. No report was available on the QTL mapping of shoot fly (*Atherigona naqvii*) resistance in maize; only those of sorghum on shoot fly (*Atherigona soccata*) resistance were. Satish et al. (2009) identified a major QTL interval *Xtxp65-Xtxp30* for leaf glossiness accounting for 14% of phenotypic variance. This genomic region is syntenic to maize chromosome 4 (4.08/4.09) and we got three major QTLs near this region accounting for leaf width, stem girth and pigmentation. Two other major QTLs accounting for dead heart between markers *Xnhsbm1044-Xnhsbm1013* and *Xnhsbm1033-Xcup16* - explaining 15.0% and 11.4% phenotypic variance respectively - were detected in sorghum. These regions were found to be syntenic to bins 9.02-9.03 of maize chromosome 9 on which QTL for oviposition and dead heart were localized, indicating the same gene block may be responsible for shoot fly resistance. Additionally, *gl15* (*glossy 15*) gene was identified as a candidate for insect resistance gene present on 9.03/9.04 bin of chromosome 9 in maize (Brooks et al. 2005; Williams et al. 2000). So, this region could be further dissected to identify the candidate gene for shoot fly resistance in maize. Co-localization of QTL for different traits might have resulted from either tight linkage of several genes (Sandhu et al. 2001), i.e. cluster of genes present in the form of multigene family controlling different traits, or the pleiotropic effect of a gene (Veldboom et al. 1994; Xiao et al. 1996). Badji et al. (2018) highlighted the existence of combined-insect resistance genomic regions in maize based on meta QTL analysis and set the basis for multiple-pest resistance breeding.

Conclusion

It is noteworthy that, to date, all published QTL mapping of maize insect resistance has involved stem borers and chewing insects. Therefore, the results of the present study are novel as they constitute a major step toward identification of genomic regions associated with shoot fly resistance. Major component traits that may be used for screening resistance against shoot fly are dead heart, oviposition, leaf injury, stem girth and seedling vigor. We identified co-localization of QTL for dead heart (*qDH9.1*) and oviposition *qEC9.1,* indicating that these traits are interdependent. The identified region of the QTL needs to be saturated with an additional set of markers. This will further help in increasing precision about the variation explained by the QTL. Also, the identification of pleiotropic QTL can help in improvement of more than one trait at a time using the same linked markers.

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Evolution of Maize Value Chains and Ex-ante Assessment of GM Crops in India

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Introduction

Maize production in India has increased from around 15 million tons (mt) in the early 2000s to 26.88 mt in 2017/ 2018 (GoI, 2018). India's share in global production is only around 2.5%, and present national productivity is very low as compared to the world average. However, some districts in the country have an average productivity of 10 t/ha, which is quite high. The country has exported 705,513.8 MT of maize to the world worth Rs. 1228.5 crores/ USD 190.3 million in 2017/ 2018 (APEDA). Major export destinations were Nepal, Bangladesh, Philippines, Myanmar and Sri Lanka.

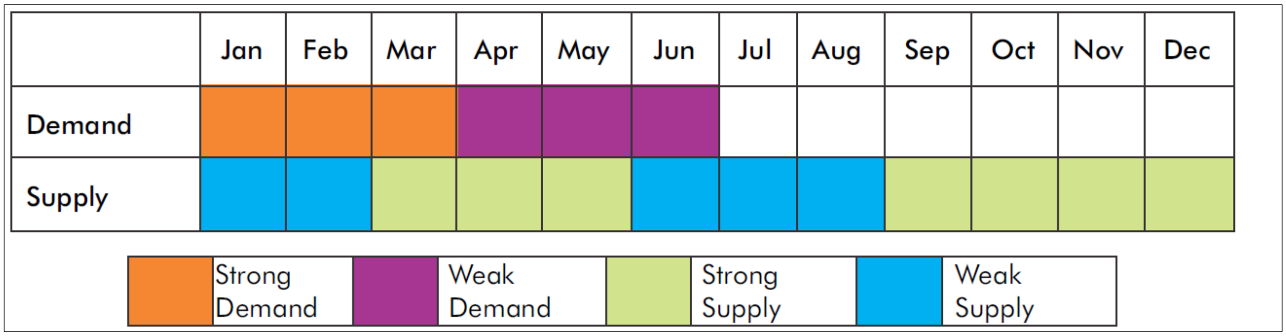
Every part of the maize plant has economic value; the grains, leaves, stalks, tassels and cobs can all be used to produce a variety of food and feed products. Not only have production and consumption of maize been consistently rising, but the consumption pattern has also changed over the years (Kumar et al., 2012). Maize is an excellent crop for biomass production. Maize straw is used as animal fodder and, as far as quality, is considered better than many other non-legume cultivated fodders. In peri-urban regions, particularly around highly populated cities, baby corn has emerged as a good source of income for farmers, and as a good quality green fodder during an otherwise lean season (Chaudhary et al., 2012).

Based on diverse range of end-products, the maize market can be grouped into three segments: poultry and cattle feed, processed products for industrial use (starch and ethanol) and human consumption. The total domestic demand for maize in India was 24 mt during FY 2016 to 2017. Poultry feed, industrial starch and ethanol production took up 13.5 mt, 1.8 mt and 1.2 mt of maize demand respectively. Per caput direct consumption of maize in rural areas has drastically reduced - from 3.7 kg per annum in 2004/ 2005 to 2.4 kg in 2009/ 2010 - while in urban areas, it remains very low at 0.3 kg per annum. If this trend continues, the direct demand for maize for human consumption would reduce to 6-7% from the current 10% by the year 2020 (Ranjit et al, 2014). As per FICCI and PWC (2018) estimates, requirements may increase to about 45 mt by the year 2022. This is in sharp contrast with the demand estimation of about 26.77 mt (Ranjit et al, 2014).

With the analysis of scenarios of maize demand and supply, few questions emerge: can the maize production, with its current pace, cater to increasing demand throughout the year? Can the value chain in its present form cater for these demand and supply scenarios? These questions arise because maize value chain depends on its production and demand outlook. Both have significant impact on efficiency of value chains.

Can the maize production, with its current pace, cater to increasing demand throughout the year?

The Government consistently tries to boost maize production through minimum support price (MSP) mechanism. The MSP intervention and technological breakthrough improved maize availability throughout the year in most states and for various purposes including grain, feed, fodder, green cobs, sweet corn, baby corn, popcorn, starch and industrial products. The sowing and harvesting pattern of maize is unique in India. The supply of fresh maize is assured for almost seven months across the country. The crop duration also ranges from 90 to 150 days. Accordingly, the arrival of maize-grain in markets show a seasonal variation. The seasonality of demand and supply is depicted in Figure 1.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Demand	🟧	🟧	🟧	🟪	🟪	🟪						
Supply	🟦	🟦	🟩	🟩	🟩	🟦	🟦	🟦	🟩	🟩	🟩	🟩

🟧 Strong Demand	🟪 Weak Demand	🟩 Strong Supply	🟦 Weak Supply

Figure 1. Seasonality of maize demand and supply. Source: Ranjit *et al.* (2014)

The early-sown crop in Andhra Pradesh comes to harvest by late September and are picked up in October. During this period, North-East monsoon starts, which most times affects grain storage, marketing and transportation. The arrivals in the south are in full flow from October to January/ February (Talwar, 2010). The peak arrivals of the *Kharif*-crop are in November, December and January. In Tamil Nadu, the crop is sown end of September and harvested in February all the way to April. The market surplus ratio of maize is around 88% (ASG, 2016), and has been so since 2010. This reaffirms that maize has become a commercial crop in India, as the marketed surplus ratio (ratio of selling quantity to total produce) of maize grain is quite high.

According to estimates by Ranjit et al (2014), maize production may reach 28.45 mt by 2020, serving maize demand in 2020. As seen above the production is spread over a large part of year. By this argument we can say that the maize supplied to the market throughout the year is keeping pace with the demand created in different verticals.

Can the value chain in its present form cater for these demand and supply scenarios?

Due to demand, the maize value chain must expand to serve diverse purposes. The pressure however, is on the value chain to be streamlined as an immediate measure to enhance the performance of the sector. Successful farmers' access to these markets depends on how the value chains are structured, the relationship between chain actors and the judicious mix of public and private provision of business development and extension services (Shiferaw et al. 2011). Most actors in India's maize value chain are from the private sector; few are from public funded institutions. Agricultural Produce Marketing Committee (APMC) is a publicly funded institution that serves as the first point in movement of grain, a rule that stifles trade and fair competition even after reforms were introduced in many states. As on 30 June 2011, there were 7,246 regulated markets and 21,238 Rural Periodic Markets in India spread across 26 states (Patnaik, 2011). In some states, APMC (Regulation) Act has been repealed, allowing trade outside the ambit of APMC rules and letting the private sector play an important role in output value chain. These private players are fast to receive and implement modern technology, thereby increasing the effectiveness and value proposition in input production, delivery, crop husbandry as well as in output market segment. The private sector is also dominant in the maize input supply chains and serve and drive most maize value chains. The usual supply chain of maize, presented in Figure 2, reveals several actors.

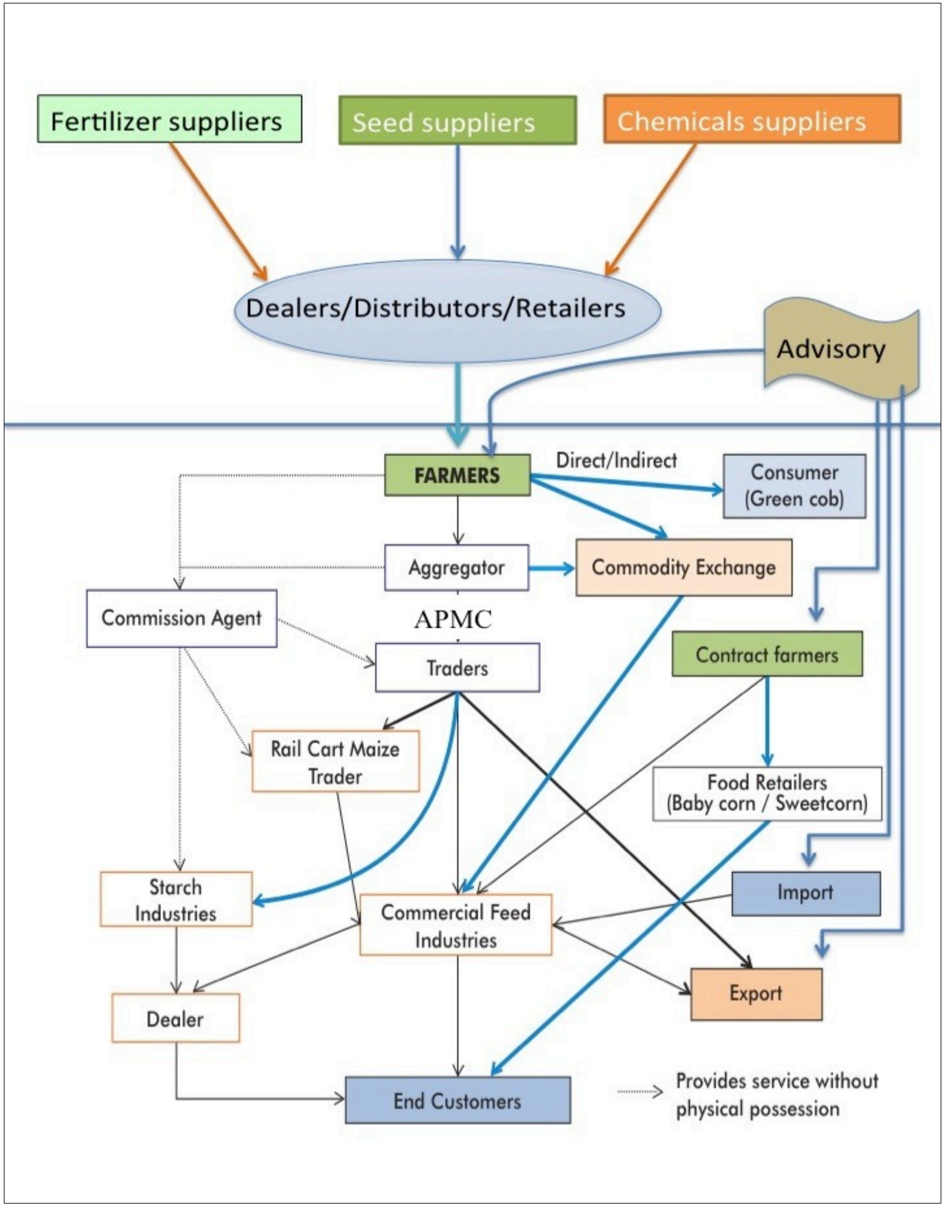


Figure 2. Value chains in maize

i. Seed and input industry

The private sector plays decisive roles in India's agricultural transformation, especially in supply of hybrid seeds of different crops including cotton and maize. The private sector covers more than 60% of maize area and supplies almost 70% of hybrid maize seed (Joshi et al., 2005; Nikhade, 2003; Spielman et al., 2011). Huge investments have been made in maize inputs by the private sector due to available market for seed and complementary material inputs (fertilizer, crop chemicals and machinery), and farmers' disenchantment with poor-performing public input-supply organizations. Efficient value chains are made possible through use of data gathered from farmers regarding inputs and creating effective delivery systems, thus ensuring equitable benefits sharing by the consumer. This was possible due to policy reforms that transfer responsibility partially to the private sector for agricultural input supply (Gerpacio, 2013). These activities increase productivity and create jobs and value in supply chains "from farm to fork" (Marco and Yuan 2017).

ii. Dealers and distributors

By maintaining proper and constant supply of inputs, fertilizer dealers and input distributors sustainably contribute to higher production and productivity of food grains. There are about 2.82 lakh practicing agri-input dealers in India. Although most of these input dealers do not have formal agricultural education (http://www.manage.gov.in), they are usually the first point of contact for many farmers, and are the prime source of farming information to the community. If their knowledge is enhanced and the ICT and other input delivery technologies are used in this segment of the value chain, efficacy could be increased. The Government of India has only recently taken the initiative to train these dealers on basic agricultural knowledge and practice.

iii. Farmers

The main stakeholders for seed in the value chains are farmers as they perform activities like sowing, crop husbandry, harvesting, processing (primary) and marketing of maize. After harvesting, most smallholder farmers sell at their farm-gates to local traders and nearby markets, regulated or otherwise. Since most maize growers are small scale, their retaining capacity is quite low as they need cash for household consumption or for next crop cultivation. This makes them vulnerable and forces them to sell at lower prices. Use of ICTs and understanding of market price structures through apps may enhance farmers' efficiency and adoption of better technologies. Many private firms have entered into advisory and output aggregation using modern technologies like block chain and artificial intelligence platforms. They also encourage proper postharvest operations.

iv. Village aggregators/ traders

These individuals play an important role in the maize supply chain, as they operate at the producer point viz. in the villages. In some cases, farmers themselves act as village aggregators and collect the grain from small growers, then sell to the big traders directly or through commission agents - depending on the volume of tradable maize in the area. They also provide price information to farmers as given to them by the commission agents.

v. Commission agent/broker

These are the middleman between farmers or traders and processors/ end-users. They decide the price of maize based on quality, and sometimes provide financial help to farmers during the growing season.

vi. Commodity exchanges

Maize is traded in large volumes on electronic commodity exchange platforms like MCX, NCDEX, and NSEL. It is being traded on Futures as well as Spot exchanges. Usually the graded and standardized lots of grains are kept in accredited warehouses, creating value for the chain.

vii. Feed industry

Here, supply of raw material and manufacture of feed takes place as per demand and quality parameters. The final product is distributed directly to customers, and through dealers or contract farming/integration.

viii. Importers and exporters

India has become a net exporter of maize in recent years, but still imports maize in small quantities mainly for popcorn and sweet corn growing. Exports are in form of grain or poultry feed, creating export value in the chain. Indian maize exports have slowed down significantly since MY 2015/ 2016 due to unfriendly international prices, unlike domestic prices which have remained firm on strong demand and rising MSPs. Improvement in yield (Ranjit *et al*, 2014) and the maize value chain is crucial for making Indian maize competitive in international markets, in terms of both quality and prices.

Policy Perspective

The food grain policies in India have been oriented mainly towards ensuring food security by encouraging production of rice, wheat and pulses. These policy changes can be divided into four phases. The first phase (1966-1972), popularly known as Green Revolution (GR) Period, during which policy focus was on modernizing and intensifying agriculture to raise yields. During the Second Phase (1973-1980), more public investment was allocated for developing new seed varieties, including developing hybrid rice. More input subsidies, mainly in the form of fertilizers, were given to encourage farmers to use them. During these two phases, major focus was on rice and wheat.

In the third phase (1980-1990), the Agro-climatic Regional Planning Approach was initiated by the Planning Commission to formulate a macro-level strategy for the 15 broad agro-climatic zones of the country. Oilseeds also caught the attention of policymakers, and consequently, the Technology Mission on Oilseeds (TMO) was launched in 1986. The fourth phase (1991 onwards) started with economic liberalization in India - which promoted integration of domestic and global economies - and affected the domestic market of several agri-commodities. The private sector has been allowed and encouraged to participate in input supplies and trade of major agricultural products (Chand *et al.*, 2003*)*. During this period, the Accelerated Maize Development Program (AMDP) was launched. The program is currently being implemented in all the maize potential districts in 26 states of the country.

Another very important policy change was introduced in 2003, when the Government of India, in consultation with the state governments, formulated a Model Agricultural Produce Market Committee (APMC) Act and advised the states to adopt it. The legislation redefined the role of the present APMC to promote alternative marketing systems and contract farming alongside the State Agricultural Marketing Boards in promoting standardization, grading, quality certification, market-led extension and training of farmers and market functionaries in marketing related areas (Patnaik, 2011). The reform also led to set up of virtual markets like Futures Exchange, Spot Exchange, Warehouse Receipt System and Web marketing. Consequently in 2003, three national exchanges, NCDEX, MCX and NMCE, were recognized with on-line trading and professional management of futures trading in several agricultural commodities.

To give further impetus to all the crops, the Seed Bill was introduced in 2004 incorporating provisions for regulating the quality of seeds for sale, import and export and to facilitate production and supply of quality seeds related matters. Once enacted, it was expected to bring a sea of changes in the maize hybrid seed market, as farmers in many rural areas expressed serious concerns over fake hybrid seeds sold in the local market.

Although these policy changes were not solely directed at maize, they created an enabling environment for the overall development of agriculture. Moreover, three important policy decisions taken by the Government of India in recent years may influence maize production significantly; these were Rashtriya Krishi Vikas Yojana (RKVY), National Food Security Mission (NFSM) and National Food Security Act.

Ex-ante Assessment of GM Maize

Introduction of broader socio-economic considerations into GMO biosafety analysis and decision-making process requires deep understanding, as there are many approaches for development and implementation of methodologies for estimation of costs, benefits, risks and tradeoffs in terms of technology use, safety, gains in knowledge and regulatory impact (Smale et al, 2006; Horna et al, 2013). It is certainly prudent for countries to consider all of these issues, starting from the most basic question of why each country wants to include socio-economic considerations into their technology decision making processes. The debate on expanded use of genetically modified (GM) crops in development has included the main clause of socioeconomic considerations in regulatory process through which these crops are approved. A strong technology-assessment methodology must reflect the understanding of all the stakeholders in the value chain of the commodity. These methodologies and implementation strategies are expected to serve as valuable and timely guides for implementing the socioeconomic assessment of these technologies. A study was conducted in Nalagonda district of Telangana for an *ex ante* assessment on probability of the adoption of genetically engineered maize crop based on farmer stated preference, using a primary survey of 125 farm households. Probit model was used to estimate the probability of adoption of genetically engineered maize which is tolerant to herbicides (Srinivas at al, 2017).

The average age of maize farmers was 42 years and about 20% of them were illiterate. Only 65.25% of the male respondents followed the recommended package of practices, resulting in productivity of 33.26 Q/ha in 2014 - 2015. These farmers grow mainly hybrids and 75% of them get seeds from private dealers. Since maize is easily infested by weeds, Atrazine (85.6%) and Paraquat (52.8%) for weed control are commonly used. The major portion of cultivation costs (14.24%) goes to weeding, including cost of herbicide, its application and manual weeding. About 76.0% of farmers are ready to accept the GM weed tolerant maize. To understand the factors determining their willingness to adopt GM, an ex-ante adoption study was done using Probit model (Kolady and Lesser, 2006). For maize, factors such as age, household size, percentage of irrigated area and presence of tube are positively influencing adoption of GM maize, and farmers with these characteristics are more likely to adopt GM crops. Other trait specific characteristics (pesticide cost) did not influence adoption of GM crop. Smallholder farmers are more likely to adopt GM crops. Trait specific variable (herbicide cost) was found to be insignificant while pesticide cost was found significant (at 10% significance level). Sensitive analysis reflects that there should also be yield advantage if benefit cost ratio is to be maintained at present level, and assuming that the cost of seeds of GM crop will be higher compared to conventional crops. Farmers were told about the desired GMOs (weed tolerant trait) and asked about their willingness to pay for the seed. About 41.6% of maize farmers were willing to pay more than 50% of seed cost as it would not only give better economic return (tangible) but also improve their lifestyle and reduce health-related problems.

Conclusion

The Indian maize sector will face a major shift in productivity due to higher demand and better adaptability. The country can provide year-round supply of fresh maize to different sectors, provided it adopts proper technology. Complicated and inefficient maize value chain networks may not match demand in near future, going by various estimates. Different players in the supply chain should examine their sector and try to adopt modern technologies to improve efficiency.

Farmers want alternative varieties (HYV, Hybrid and GM) for different crops, and are willing to pay more for seed as long as the crop increases the profitability. These can be done either by reducing the cost of cultivation or by increasing yield. Many farmers feel that GM crops can be useful in enhancing profitability and reducing labor requirements if they are made aware of all necessary precautions needed to raise a genetically modified crop. Farmers also believe that the new varieties (GMOs) should have proper environmental safety precautions and that government should be very strict in observing them.

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Debunking the Myths around Gender Norms in Agriculture in Bangladesh

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Introduction

This paper is based on a global study, commissioned by CIMMYT, on Innovation and Development Through Transformation of Gender Norms in Agriculture. By and large, gender norms refer to rules that prescribe women and men roles in the society. Often times, these gender norms are discriminatory and provide advantage men over women that leads to gender inequality in the society. Innovations in agriculture and Natural Resource Management (NRM) that ignores these gender norms can have limited impact and also runs the risks of amplifying existing poverty, workload and well being of women especially the most marginalized women in the society. More specifically, the paper aims at debunking some existing myths around gender norms in agriculture in Bangladesh by providing extensive evidences from a participatory research conducted in 2015. The paper focuses on the changing paradigm in wheat and maize production in Bangladesh in the context of gender structure, wheat and maize innovations, different capacities of women and men to uptake new innovations and the impacts of new innovations in existing gender norms.

Methodology

The paper is based on six case studies; Barakpur (Meherpur), Beelmahmudpur (Faridpur), Dharmapur (Rajshahi), Matiakura (Dinajpur), Kolkondo (Rangpur) and Begunbari (Maymensingh). The rationale for site selection is guided by the principles of maximum diversity sampling. Two key dimensions informed the sampling framework, the economic dynamism and gender gap in asset and capacities. To generate data from each case, fifteen (15) exercises were conducted such as a profile of the village, six focus group discussions with poor and middle class women, men and youth; four in-depth Interviews, four key informant interviews. Considering the heterogeneous rural community in Bangladesh, data were gathered from different classes, age groups, and ethnicities to identify their roles and capacities in wheat and maize innovations.

Results and Discussion

The findings suggest that women's role in agriculture and more importantly in wheat and maize production is significant. However, their contribution is often unrecognized and considered as auxiliary. It is observed that strong gender norms in rural Bangladesh often informed by social, cultural and religious factors hinder the possibility of women to take the central stage in agriculture, wheat and maize innovations and become more productive. As culture norms are not always 'fixed' and 'static', evidence suggests that sometimes economic reasoning overrides over strong gender norms in rural Bangladesh.

The existing myths around wheat and maize innovation include: 1) Women's roles in agriculture including wheat and maize innovations are low. However, field data suggest that despite myths around gender, a large number of extremely poor, poor widows and women from lower-middle class are heavily involved in wheat and maize production especially in postharvest, homestead gardening and NRM activities in Bangladesh. Historically, agricultural activities are male dominated in Bangladesh (Naveed 2011). The men-dominated agricultural activities are due to various social, cultural and religious norms that are been practiced by rural communities for years. Culture as a phenomenon changes over time, therefore, a fluidity in cultural norms are also visible in women's role in agriculture and wheat and maize innovations. This paper aims to show that existing myths around women's participation in agriculture including wheat and maize innovations are low. The popular myths also include that women are not capable of adopting new agricultural technologies and men are the ultimate farmers who holds the knowledge of agriculture, women's restricted mobility and work space is mostly due to protect the family honor. Data from six cases show how directly and indirectly women are involved in agriculture including wheat and maize cultivation. Data also show what crucial roles women are playing in agricultural and NRM activities especially in postharvest activities and homestead gardening. In addition, due to dire economic situation how extremely poor, poor, widows, ethnic minorities and a few women from lower middle-class strata are also being engaged in field-based agricultural activities in some case areas.

Data from all six cases show that women play a secondary role in agriculture and NRM activities. The nature and degree of involvement varies between men and women. These differences are evident within and across households and communities. Field data show that middleclass and large land holder household heads, mostly males, do not allow their women to be involved in agricultural work outside their home compounds. However, their close family members appreciate them when they grow vegetables through homestead gardening for household consumption and selling the produce through other male members for an additional income for the family. In contrast, involvement of lower middle class, and woman-headed households in non field-based agriculture and wheat cultivation are quite high. Widow, poor adult and some extremely poor adult women are engaged in field based agricultural activities as a daily labor er and sharecropper. These roles for women in agriculture and maize are common in all six cases.

Thus, the study reflects an intersection of gender and class/economic status in agriculture and wheat innovation(s). Some exceptions for middle class women are also found in one case study.

Women's position in her life cycle such as unmarried, married, widow also plays a part in determining their role in agricultural activities. Similarly, relatively older women from a lower economic class enjoy more mobility than unmarried and newly married women from the same economic class. Local norms also vary for younger versus older women and men. It is evident from the field data, agriculture labor for younger versus older men do not vary much. However, young unmarried women are not allowed to work in the agricultural field. The community has zero tolerance for that. Even the poor husband does not allow their newly married wife for waged work. The mobility of the newly married women is very restricted by the in-laws family. Newly married women seem to be the holder of family dignity and honor at least for first few years.

There are differences in barriers between men and women towards hard and soft wheat innovations. The popular and dominant hard innovation identified are the strip-tillage, bed-planter (PTOS), reaper/harvester, power thresher and irrigation devices, improved seed varieties and the soft innovation includes training, updated knowledge and information on seeds, fertilizer, and pest control. These differentiated barriers such as for women among others are- gendered division of labor, restricted mobility, access to new agricultural technology, information, lack of household decision making power etc., and for men among others as described are- access to finance, appropriate price for produce, and availability of good land and pesticides etc. These barriers are found between men and between women and within intra and inter households across all six cases. The root causes of differences are complex and manifold for both men and women.

The barriers towards innovations are different for women across class, age, and other social categories. Given the reality around women's role in agriculture, it seems that there is a clear division of labor between men and women in rural Bangladesh. Women's work is primarily associated with household work such as child rearing, cooking, cleaning, homestead gardening, and raising the livestock. These traditional gender roles and perceptions of women who should not be allowed to work field based agriculture and wheat restricted their ability to test their true potential in agricultural innovations including the wheat innovations.

Despite barriers, 12 successful female innovators were interviewed across six cases deployed their agency to overcome those barriers. Study findings show that women from lower middle class were highly motivated and confident and were able to weigh their options for wheat innovations to utilize their agency around wheat innovations. Despite restrictions on their physical mobility, some women managed to participate in agricultural trainings offered by the local NGO and government offices, adopted hard and soft innovations and received loans to increase their agricultural productivity in order to improve their economic conditions. In one case, the resilient successful female innovators reached out to another successful male innovator over the phone for suggestions on how she can adopt the wheat innovations. The dream for success and the personal inner motivations and drive among the successful innovators was so strong through which they were able to overcome the barriers. For some female innovators it was driven by their economic needs and for others it worked out of pure interest.

Women in the community are willing to overcome this situation and some women tried to adopt new agricultural technologies and became successful. The Union Federation established by a consortium of NGOs was very effective for bringing women in agriculture and NRM activities. The federation worked as a platform for women at village level where they were given agricultural supports such as training, inputs and updated knowledge to help improve their agricultural production. Among other components, inclusion of women as their direct beneficiary and pragmatic program strategy of the Federation appears to be the reason for the program success.

Barriers for men are different than that of women. It is important to unpack how men overcome barriers with innovation compare to women. For men, as the data shows the barriers are the lack of money, suitable land, availability of good seeds and pesticides and uncertain weather conditions. Although these barriers are equally applicable for women innovators, however, women were able to identify other larger factors that seem more immediate to ensure their participation in field-based agriculture and wheat. Often, men reach out to their women for money to buy the agricultural inputs needed for wheat innovations. Although women do not have permanent source of income, but as a cultural practice, most women try to maintain a savings through selling of their chickens, ducks, eggs to their neighbours, and cash gifts they receive from their relatives during different religious and social festivals. Men usually seek out suggestions from other experienced men in agricultural cultivation such as adopting of new innovations.

Another popular myth is that women's restricted mobility and workspace is mostly to protect the family honor. This 'myth' derived from orthodox Islam and accepted by society at large also shapes gender division of work in agriculture and wheat. This myth also sometimes guides the dominant agricultural development initiatives and programs. Our findings demonstrated that despite many challenges, some women were exemplary in becoming a successful wheat innovator without compromising family honour and dignity. Further that the larger community celebrated the success of female innovators. Women were able to adopt hard and soft innovations, took important decision to manage the agricultural production cycle and the selling of produce at good profit margins. Successful women innovators were also able to make a positive impact to their lives and that of their families. Some women also extended their cooperation by providing moral and financial support and expert opinions to their male counterparts to ensure a good harvest for the household.

Data show that among the youths, the sense of equity in gender in everyday life is increasing. Today's youth with higher exposure to formal education, the Internet, mass media, and NGO-led development programs, have begun to challenge the myths around gender. Although development is 'slow' it can be assumed that with careful planning and pragmatic strategies, the myths around gender in agriculture/wheat/Maize cultivation can be effectively overcome.

New agricultural innovations are very hard to get access to for both poor men and women in the village, which was observed in all the case areas. Across six cases, data suggests that the hard system related to innovation as agricultural machines, processing technologies for key commodities and improved seed varieties introduced by global institutions like CIMMYT by design favours the middle class or rich farmers who meet primary 'eligibility criteria'. This study finds this trend as non-inclusive by design. The study data suggests that the dissemination of agricultural knowledge through the event called 'field day' by local NGOs including CIMMYT was very effective for both men and women. The field data reveals that women get opportunities to participate in 'field days' organized by local NGOs as part of their program activity of which CIMMYT is not a part in Meherpur and Faridpur but in all other cases women were not targeted as beneficiaries. In terms of inclusive development, both women and men should be included as potential beneficiaries.

With regards to wheat and maize innovation, the study findings suggest that from the introduction of varieties/ technology in the community, only the upper and middle class men benefited most from the new wheat innovations in agriculture. Poor men and women were not targeted as potential beneficiaries of wheat innovations. So far, innovations in wheat especially the hard innovation in Bangladesh is clearly designed and favoured men over women and was not gender inclusive.

Conclusion

Agricultural innovations, both hard and soft, promise to solve problems of food security through potentially increased productivity of wheat cultivation. However, the benefits of innovations fail to reach majority of farmers including women and the socially marginalized. This paper argues that to ensure sustained positive impacts from technological innovation, it is important that we take consideration the processes and socio-political and institutional set-ups where the technology is being incorporated.

Maize Market Development in Nepal – Challenges and Prospects

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Introduction

Maize is the leading cereal in terms of production, with 1.06 billion tons produced on 187 million hectares (M ha) globally, with a productivity of 5.6 metric tons/hectare (MT/ha) (FAOSTAT, 2018). It is one of three leading cereals that feed the world (Shiferaw *et al.,* 2011). Aside from its staple food use, it makes a significant contribution to animal feed (especially poultry), bio-fuel and for industrial uses (Hellin and Erenstein 2009). Population growth, changing diets and a rapidly growing poultry sector are contributing to a sharp increase in maize demand (Erenstein, 2010). During 1991-2011, total utilization of maize almost doubled in Asia. With its multiple uses, maize is the world's most multi-purpose crop. In Nepal, maize is the second most important crop after rice in terms of area, production and yield (Subedi *et al.,* 2017; MOAD 2017). It is a traditional crop grown for food, feed and fodder. Maize occupies 43% of cereals' area and contributes 53% of its production. The share of cereal crops to Agriculture GDP is about 49%, and maize alone contributes about 7% (Sapkota *et al.,* 2016). The total area, production and yield of improved maize in Nepal have been reported at 0.89 M ha, 2.23 million MT and 2.5 MT/ha respectively (MOAD, 2017). Mid hills represent more than 70% of area and production, whereas high hills occupy 20% of area and 10% of total production. The Terai occupies 10% area, contributing 20% to national maize production (Gurung *et al.,* 2011). In Nepal maize is grown in three seasons: summer, spring and winter with 74% (mainly in mid-hills), 14% and 12% respectively (Gurung *et al.,* 2011).

The International Maize and Wheat Improvement Center (CIMMYT) is working with its partners in Nepal to promote new maize varieties, including hybrids and bio-fortified maize. This paper discusses the challenges and prospects for maize market development in Nepal using literature review, household survey and key informant interviews with stakeholders. By using stratified multi-stage random sampling, 600 households were surveyed in Central, Western, Mid-western and Far-western regions of Nepal in August 2017. A total of 13 seed companies, 95 agro-dealers and 13 District Agriculture Development Officers (DADOs), were interviewed in the four development regions to analyze their respective roles, functions, constraints and opportunities in input value chain including maize grain. Six focus group discussions were organized with key stakeholders comprising seed companies, agro-dealers, feed mills, Nepal Agricultural Research Council (NARC), Ministry of Agriculture and Livestock and farmers between August 2017 and April 2018.

Utilization and Commercialization of Maize of Nepal

Nepal produces about 2.23 million tons of maize annually, against a national requirement estimated at 2.6 million tons (MoAD, 2016; Bhattarai, 2017), with the deficit being fulfilled by imports. While about 86% maize production in the hills is used for human consumption, about 80% of the production in Terai is used for poultry and animal feed (Gurung *et al.,* 2011). Maize demand has been consistently growing by about 5% annually in the last decades (Sapkota and Pokhrel, 2010). In 2014, per capita maize consumption in Nepal was 67.7 grams per person per day, and the total quantity of maize required for food per year was around 0.69 MMT (CBS, 2016). An estimation of the amount of maize used at household and unorganized feed industry is lacking. Timsina *et al.,* 2016, reported that in Kavre and Lamjung districts 60%, 25% and 3% of the maize were used for feed, food and seed purpose respectively at the household level. CIMMYT 2018 reported that only 14% of the households sold maize in Nepal. The same study reported that only 11.4% of the total production comprising 19% Terai, and 10% hills was sold, and the rest used for household consumption (as food or feed). The consumption of maize as food is decreasing (CDD, 2011, Timsina *et al.,* 2016, Tripathi *et al.,* 2016) compared to the situation a decade ago. There is an increasing trend in diversification of maize products for human nutrition such as soups, vegetables, maize grits and edible oils (Tripathi *et al.,* 2016). Increasing trend of poultry and livestock business, along with increasing population and rising income, has led to an increase in the demand for maize grains (Tripathi *et al.,* 2016).

The use of maize in commercial feed manufacture is increasing (KC *et al.,* 2015b, Timsina *et al.,* 2016). Timsina *et al.,* 2016 reported a 13% and 8.5% per year increase in demand of poultry feed and animal feed respectively over the last five years. These authors also estimated that out of total maize used in feed production, 87% of the maize was imported from India each year by feed industries. According to Nepal Feed Association, 127 feed mills with capacities ranging from 2 MT to 400 MT/day are registered in Nepal (Personal Communication, Mr. Narayan Khatri, Chairman, Nepal Feed Industries Association, April 2018). About 80% of these mills are concentrated in Kathmandu, Bigunj, Hetauda, and Narayangarh. The distribution of the mills is in line with the adoption of hybrid maize area.

Challenges in Maize Market Development

Maize Production

Maize yields fluctuate seasonally and annually especially in the hills (Poudyal *et al.,* 2001). CIMMYT studies showed that there are enormous diversities in the way maize is cultivated among different maize production environments in terms of timing of crop establishment, inputs and output levels, varieties preferred, crop rotation and crop management practices. Majority of maize farmers use local varieties and use farm saved seeds. Factors that influence a farmer's choice of variety are the level of productivity, maturity period, harvesting time, quality and quantity of foliage and the belief that a certain variety produces a minimum quantity despite adverse weather (Poudyal *et al.,* 2001, CIMMYT 2018). The yield of local maize in the Terai ranged from 0.20 MT/ ha to 2.00 MT/ha, and that of improved OPVs from 1.35 MT/ ha to 2.83 MT/ha (Poudyal *et al.,* 2001). Farmers on average produced 0.49 MT on an area of 0.25 ha, with an average yield of 1.96 MT/ha which is lower than the national average yield of maize in 2016 of 2.43 MT/ha (CIMMYT 2018). There is a gap of about 5.5 MT/ha between the potential yield at national level trials and farmers' level (MOAD, 2014; KC *et al.,* 2015b). Lamichhane *et al.,* (2015) reported that the improved varieties such as Rampur composite, Arun 2, and Manakamana 6 were getting popular in the western hills, indicating adoption of improved varieties substituting the local. The Government of Nepal (GoN) has been heavily subsidizing the seeds of such improved varieties. Unfortunately, the few hybrids developed by NARC are yet to be commercialized and new OPVs are not being popularized by both public and private sectors.

Seed Systems

Several studies have reported problems in maize production (Poudyal *et al.,* 2001; KC *et al.,* 2015b; Subedi *et al.,* 2017; CIMMYT 2018). Across all agro-ecologies, farmers mentioned the lack of quality seed as the single most important factor affecting maize productivity. Improved quality seeds contribute to about 20–30% increase in yield (MoAD, 2015). The maize seed sector in Nepal is handicapped by low domestic research and production capacity, which resulted in the poor supply of breeder and foundation seed for its multiplication (Sapkota *et al.,* 2017). Up to April 2018, NARC has released/registered 73 improved maize varieties including 24 OPVs and 49 hybrids. However only 12 OPVs were under production at NARC stations, with three varieties viz., Rampur Composite, Manakamana-3 and Arun-2 (Figure 1) constituting 81% of the total source seed production of 75 MT (SQCC, 2018). Total annual demand of maize seed in Nepal is 19,552 MT, but the seed replacement rate (SRR) is only 15.3% (SQCC, 2018).

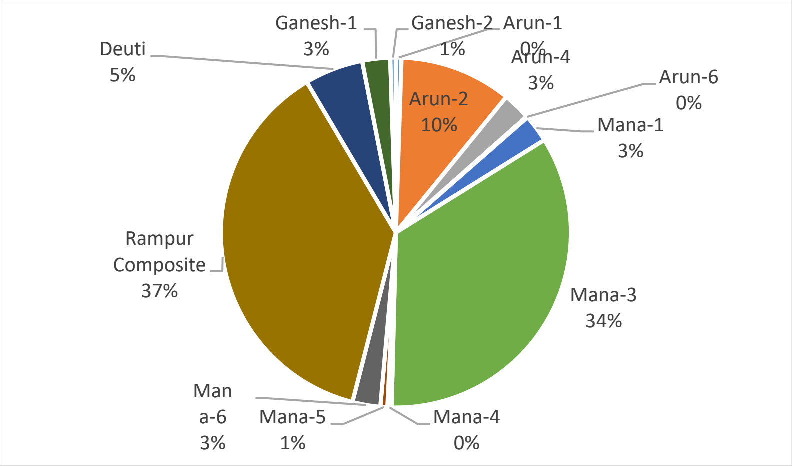

Figure 1. Source seed by different maize varieties projected to produce at NARC stations

Nepal has released seven maize hybrids namely Rampur hybrid-2, -4, -6, -8, -10 and Khumal Hybrid-2. One hybrid variety, Gaurav, which was released in 2003, has been removed from the list due to poor seed setting. The Rampur series hybrids were released for Terai region and Khumal Hybrid-2 for the mid-hills. As of 2015, Khumal Hybrid-2 was under partial commercialization by two private seed companies with a total seed production of 10 MT, which remained at 4 MT in 2016 and 2017. These companies also sold 2.7 MT seed of this hybrid during the summer season of 2018. Many companies are reluctant to invest in commercializing Nepali hybrids as there is a lack of clear guidelines from SQCC on exclusive licensing to produce and market these hybrids. In general, there is an increasing trend in hybrid seed use in Nepal. The Seed Entrepreneurs' Association of Nepal (SEAN) estimates about 1,500 MT of hybrid maize seed were imported through both formal and informal channels in 2017. The illegal and informal hybrid maize seed import from across the Indian border is common.

In Nepal most of the seed companies are in the Terai. Seed cooperatives and farmers' groups promoted by Hill Maize Research Program (HMRP) under Community-Based Seed Production (CBSP) program in the year 2000 produce and supply about 90% of the seeds in the hills (KC *et al.,* 2015a). Many of these institutions may not follow the certification and truthful labelling procedures mandated by SQCC due to limited number of seed laboratories and technical staff. Seed producer groups have limited access to and availability of quality source seeds, leading to the deterioration of seed quality.

Postharvest

In developing countries, people try to make the best use of food produced. However, a significant amount of produce is lost in postharvest operations due to a lack of knowledge, inadequate technology and/or poor storage infrastructure (Kuman and Kalita 2017). The major problem with postharvest handling is the difficulty in drying maize, as most farmers do not have drying equipment (Rajbhandari *et al.,* 2015). The summer maize harvesting season coincides with the late monsoon when cobs have a relatively high moisture content (between 23-28%) - Poudyal *et al.,* 2001. Maize must be dried to at least 12% for it to be stored safely for any period of time (Ransom 2001). However, because of humid rainy days during and immediately after harvest, maize is usually not dry enough to be safely stored (Poudyal *et al.,* 2001). A major reason for postharvest losses is traditional storage practices by farmers (Rajbhandari *et al.,* 2015). Mycotoxins (e.g., aflatoxin) produced by fungi in insufficiently dried food commodities affect 4.5 billion people worldwide (Bradford et al., 2018). Farmers normally store the maize after just 3-4 days of sun drying or, in some cases, keep the husked cob in piles for weeks (Pokhrel, 2016). This provides conducive environment for growth of mold. Pokhrel, 2016, reported that 20% of the maize samples received by the Department of Food Technology and Quality Control Center (DFTQC) had aflatoxin above threshold level

(20ppb). This suggests necessity of increasing awareness among farming communities about aflatoxin smart technologies.

Access to services

In Nepal, as little as 24% of farmers are reached by the formal extension services in Nepal (ADS, 2014). The main problems faced by farmers relate to weather, input availability and use-related risks and problems. Multi-cropping is the most common mitigation strategy (CIMMYT, 2018). The same study revealed that farmers apply on average 42 kg less nitrogen, 23 kg less phosphorous and 30 kg less potash per hectare on their maize than recommended government rates (CIMMYT, 2018). An emerging challenge faced by maize farmers is the quantity and pattern of rainfall. Few farmers receive advisory services to cope and adapt to such challenges (CIMMYT, 2018). The presence of private sector agricultural inputs and service providers in remote rural areas is almost non-existent (Gurung *et al.,* 2011). The involvement of NGOs in supporting agricultural development is common, mainly through extension and subsidized input supply in projects funded by donors (Joshi et al., 2012). Most smallholders in remote rural areas of hills, mountains and Terai have limited access to NARC research (Joshi *et al.*, 2012). Nepalese agriculture is increasingly facing a shortage of labor, and women have become more responsible for making on-farm production decisions. This suggests the need for targeted interventions to address rural women's needs.

Marketing of Maize

The maize grain value chains in Nepal is unorganized and lacks coordination mechanisms. Not many value chain actors are involved in grain marketing in the mid hills. Rural traders collect surplus maize from large scale and smallholder farmers and supply it to the local traders (Gurung *et al.,* 2011). Due to lack of an efficient market mechanism and competitive market structure, farmers are not able to benefit from increased production (Koirala, 2002). Small producers are unable to market their produce individually due to small volumes and long distances. They sell produce in local markets where the prices are low. Timsina *et al.,* 2016, reported a negative correlation between scale of feed production and use of domestic maize. It is common understanding in Nepal that white varieties are used for food and yellow varieties are used for feed, but none of the farmers had planned to produce different varieties based on their utilization such as food, feed and seed (Timsima *et al.,* 2016, CIMMYT, 2018). Feed companies are interested to buy Nepalese maize if these could be delivered in required time, quantity and desired quality.

Market Development of New varieties

The highest volume of maize seed sold by agrovets in 2016 were the Rampur Composite and Arun 2 varieties (CIMMYT, 2018). Only 3% of sampled maize-growing households reported using hybrid maize seed. Farmers and agro-dealers have limited access to information on new varieties and their traits. A major reason for this is poor on-farm demonstration of the new varieties and their management practices. Use of innovative techniques for popularizing new varieties such as advertisements on FM radio in regional languages, use of multi-stakeholder meetings, and large numbers of demonstrations is uncommon (Joshi *et al.,* 2012). Seed companies never conduct varietal demonstrations, farmer field days, fairs etc. to disseminate varietal information for promoting their products. Seed companies do not strive to develop markets for their brands, hence there is hardly any competition in the market. DADOs use conventional methods of promoting new varieties, such as a few mini kits, but without any follow-up or feedback. Moreover, researchers do not find interest in promoting new varieties as it is beyond their mandate. Timsina *et al.*, 2016, reported that 60% of the feed industries do not know about the quality protein maize released by NARC.

Policy and Institutional Arrangements

The Agriculture Development Strategy (ADS), which is the flagship policy of the GoN, aims at commercializing the agriculture sector in Nepal to move from subsistence to commercial production. However, in Nepal, small size and fragmented farms make it more difficult to realize economies of scale. Staple commodities such as rice, wheat, potato and vegetables have higher commercialization rates (30-50%) than maize and fruits (15-25%) (ADS, 2014). Maize commercialization is hampered by lack of value chain coordination mechanisms for grains. Unavailability of competitive hybrid cultivars within the country and underdeveloped seed industries caused dependency on imported hybrid maize seed every year (Gurung et al., 2011). In the case of hybrid maize varieties most of these are registered for cultivation in the central Terai (East of Narayani River) resulting in unmet demand in other potential areas (CSISA, 2017). The GoN has launched the maize super zone and zones program for focused maize production in the country, under the Prime Minister Agriculture Modernization Project (PMAMP). The Department of Agriculture (DoA) also implements the mega maize program. Although various research and development organizations are working in maize sub-sector, there is huge yield gap and its value chain remain disorganized. These challenges demand an approach to agricultural promotion and competitiveness that acknowledges the vital role of the private, public and cooperative sector and better implementation of policy priorities.

Prospects of Maize in Nepal

The feed sector is driving the commercial maize markets in Nepal. With reduced consumption of maize for food and increased use for feeding animals at the household level, there is tremendous scope to link smallholder farmers in commercial value chains and explore the crop livestock/poultry integrated value chain linkages. This will however need a strong alignment of the production system, markets and polices. It is obvious that land area cannot be increased to meet the industry requirements for maize and much of the demand should be met from productivity gains and diversifying maize production seasons and cropping intensity in Terai, inner Terai and foothills. Domain expansion of registered hybrids can be an effective way to increase area under maize production. The increment of winter maize area two-fold under hybrid in Terai may help to reduce the current grain deficit in the country's feed industry (Tripathi et al., 2016). Though various research and development organizations are working in the maize sub-sector, productivity of maize is still low, and its value chain remains disorganized. Even if productivity of OPVs is low compared to hybrids, there is ample opportunity for sustainable intensification of maize in the hills of Nepal. However, this needs a complete package of technologies and their efficient deployment through market actors.

Over 40% Nepalese children are chronically malnourished (MOHP, 2012). Similarly, Nepal is among the countries with over 40% prevalence of Vitamin A Deficiency (VAD) among preschool children. Another nutrition-related problem in Nepal is zinc deficiency. This calls for concerted interventions to improve child and maternal nutrition. Maize is a dietary staple and often a source of protein energy for millions of Nepalese. However, normal maize is deficient in essential amino acids, lysine and tryptophan – key protein building blocks that cannot be synthesized by the human body and must be acquired from food. Hence there is immense scope in the introduction and promotion of biofortified maize products to meet rapidly evolving demand for nutritious food and animal feed in Nepal.

CIMMYT and its partners are working on increasing the competitiveness of seed and fertilizer value chains in Nepal, particularly working with the PMAMP to develop the maize sector coordination strategy in collaboration with other stakeholders. Transformation towards an upgraded maize market system requires a set of measures that focus not only on farmers, but, fundamentally on agro-enterprises involved in the commercialization of agricultural products and services (ADS, 2014). To upgrade the maize sector to target both food and feed sectors, there is need to focus on core elements that can contribute to strengthening the national maize development strategy. These are explained in Table 1.

Table 1. Components and Actions for a Competitive Maize sector in Nepal

Component	Actions
Research and Development	• Develop and launch new and competitive hybrids suitable for various agro-ecological regions of Nepal. • Develop and launch stress tolerant varieties for the different agro-ecologies of Nepal. • Develop nutritionally rich maize varieties fortified with zinc, protein and vitamins. • Update research on best management practices for optimum economic returns. • Increase research focus on medium and short duration varieties for mid-hills. • Improve the maintenance and production of source seeds by including the private sector. • Enhance capacity of breeders in public institutions and the private sector to develop new varieties.
Market Development and Extension	• Increase farmers' awareness of new varieties by conducting fairs, demonstrations and farmer field days. • Motivate private seed companies to develop competitive brands and popularize them to farmers. • Develop and educate the agro-dealers on varietal traits, performance and crop management. • Educate feed mills/ food processors on varieties available in Nepal and their potential in feed and food processing. • Use digital tools for information dissemination at various levels.
Access to inputs	• Engage in alternative options of input financing at the farmer level, for instance, tripartite engagements between banks, seed companies and farmers. • Promote the use of seed and fertilizer vouchers for delivery of quality inputs. • Develop and disseminate domain specific input recommendations based on assessment of soil characteristics and crop trials through extension agencies and the private sector. • Strengthen the informal seed production and delivery in the mid-hills through technical assistance. • Strengthen quality control at seed enterprises. • Strengthen federal and regional inspection and certification capacity.
On-farm production	• Increase awareness of recommended input and agronomic management methods through the local agriculture units. • Make appropriate crop management inputs available with agrovets and extension agencies. • Provide crop advisories though distal tools and social media platforms.
Postharvest handling	• Generate/ adapt and fast-track the dissemination of improved postharvest technologies. • Increase access to postharvest processing equipment and technologies through custom hiring facilities, rural enterprises and cooperatives. • Increase farmer awareness of, and access to, effective on-farm storage. • Increase farmer access to community-level storage facilities with skilled personnel. • Support investment for decentralized storage facilities at various locations across the country through the PMAMP managed by the private sector.

Market Coordination	• Develop tripartite arrangements between farmer groups, feed mills and grain aggregators. • Tap into large-scale maize demand of food aid agencies.
Policy and Enabling Environment	• Implement the provisions of the ADS to support commercial agriculture. • Discontinue subsidies on old maize seed varieties. • Promote Nepali hybrid maize through the national programs. • Upgrade domains of registered hybrids to enable seed access to all potential farmers. • Create and reinforce a predictable and responsive policy environment for investments in maize aggregation, processing and for feed production.

Conclusion

The maize sector in Nepal is disorganized and needs coordination, investment and technical support. It is imperative that local measures be developed, implemented and monitored to promote growth in the maize sector. Similarly, the nutrition gains offered by bio-fortified maize should be promoted through a public-private partnership (PPP) approach. The capacity of both the public and private sector is weak, and these entities will need continued support and capacity development. A PPP approach led by the PMAMP can yield considerable value and results to implement a holistic maize market development framework. Implementation of the provisions of the VC flagship component of ADS, which unfortunately have been slow, can certainly lead the country to increase production and commercialization and progress towards self-sufficiency in maize.

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Technical Efficiency of Smallholder Maize Growers in Nepal and its Implications on Value Chain Promotion and Extension

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Abstract

The paper, in the first stage, uses a cross-sectional stochastic production frontier function to estimate the level of technical efficiency of small-scale maize growing household using data collected in 2017 from 13 districts of Nepal. In the second stage, the level of inefficiency of the maize growers was specified to eight different variables related to household characteristics, biophysical environment, input use and access, and agricultural literacy to explain the variations in the inefficiency among maize growers. The study finds that maize yield responds positively and significantly to area, quantity of seed, fertilizer and labor inputs used in maize production. The technical efficiency of maize growers ranges from 16% to 100% with an overall average efficiency of 82%. Around 3/4th of the sample household had efficiency 74% or above, while 5% of the household had efficiency below 50%. We find sex, caste, type of seed used, and distance to the point of fertilizer purchase, soil pH, and rainfall shock to be significant in explaining the variation in level of inefficiency. These results imply that the efficiency, on an average, could be improved by 18%. The result also suggest that access to high yielding, stress tolerant varieties and fertilizers, provision of soil testing services along with recommendation of domain specific soil/ plant nutrient management practices and their adoption have potential of enhancing efficiency of small holder maize growers in Nepal.

Key words: Maize, technical efficiency, stochastic frontier, Nepal

Introduction

The level of agricultural productivity in general and that of crop in particular is very low in Nepal. Maize is the second most important crop after rice in Nepal but its productivity is low, the lowest among its neighbors (FAOSTAT, 2016). The yield gap is substantial when the current maize yield at the national level (2.5 MT/ha) is compared with the potential yield of major open pollinated varieties of maize (around 4 – 6 MT/ ha) released from the national research system (AICC, 2016). This gap widens when compared with the potential yield of imported hybrids gaining popularity in the country. If low productivity is due to low input use then increase in productivity require more (new) inputs (technology) to shift the production possibility frontier upward. On the other hand, productivity increase is possible with efficient use of current production inputs (technology) when there are opportunities to eliminate the inefficiencies through judicious use of inputs. In this regard, assessment of the level of efficiency of the smallholder maize growers and determining the factors that explain the level of inefficiency is prerequisite for devising intervention points for agricultural program that aim at productivity gains.

While there exists some literature on level of efficiency in maize cultivation in developing countries (Baha 2013; Chirwa, 2007; Mango *et al.*, 2015; Seyoum *et al.*, 1998; Tefaye and Beshir, 2014), there is not enough information on this in Nepal. We found two studies that looked at efficiency of maize production in Nepal. First one focused on cost efficiency (Poudel and Matsuoka, 2009) using data collected in 2005 and second one focused on maize seed production (Bajracharya and Sapkota, 2017). Both studies have restricted geographical coverage (only one district) limiting the generalizability of the findings to different agro-ecologies in the country. To improve the generalizability of the findings across agro-ecology of the country, this study uses data collected from 364 maize growers from 13 districts, six terai (southern plain bordering India) and seven mid-hill districts.[1] This study will primarily address the following two objectives:

1. Estimate the technical efficiency of small holder maize farmers
2. Identify factors that best explain the variation in the level of inefficiency of maize farmers and derive value chain and extension implications based on the results.

Econometric method

Measuring agricultural productivity and efficiency is an important but a challenging task. It is important as farm productive resources are scarce, especially for small holders, and need to be put to prudent use. It is challenging because of multitude ways, each with its pros and cons, to estimate efficiency. There is a large body of literature on efficiency analysis where two competing efficiency analysis methods are predominant. These are: (i) non-parametric method using Data Envelopment Analysis (DEA) and (ii) parametric methods using Stochastic Frontier Analysis (SFA). There have been numerous applications of parametric SFA models ever since Aigner *et al.* (1997) and Meeusen and van den Broeck (1977) almost simultaneously but independently introduced the theoretical concept for these models. The idea behind the SFA is that the empirical specification consists of a response function (in terms of production, cost, revenue, profit, etc.) and a composite error term. The composite error term consists of a two-sided error representing random effects and a one-sided error representing technical inefficiency (equation 1).

Overview of empirical application and development of SFA models is well documented by Bauer (1990), Greene (1993), and Kumbhakar and Lovell (2000) and may other authors. The empirical application of SFA is common across sectors including agriculture (Bravo-Ureta, 2007; Mekonnen *et al.*, 2012; Thiam *et al.*, 2001) where they discuss two groups of studies that have applied SFA to study efficiency in agriculture. First group includes studies that only report the efficiency levels while the second group of studies use a two-step approach. The first stage estimates the efficiency of each of the Decision Making Units (DMUs) followed by specification of a regression model where the level of efficiency of the DMUs is expressed as function of set of variables in the second stage.

This study uses a two-stage approach to estimate the efficiency/ inefficiency of the DMUs (these are maize growing households in our case). In the first stage, a Cobb-Douglas form of the cross-sectional stochastic frontier production model with log-log specification was used to estimate the level of technical efficiency across the maize growing households. This form of model specification is commonly found in literature to assess the efficiency of smallholder farmers in developing countries (Bempomaa and Acquah, 2014; Binam *et al.,* 2004; Mango *et al.,* 2014). The production function as indicated in equation (1) was estimated using the Maximum Likelihood method assuming a half-normal distribution of the inefficiency variance.

$$\ln(yield)_i = \alpha_0 + \alpha_1 \ \ln(area)_i + \alpha_2 \ ln(seed)_i + \alpha_3 \ \ln(labor)_i \\ + \alpha_5 \ \ln \ (fert)_i + \ \mathcal{E}_i \ \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where: yield = maize production in kg per hectares; area = hectares under maize; seed = quantity of seed (kg/ha); labor days per hectare (1 labor day = 8 hours work of a human labor)[2]; fert = fertilizer (Urea, DAP, MOP) in kg/ ha; and $\mathcal{E}$i is the error term equal to $(V_i - U_i)$ where V_i is a two-sided random error component beyond the control of the farmer; U_i is a one-sided inefficiency component.

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[1] The survey data collected by Feed the Future's Nepal Seed and Fertilizer project is made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents of this technical paper are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

[2] $Labor = \frac{\text{(cost of human labor + cost of animal labor + cost of mechanical labor)}}{\text{Wage rate per human labor per day}}$

The farm-specific technical efficiency (TE_i) of the i^{th} farmer is then estimated using the expression of U_i conditional on the random variable $\mathscr{E}_i$ as in equation (2).

$TE_i = Exp(- U_i)$ where $0 \geq TE_i \leq 1$; and technical inefficiency = $(1 – TE_i)$ …..…………..…….. (2)

In the second stage, eight different variables were used to explain the variation in the level of inefficiency, among the maize growing households using maximum likelihood estimation as specified in equation (3). These eight variables can be broadly grouped into three broad categories. First group consists of two variables related to household characteristics (sex and caste). Second group consists of three variables related to access and use of critical production inputs (type of seed and distance to the point of fertilizer purchase) and knowledge about agriculture (level of agronomical literacy). The third group consist of three biophysical variables that are important for agricultural activities (soil pH, rainfall shock, and altitude). The name and definition of these variables in presented in Table 1.

$$\ln(U)_i = \beta_0 + \beta_1\ (Sex)_i + \beta_2\ (Caste)_i + \beta_3\ (Seed\_Type)_i + \beta_4\ (Fert\_Distance)_i + \beta_5\ (Ag\_literacy)_i + \beta_6\ (Rainfall\_Shock)_i + \beta_7\ (Soil\_pH)_i + \beta_8\ (Altitude)_i + e_i \quad \ldots\ldots (3)$$

where: U is technical inefficiency, β_i (i = 1, 2, ….8) are the parameters to be estimated for vector of variables as presented in the equation and e_i is a random error term.

Table 3. List of variables for considered for second stage stochastic frontier model (equation 3)

Symbol	Variable Definition
Sex	Sex of the household head i.e. the major decision maker for agriculture related matters of the household (1 = male, 0 = female)
Caste	Caste of the household (1 = upper caste, 0 = other caste), upper caste includes Brahmin and Chettery households and remaining are considered other caste
Seedtype	Type of seed (1 = hybrid, 0 = non-hybrid i.e. improved and local varieties)
Distance	Distance to usual fertilizer purchase point from the household, measured in kilometers
AgLiteracy	Agronomical literacy score calculated based on the percentage of correctly answered questions out of total 13 multiple-choice questions (related to varieties, chemical fertilizer, micronutrient, government extension and subsidy on seed etc.)
Rainfall[2]	Deviation of mean monthly rainfall for June 2016 to June 2017 from mean monthly rainfall of June 2008 to May 2016. The average monthly rainfall is based on Climate Hazard InfraRed Precipitation with Station (CHIRPS) version 2.0 data with spatial resolution 0.05 degree (5 kilometers approx.).
Soil_pH[3]	Soil pH is the measure of acidity or alkalinity in soil and the soil pH data was obtained from SoilGrids where pH is calculated as: (pH x 10 using water based solutions at soil depth 0.30 m). The SoilGrids pH values were divided by 10 to bring the pH values in the range from 0 – 14.
Altitude	Altitude of the respondent farmstead for which information is collected during the survey

[2] Source: Climate Hazard Group (ftp://ftp.chg.ucsb.edu/pub/org/chg/products/CHIRPS-2.0/)

[3] Source: https://soilgrids.org

Results

Summary statistics

This paper uses four production variables (input variables) along with yield of maize (output variable) to estimate the stochastic frontier analysis model (using equation 1) to predict the household level efficiencies/ inefficiencies in maize production. To explain the variation in technical inefficiency (using equation 3), a set of eight variables were used (Table 1). The summary statistics of the variables used in equation 1 and equation 3 is presented in Table 2. An average maize producing household surveyed grew maize in 0.25 ha and produced 2.0 MT/ha of maize using 37 kg of seed, 44 kg of chemical fertilizer and 145 labor days per hectare but with considerable variations for each of these variables across households.

The sample respondents consisted of 43% male and 57% female household heads (i.e. major decision makers on agriculture related matters for the household). Around 44% of the respondents belong to upper caste (i.e. Brahmin / Chettery caste). About 16% of the households used hybrid maize seed. The average distance to the usual point of fertilizer purchase was little over two kilometers from farmstead. The average soil pH ranges from 6.2, and the average altitude of maize farms was 972 meters from the sea level. The agronomical literacy was poor with agricultural literacy score of 15%. The average rainfall shock was 0.005 ml.

Table 4. Summary statistic of the variables used in stochastic frontier model (equations 1 and 3)

Variable	Mean (n=364)	Std. Dev	Min	Max
Production function Variables				
Yield (MT/ ha)	2.00	1.15	0.17	7.9
Area (ha)	0.25	0.21	0.34	2.29
Seed (kg/ha)	37.02	21.62	8.43	147.45
Fertilizer (kg/ha)	43.73	76.27	0.00	442.35
Labor (man-days/ ha)	144.57	75.88	21.84	468.45
Inefficiency effect model variables				
Distance	2.27	1.58	1	5
Soil_pH	6.2	0.43	5.7	7.3
Rainfall	0.005	0.08	-0.15	0.16
Altitude	972	580	107	2472
AgLiteracy	15.17	13.54	0	71.43
Sex (dummy)	Male: 43%; Female: 57%			
Caste (dummy)	Upper caste: 46%; Other caste: 54%			
Seedtype (dummy)	Hybrid: 16%; Non-hybrid: 84%			

Stochastic Frontier Estimation

The results of the stochastic production function, based on Maximum Likelihood estimation, shows that the coefficient of four factors of production (area, seed, fertilizer, and labor) have expected positive and significant relationship with yield. Based on the coefficients (elasticities), 1% increase in area, seed, fertilizer and labor would result in 12%, 14%, 2% and 34% increase in yield respectively (Table 3). The mean technical efficiency is 82% (Std. Dev.= 15) but at the individual household level it ranges from 16% to 100% indicating considerable variability among the households (Figure 1). Fifty-four percent households are above average, 34% of the households fall in the third quartile, and 5% of the households have less than 50% efficiency.

In the second stage, eight different variables (Table 1) were used to explain the variation in the level of inefficiency among the maize growing households using Maximum Likelihood estimation as in equation (3). The results are presented in Table 3 and the results are discussed in subsequent sections.

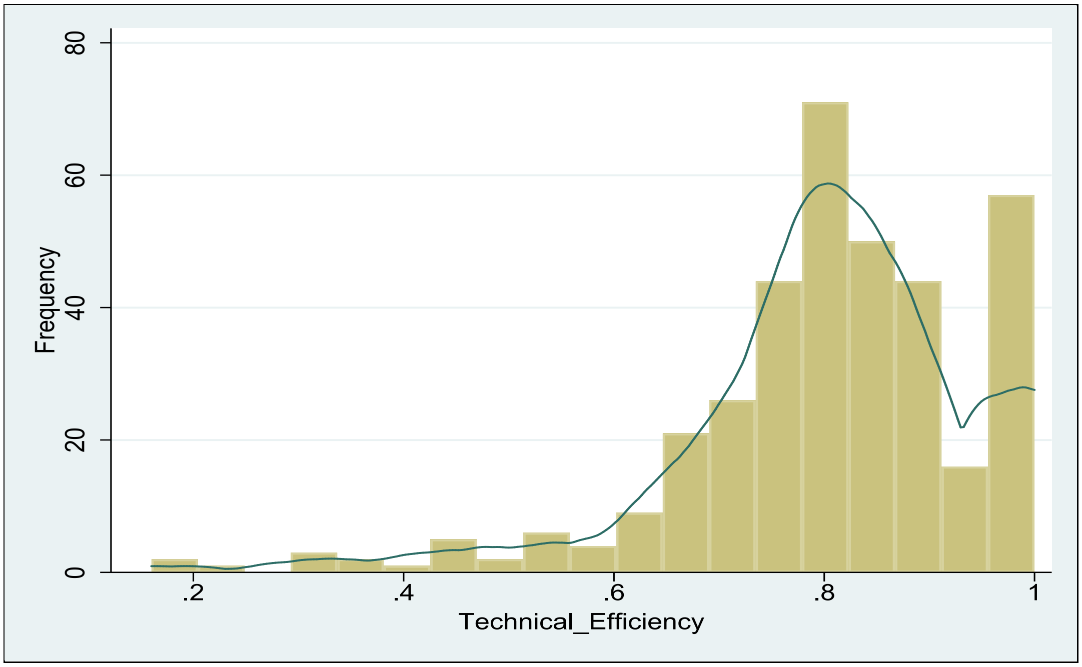

Figure 5. Frequency and density estimates of the technical efficiency of maize farmers.

Sex, caste and technical inefficiency

The positive elasticity for sex indicates the households with male heads (decision makers) are inefficient relative to the female-headed households, which is contrary to common belief in most developing countries including Nepal. However, for our sample, we find higher percentage of female-headed households purchased seed (generally better germination and yield), applied urea in split doses (more efficient use of fertilizer), lived closer to extension services (average of 46 and 62 minutes for female-headed and male-headed household), and lower percentage of these faced shortage of seed and fertilizer in the year. All of these factors can potentially contribute to maize productivity and in turn to greater efficiency of female-headed households. The relationship between sex of the household and the level of efficiency in maize production has been shown to be significantly greater for male headed households in Zimbabwe (Mango *et al*., 2015) but contrary was reported in Ghana (Bempomaa and Acquah, 2014). Though our finding is not unique, further investigation of the underlying causes will help better explain the differences across studies.

The negative and significant elasticity for upper caste households is consistent to expectation as these household in Nepal are generally more educated, and have better access to productive resources. For the households in our sample, almost 42% of the household heads from upper caste had completed class 10 schooling while only 27% of the households from other caste had completed class 10 schooling. In addition, less percentage of upper caste households reported lack of money to buy fertilizer (30% vs 37% households) and this makes sense as the upper caste households in our sample, on an average, had NPR. 136, 000[3] more annual income than households of other caste. Literature also suggest evidence of positive spillover effects of off-farm income on farm production and productivity (Bojnec Š, Fertő I, 2013).

Table 5. Estimation results of two-stage Stochastic Frontier Analysis model (bootstrap, 50 replications)

Variables	Bootstrap Co-efficient	Std. Err	P>z
Productive factors			
ln Area	0.12	0.05	0.03**

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[3] USD 1 = NPR 103, which was the approximate average rate when the survey was done in September 2017.

ln Seed	0.14	0.06	0.01**
ln Fertilizer	0.02	0.01	0.09*
ln Labor	0.34	0.09	0.000***
_cons	5.72	0.41	0.000***
Inefficiency effects			
Sex	0.70	0.29	0.02**
Caste	-0.90	0.48	0.06*
Seed Type	-28.38	9.87	0.004***
Distance	0.26	0.11	0.01**
Soil pH	0.24	0.08	0.004***
Rainfall	9.06	3.72	0.02**
Altitude	0.001	0.001	0.60
Agricultural Literacy	0.001	0.02	0.97
_cons	-18.10	6.02	0.003***
Random error			
_cons	-1.73	0.12	0.000***
Other statistics			
Log Likelihood Ratio -241.83; Prob > χ^2 0.0000*** ; Wald χ2 (4): 38.4			
*p <0.10, **p <0.05, and ***p <0.01			

Hybrid seed, fertilizer access, level of agronomical literacy and technical inefficiency

The elasticities for type of seed used, as expected, is negative and significant indicating that the households that used hybrid seed had significantly lower inefficiency (more efficient) compared to those using non-hybrid seed. The average yield of maize is considerably low for our sample, compared to potential yield of maize varieties used in Nepal, irrespective of the type of seed used. The average yield for hybrid seed users in our sample is 2.87 MT/ha (range: 0.59 to 78.78 Mt/ha) while that for non-hybrids users is 1.84 MT/ha (range: 0.17 to 6.65 MT/ha). Again, 17% of the non-hybrid users had yield less than one Mt/ha while only one hybrid user had yield lower than one MT/ha. In addition, majority of the hybrid seed users (75%) were from terai districts (closer to market and agro-vets) and applied around 7 kg fertilizer (6 kg DAP and 1 kg urea) more per hectare than the non-hybrid users. These probably help explain the lower inefficiency of the hybrid users.

The distance to usual fertilizer purchase point is positive and significant to level of inefficiency of maize farmers as expected. In Nepal, the major fertilizers like urea, DAP and MOP are subsidized and sold through authorized co-operatives with no private sector (agro-vets/ dealers) involved. On an average, the time taken to travel to the nearest co-operative, for the households in our sample, is around 37 minutes and at the upper end it can goes up to 4 hours. Around 11% of the households included in this study reported facing shortage of fertilizer. In that sense, the proximity to point of fertilizer purchase does explain the difference in the level of technical inefficiency among the maize growing households. The level of agronomical literacy was not significant. This probably is due to the fact that the our agronomical literacy score is constructed using 13 different multiple choice questions covering broad range of subjects in agriculture (seed, fertilizer use and nutrient composition, micronutrients, government extension, seed and fertilizer subsidy etc.), rather than questions specific to maize crop alone. Another probable cause for this might be that when the households have poor access to seed and fertilizer (due to availability and affordability), the agronomical literacy alone would not as such translate in to good crop management and higher efficiency.

Environmental variables (soil, rainfall, and altitude) and technical inefficiency

The elasticities for soil pH, rainfall shock and altitude are positive but only soil pH and rainfall shock are significant. For our sample households, the soil pH ranges between 5.7 and 7.3 with a mean of 6.2. The ideal soil pH range for most crops is from 5.5 to 7.5 and for maize 5.5 to 6.5 is considered ideal[4], however, ideal varies on other soil characteristics as well. The soil pH affects many processes necessary for crop growth and yield as high soil pH restricts use and availability of iron (leading to iron chlorosis), most micronutrients, and phosphorus becomes unavailable to plants at high pH (Adeoye and Agboola, 1985 and Mallarino *et al.*, 2011). In our sample, about 17% of the households were in areas with pH above 6.5 and this might, to some extent, explain the positive and significant relationship of soil pH and technical inefficiency.

In Nepal, based on government statistics, more than 80% of maize cultivation is under rain fed conditions and most of this rain fed condition is in hills where almost 73% of the total maize production of Nepal comes from. In our sample, 75% of the households are from the hilly districts. The positive and significant relationship between rainfall shock and technical inefficiency that we find is in line with the findings that suggest rainfall shocks/ variability tend to decrease agricultural productivity of maize (Koo and Cox, 2014; Amare *et al.*, 2018). This paper uses the altitude (continuous variable) of the farm location rather than dummy for district (hill and terai districts) to reflect the variability in altitude across hilly districts to check if the locality of the maize growing area has any effect on the technical inefficiency of maize growers. The elasticity for altitude is not significant, even though yield in higher altitudes, in general, reported to be low relative to teari in Nepal. About 28% of the farms in the hill districts are located below overall average altitude of 972 m from the sea level indicating not all hilly farmers grow maize in the hill slopes but also in fertile river basin (altitude similar to terai districts). This may be the reason for altitude to come as non-significant in the model.

Conclusion and recommendation

The analysis in this study provide few important tips for maize value chain and agricultural extension interventions for improving the technical efficiency of smallholder maize growers in Nepal. We find the female-headed households had 4-point advantage on the level of technical efficiency over male-headed household. However, further analysis by caste and sex shows that female-headed households that belong to upper caste have almost 10 and 15-point advantage over female-headed households and male headed household from other castes respectively. Our results show higher efficiency of female headed household from upper caste (compared to female headed households or male headed household of other caste) is in conformity to the fact that caste/ethnic identity affects the degree to which rural women benefit from development activities in Nepal (Bennett, 2005). In this regard, sex together with caste of farmers need to be considered when designing maize value chain and extension interventions.

Our results suggest that use of hybrid seed puts farm households in advantage in terms of technical efficiency of maize production. Our results is as expected and in agreement with other studies that show the elasticity of hybrid seed on technical efficiency to be positive and significant (Aye, 2010). In our sample, the farmers who used hybrid seed were those who purchased seed. On the other hand, 80% of the farmers who used non-hybrid varieties did not purchase seed meaning most of them used farm saved seed. The farmers who bought seed (replaced seed) had about 10-point advantage in technical efficiency over those who did not. In this regard, easier access to affordable hybrid or good quality improved seed for maize growers in key to enhancing technical efficiency of maize growers.

Three major fertilizers (urea, DAP and MOP) used in Nepal are imported and distributed only by authorized co-operatives under subsidy scheme with no participation of the private sector dealers/ distributors. This

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[4] http://www.cropnutrition.com/efu-soil-ph

limits the number of points from where the fertilizer is distributed and farmers often have to travel long distances for fertilizer (on average 37 minutes of travel to co-operatives). Again, in some instances fertilizer shortage is reported at critical stages of crop growth. The distance to the point of fertilizer potentially limits the purchase and application of fertilizer reducing the yield and efficiency of maize growers. In this regard, our result that suggest positive and significant relationship between proximity to point of fertilizer sale and inefficiency is logical. A policy that eases timely access to fertilizer, which may include involvement of private sector in fertilizer distribution, is suggested for improved efficiency of maize growers.

Our result suggests that rainfall shock (rainfall variability, high or low rainfall) has positive and significant relationship with inefficiency. Low rainfall is a problem for maize growers in Nepal given maize is mostly produced as rain fed crop, especially in the hills. To reduce the risk of negative rainfall shocks, it is suggested that irrigation projects be considered in dry areas suitable for maize cultivation and promote stress tolerant varieties for areas that are hot spots for rainfall shocks. Our results suggest that the soil pH and technical efficiency have inverse and significant relationship for maize growers. In Nepal, there is a single fertilizer dose recommendation for the entire country for maize, irrespective of soil type, which does not make sense for a country that is so agro-ecologically diverse. For this, soil-testing services should be integral to the agriculture extension approach and current blanket fertilizer recommendation should be replaced with a site/ domain specific fertilizer recommendation based on soil testing results. In Nepal as little as 24% of the farmers are reached by the formal extension services. About 86% of the sampled household for this study reported that training related to soil fertility management would be useful for them showing the unmet demand for soil management related extension services. Since, private sector led soil testing services are not available in Nepal, majority of farmers do not have easy access to soil testing service. A mobile soil-testing model tested in Nepal by Pandey *et al.*, 2018 has the potential to motivate farmers to apply fertilizers in both needed quantity and time to enhance the efficiency of maize growers.

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Determinants of the Maize Street Vendors Livelihood: Empirical Evidence from Pakistan

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Introduction

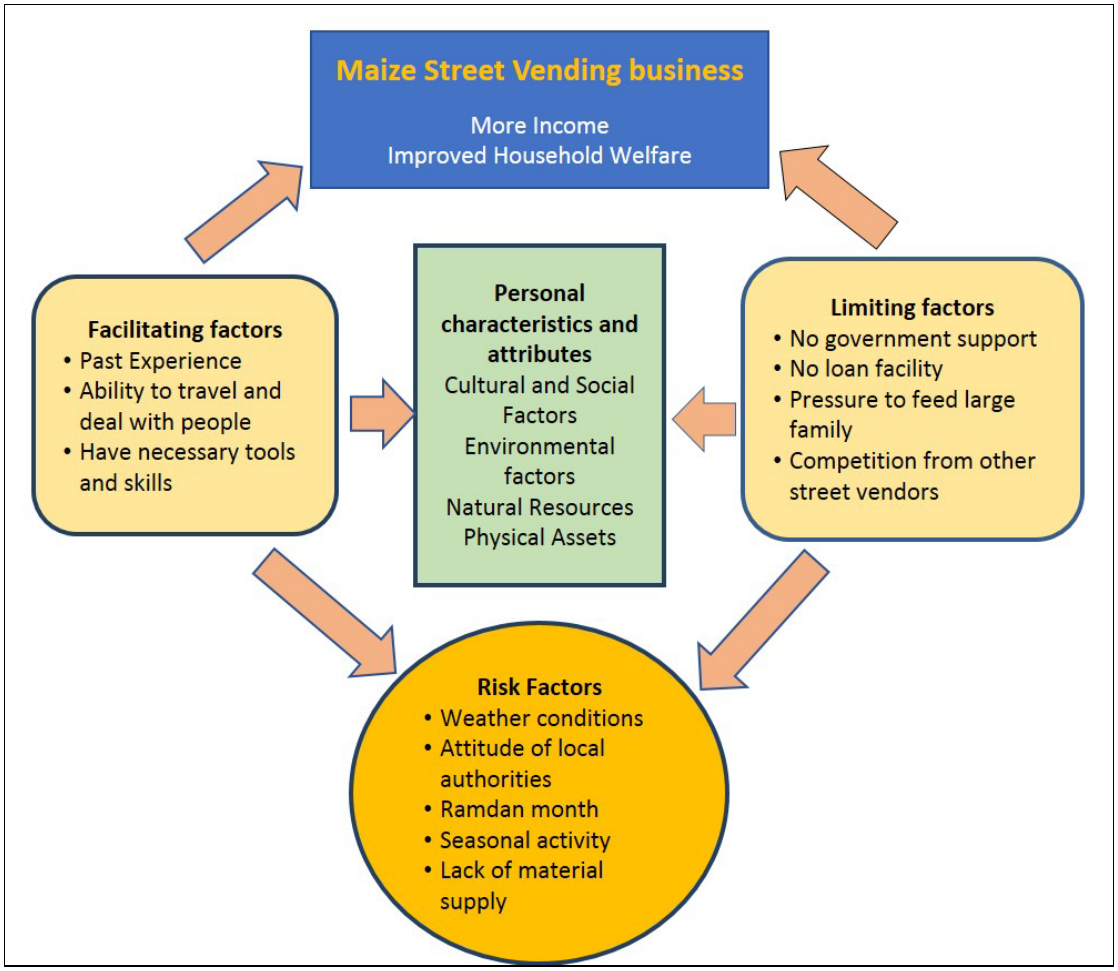
In any human society, employment plays a crucial role in the overall development. In Pakistan majority of the poor people are mostly involved in the informal sector. In Pakistan the informal business sector exists on large scale as Pakistan is the 6th most populous country of the world with total population of more than 210 million (Economy Survey of Pakistan 2016-17). Globally street vending is major source of employment and income for people living in the cities. Due to ever-increasing rate of unemployment and inflation in Pakistan, people were forced to join informal sector to earn their livings. In Pakistan, the number of street vendors is increasing continuously. Business opportunities for maize vendors are bright at public places; mobile maize vendors usually visit schools, hospitals, streets, markets and parks etc. In developing countries a large variety of food is sold by street vendors fulfilling the demand of millions of people. These street foods cater the dietary requirements of many sectors of the population at an affordable price (Ohiokpehai, 2003). Street foods are ready to eat foods and beverages prepared and sold by vendors and hawkers from pushcarts, stalls, baskets, balance pole or from shops (FAO, 1989; Tinker, 1987).

The street vendors can be classified into three main categories i.e. mobile vendors, semi mobile vendors and stationary vendors. The mobile vendors continuously move from one place to the other, the semi mobile vendors occasionally move while the stationary vendors stick to one place (Escalante, 2003). Majority of street venders in urban area are those who have low working skills and they also have migrated to large urban areas from rural areas. These people take street vending as a business when they do not find other way of earning (Bhowmik, K. 1998). The investment in this business is too low, not like other businesses, which need a lot of investment. This business does not require any type of special skills or training.

In any urban society the street vendors are the integral part of the daily life. However, the street vendors livelihood is affected by number of constraints and factors. No doubt street vendors are marginalized community and needs attention and support to improve their livelihood, the current paper is small step in that direction. The main purpose of the current study is to document to livelihood of the maize street vendors in Pakistan and the study has couple of novel aspects for being the first study focusing on the livelihood of the maize street vendors community in Pakistan.

Conceptual Framework

The available options with the street vendors are either to engage himself in the street vendor business or do the daily paid labor. In case of street vending business there is more independence, self-respect and flexible timing. Regarding street vending business there are many options i.e. either to engage in maize street vending or vegetables/fruits/cooking etc. street vending business. The street vending business is facilitated and limited by a number of factors, as shown in the figure below.



Data Collection

The current study is based on cross sectional data set collected from the four big cities of Pakistan i.e. Rawalpindi, Islamabad, Lahore and Faisalabad. Detailed comprehensive questionnaire was used for data collection. Questionnaire included detailed information about the socioeconomic, demographic and business related variables maize street vendors. A team of well-trained enumerators carried out the survey. Before carrying out the formal survey pre testing of the questionnaire was carried out and the questionnaire was improved in the light of the pre testing results. The analysis was carried out by using STATA statistical software. A number of econometric models and techniques were used for estimating the livelihood determinants of maize street vendors.

Results and Discussion

Determinants of Continuing in Maize Street-vending Business

For the determinants of the continuity in the maize street vending business Tobit model was estimated and the results are presented in table 1. Tobit model is censored regression model and it can censor the data both at lower and upper limits. In the current analysis the data has been censored at the lower level i.e. 0 years of experience. The dependent variable was the number of years of experience in the maize street vending business. A number of household and business level variables were included in the model. The age coefficient was positive and significant at 5 percent level of significance. The education was negative and significant at 10 percent levels of significance. The origin was included as dummy variable and the coefficient was positive and significant at 1 percent level of significance. The household head was positive and significant at 1 percent level of significance. The marital status was also positive and significant. The native language was included as dummy variable and the coefficient is positive and significant at 1 percent level of significance. The family size was positive and highly significant at 1 percent level of significance. The family system was also positive and significant at 10 percent level of significance. The coefficients fixed locations, gender, credit and market distance were non-significant. The transport fare was negative and significant at 5 percent level of significance. The cart ownership was positive and significant at 10 percent levels of significance. The weather effect was negative and non-significant. The quality of maize was positive and significant at 10 percent levels of significance. The LR Chi square is highly significant at 1 percent level of significance indicating the robustness of the variables included in the model. The findings indicated that mostly the respondents having less education and were self-household head and have higher family size mostly continue in the maize street vending business.

Table 1. Determinants of the Continuing is the maize street vending business (Years of experience) (Tobit estimates)

Variable	Coefficient	t-values
Age (Years)	0.02**	2.19
Education (Years)	-0.01*	1.85
Origin (1= Rural)	0.009***	2.86
Household head (1=Self)	0.05***	3.22
Marital status (1= married)	0.02***	2.07
Native Language	0.004***	2.71
Family size	0.01***	2.55
Family system	0.003*	1.88
Fixed Location	0.02	0.96
Gender (1=Male)	0.004	1.34
Credit	0.002	1.07
Market Distance	0.05	1.33
Transport fare	-0.03**	-2.14
Cart	0.01*	1.45
Weather affect	-0.03	-1.26
Maize quality (1=Good)	0.01*	1.72
Constant	0.006**	2.13
Numbers of Observations	203	
Value of R^2	0.21	
LR Chi square	264.24	
Prob> Chi square	0.000	

Note: Results are significant at ***, **, * i.e., 1%, 5% and 10% levels, respectively.

Business Cost and Profit

In Pakistan maize street vendors led a very simple life. They have limited income and expenditures. The major monthly expenses indicate that average house rent is about rupees 2378 per month and its almost same in all the four big cities of Pakistan i.e. Islamabad, Lahore, Rawalpindi and Faisalabad.

Monthly expenditure on the kids' education was rupees 1055. The average monthly medical expenditure was rupees 695 per month. The average food expenditure per day were about rupees 303. The average per day profit earned by the maize street vendors were rupees 689. On average the daily sale volume was rupees 1820 and the daily operational cost was rupees 1196. The operational cost comprises of cost of the fuel wood, cost of cob material and other supporting costs.

The fuel wood cost on average was rupees 251 per day and the average daily expenditure on buying green cobs and other material was rupees 842. The other supporting costs were about rupees 101 per day. The other supporting costs include expenditure on the purchase of salt, packets and spices etc.

Conclusion

The current study was carried out in the big cities of Pakistan i.e. Islamabad, Rawalpindi, Lahore and Faisalabad. The empirical findings indicated that maize street vendors earn more as compared to daily paid labor ers. However, in addition they enjoy business freedom. A number of constraints were faced by the maize street vendors especially from the harsh weather conditions. The business volume is decreased due to harsh weather condition. The maize street vendors shift to alternate business during the fasting month of Ramadan. Mostly the street vendors do labor during Ramadan month.

The street vendors mostly do not receive any institutional support from the local authorities. The policy implications from the empirical findings suggest that the enabling business environment needs to be provided to maize street vendors. The institutional support can help the street vendors to do business in more comfortable environment. The local and district administrative authority normally don't facilitate the street vending business and the street vendors were scare from the local authorities as well as from the police. Hence enabling business environment needs to be provided so that the street vendors can earn their living without any fear. The findings of this study can be helpful in identifying peak market seasons for green maize cobs in the major cities of Pakistan. The enabling business environment can facilitate the maize street vendors to increase their business sales and in turn can improve the livelihood. This can help to enhance the overall community development in any developing country.

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Dynamics of Maize Consumption and its Implication in Maize Technology Demand in Nepal

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Introduction

Maize is considered a staple food for hill communities, whereas wheat and rice are staple foods for Terai people of Nepal. Maize is the second most important cereal crop after rice in Nepal which is used as food, feed, fodder and industrial raw material. It contains 11.1% protein, 3.6% fat, 2.7% fiber, 66.3% carbohydrate and 1.5% minerals (Calcium, phosphorous, Iron) and Vitamins (A, B, E) (Joshi et al., 2017). It is grown under diverse agro-ecologies in the country. Of the total maize area, Terai occupies 17.34%, mid hills 72.85% and high hills 9.81%, with average productivity (improved varieties) of 2.79, 2.51 and 2.11 tons per hectare (t/ha) respectively (NMRP, 2017). Although there were some fluctuations, maize was grown in about 0.89 million hectares of land in 2015/2016, with productivity of 2.5 metric tons per hectare (Mt/ha), and 2.2 million metric tons (Mt) of maize produced. Except for the few years (2009/2010, 2012/2013 and 2014/2015) during which maize production declined compared to the previous year, the production, area and productivity of maize is increasing at compounded annual growth rate of 2.91%, 0.57% and 2.35% respectively in the last 15 years (Figure 1). Despite increasing rice eating culture replacing maize based food, the import of maize has been growing for a few years.

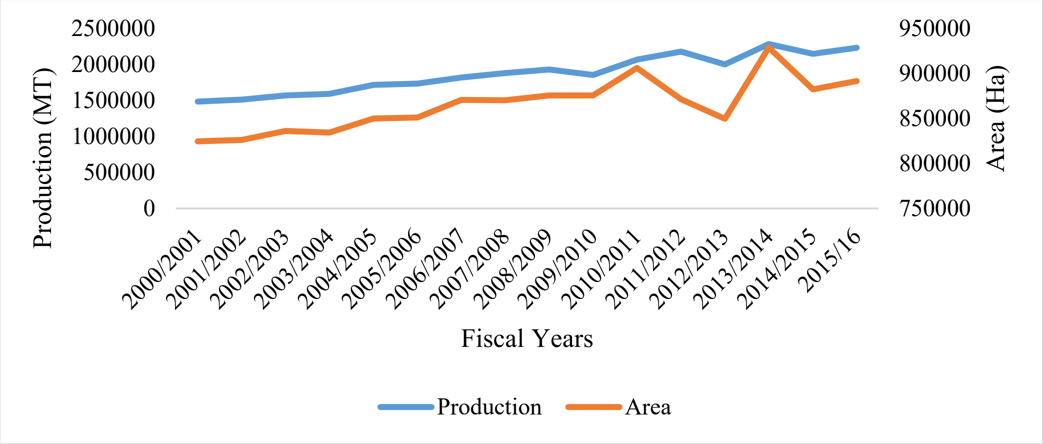

Figure 1. Trend of maize production in Nepal; Source: MOAD, 2017 (Statistical Information on Nepalese Agriculture 2015/2016.

An in-depth study was carried out in 2017 to investigate changes in the pattern of maize consumption and suggest appropriate technology development strategies to cater to the changing needs of the country. A total of 682 maize growing households were randomly selected and surveyed for this study purpose from six districts (Chitwan and Dang from Terai, and Khotang, Sindhupalchock, Lalitpur and Dadeldhura districts from the hills). Review on livestock and poultry development projects was also carried out.

Maize variety development strategy and varieties developed in Nepal

National Maize Research Program (NMRP) envisions generating high yielding maize based production technological packages that contribute to food, feed, nutritional security, employment generation and livelihood improvement of the Nepalese people (NMRP, 2017). Maize research activities to realize this vision include germplasm conservation, development of stress (drought, heat, cold and low nutrient) resilient hybrids and open pollinated varieties, low cost & resource-conserving production technologies, and development of quality protein maize for nutritional enhancement. Also included is the development of specialty maize cultivars such as Quality Protein Maize, baby corn, sweet corn and popcorn for diverse uses. Implementation of these activities has led to the development of different maize varieties in Nepal.

Until 2017, Nepal Agricultural Research Council (NARC) has developed 32 maize varieties (NMRP, 2015; NMRP, 2017); seven hybrids (Gaurav, Khumal Hybrid-2, Rampur Hybrid-2, Rampur Hybrid-4, Rampur Hybrid-6, Rampur Hybrid-8 and Rampur Hybrid-10) and six were de-notified (Makalu-2, Janaki, Sarlahi Seto, Hetauda Composite, Kakani Pahelo and Rampur Pahelo) (NMRP, 2015). Thirty-four imported hybrids of maize were registered in Nepal (NMRP, 2013). Based on two years' multi-location trials conducted by NARC, 13 multinational company hybrids were registered by the National Seed Board in 2016 (NMRP, 2017).

These improved varieties have been scaled up through source seed production. In FY 2016/2017, 43 tons of source seed of maize were produced. Only eight varieties (Rampur Composit, Arun2, Arun 3, Arun 4, Arun 6, Deuti, Manakamana 3, Poshilo makai 1) were used for maize seed production (NMRP, 2017). NARC has been producing source seed for different special projects like the Agriculture and Food Security Project (3000 kg breeder seed and 2600 kg foundation seed) and *Kisan ka lagi Unnat Biu bijan Karyakram* (3000 Kg of breeder seed) for seed multiplication. Seed maintenance has been carried out for released maize varieties. In 2016/2017, grid selection was applied in Rampur Composite and Deuti. Half sib family selection was used in Arun-2, Manakamana-3 and Poshilo Makai-1, and 3 kg, 4 kg and 3.5 kg nucleus seed of these varieties, respectively, was produced (NMRP, 2017). In the case of Rampur Composite, 7.37 tons of breeder seed and 13.02 tons of foundation seed were produced. In case of Arun-2, 1.34 tons of breeder seed and 3.93 tons of foundation seed were produced. For Manakamana-3, 3.19 tons of breeder seed and 11.10 tons of foundation seed, while for Deuti, 1.24 tons of breeder seed and 2.60 tons of foundation seed were produced. Finally, 0.06 tons of breeder seed and 0.11 tons of foundation seed of Poshilo Makai-1 were produced. However, Ghimire et al (2003) reported that seed cycle was not maintained, leading to low and slow adoption of newer maize varieties in Nepal.

Joshi et al. (2017) reported that the number indicated after any maize variety refers to Kernel Color. White maize varieties have odd numbers (e.g. Manakamana-1, 3, 5; Poshilo-1; Arun-1, 3; Ganesh-1 etc) and yellow varieties have even numbers (e.g. Arun-2, 4, 6; Rampur Hybrid-2, Rampur Hybrid-4, Rampur Hybrid-6, Khumal Hybrid-2, Rampur Hybrid-8 and Rampur Hybrid-10 etc.). It is common understanding in Nepal that white varieties are for food and yellow varieties are for feed; but farmers in Kavre and Lamjung do not produce different varieties based on their utilization such as food, feed and seed purpose (Timsina et al., 2016). These varieties have been scaled up in different parts of the country, leading to increased maize production.

Utilization of maize grains in Nepal

Maize produced in the study area was used by households for food, feed, seed purposes and income generation (Table 1). Since the survey areas were major maize growing areas, farmers sold maize grains mainly to poultry feed companies. The number of farmers selling maize for poultry feed was higher in Terai districts, particularly where poultry businesses flourish, while sale of maize as seed was found higher in hilly districts. A significant number of farmers used grains as household food and sold sizeable volumes at the market.

Table 1. Distribution of farm households in maize utilization in the study area, number of respondents

Utilization of Maize by Households										
Districts	**Sale of maize**	**Sale of maize for poultry feed**	**Sale of maize as cattle feed**	**Sale of maize as seed**	**Sale of maize as food grain**	**Use of maize for backyard poultry**	**Use of maize as cattle feed**	**Saved maize for seed purpose**	**Maize consumed as food**	**Total**
Chitwan	63 (65)	46 (47)	17 (18)	1 (1)	11 (11)	9 (9)	77 (79)	17 (18)	57 (59)	97
Dang	111 (78)	96 (67)	31 (22)	30 (21)	12 (12)	28 (20)	116 (81)	107 (75)	133 (93)	143
Khotang	42 (38)	0 (0)	0 (0)	32 (29)	20 (18)	66 (60)	101 (92)	81 (74)	109 (99)	110
Lalitpur	45 (41)	33 (30)	4 (4)	7 (6)	4 (4)	0 (0)	107 (96)	71 (64)	110 (99)	111
Dadeldhura	89 (81)	6 (5)	1 (1)	74 (67)	19 (17)	7 (6)	72 (65)	62 (56)	86 (78)	110
Sindhupalchock	37 (33)	1 (1)	0 (0)	22 (20)	16 (14)	18 (16)	92 (83)	25 (23)	96 (86)	111
Total	**387 (57)**	**182 (27)**	**53 (8)**	**166 (24)**	**82 (13)**	**128 (19)**	**565 (83)**	**363 (53)**	**591 (87)**	**682**

Source: Field Survey, 2017; Figures in parentheses indicate percentage.

While analyzing the use of total maize produced by the respondents for different purposes (Table 2), the study revealed that; 36% of the total maize produced by farmers was sold, 43% was used as livestock feed, 21% was sold to poultry feed enterprises, 18% was for household consumption, 6% was sold as seed, 5% was sold as food grain, 4% was sold as cattle feed (4%), and 2% each was saved as seed, and fed to backyard poultry (Table 2).

Table 2. Percentage use of maize produced by respondents for different purposes.

Total maize utilized by the households (%)										
Districts	**Maize sale**	**Sale for poultry feed**	**Sale as cattle feed**	**Sale as seed**	**Sale as food grain**	**Consumed as food grain**	**Used for poultry feed**	**Used for cattle feed**	**Saved for seed purpose**	**Total**
Chitwan	58.3	44.1	7.0	0.0	7.1	7.9	1.7	31.4	0.7	100
Dang	60.5	41.1	5.1	10.4	4.0	14.9	0.7	22.1	1.9	100
Khotang	16.1	0.0	0.0	8.0	8.0	27.0	9.5	45.0	3.0	100
Lalitpur	20.7	14.2	3.4	2.2	0.9	20.5	0.0	57.5	1.2	100
Dadeldhura	53.1	1.0	0.4	44.0	7.7	24.5	1.0	18.2	3.3	100
Sindhupalchock	9.4	0.1	0.4	1.8	7.0	19.4	1.7	63.1	6.5	100
Total	**35.7**	**21.2**	**3.5**	**6.1**	**4.9**	**17.5**	**1.9**	**42.7**	**2.3**	**100**

Source: Field Survey, 2016.

Nepal has a per capita maize consumption of 98 g/person/day, which is the highest in South Asia (Ranum et al, 2014). In the eastern, central and western hills, maize is prepared as Bhaat (grits cooked much the same way as rice) or Dhindo (porridge) whereas in the mid and far-western hills, maize is prepared as Roti (home-made bread) and people prefer a soft and floury maize grain (Poudyal et al., 2001). In the mid hills and high hills of Nepal, more than 86% of the maize produced is used for human consumption and very little is fed to the animals (Paudel, 2008; Gurung et al, 2011). However, Timsina et al. (2016) revealed that 60%, 25% and 3% of the maize grains were used for animal feed, food and seed respectively in hill districts, and the remaining 12% of the total maize produced was sold to different buyers. In Terai, more than 80% of the total maize produced is utilized for poultry and animal feeds, and 10% each for industries and human consumption (Gurung et al., 2011; Timsina et al., 2016). In central Terai, 95% of maize production goes to the market and the rest used for domestic animal feed (Poudyal et al., 2001). We found that even in the hills major maize production goes to cattle and buffalo feed, and in the Terai, to poultry feed production.

The rate of demand for feed increased to 11% per annum (pa), while the poultry industry expansion rate is 8.7% pa (CDD, 2011; KC et al, 2015). Over the last five years, the demand for poultry feed and animal feed is increased by 13% and 8.5% respectively (Timsina et al., 2016). The poultry industry is likely to expand at least three-fold in the next decade. The existing poultry industries in Nepal need about 646,000 metric tons of feed, whereas only 500,000 metric tons of feed is produced annually by the 114 feed businesses operating under National Feed Industry Association (NFIA) in Nepal (Bhattarai, 2011; CDD, 2011; KC et al., 2015).

In Nepal, maize demand has been growing by about 5% annually (Sapkota and Pokhrel, 2010). The feed businesses affiliated with NFIA in Nepal require 1.5 million tons of maize each year, 87% of which is imported from India (Timsina et al., 2016). The overall demand for maize, mostly driven by the feed industry, is expected to grow by 4-6 % pa over the next 20 years (Poudyal et al., 2001).

In the maize super zone of Prime Minister Agriculture Modernization Project, Dang district, maize consumption in the poultry sector increased from 5,000 mt in 2006 to 20,750 mt in 2010, and poultry businesses increased from 100 to 416 in the last five years. In other sectors (cattle, pig and fish industries), maize consumption increased from 5 mt to 107 mt, while industrial growth rate went from 3% to 64% in the last five years. The price of maize grain increased from Rs. 13.8/kg to Rs.18/kg. Maize imports within the district increased from 1000mt to 5000 mt (CDD, 2011).

Consumption of protein and micronutrient rich food items in Nepal has gone up over the decades (MOAD/CBS, 2016), as has the average per capita consumption expenditure for grain and cereals (from NRs. 9478 in 2013/2014 to NRs.9896 in 2015/2016), and for meat and fish, from NRs. 4807 in 2013/2014 to NRs. 5354 in 2015/2016 (AHS, 2014; AHS, 2016). The average per capita food consumption expenditure in 2015/2016 for grains and cereals, meat and fish, and egg and milk products accounted for 32.1%, 14.5% and 8% respectively (AHS, 2016). With the change in income, a rice-eating culture has developed. In 2014/2015 the average per capita consumption of coarse rice was 90.52 kg per year in rural areas, majority representing hill areas (AHS, 2015); this has increased to 109.4 kg per year in 2015/2016 (AHS, 2016). The lower social value attached to maize-based food has caused the decrease in the average per capita consumption of maize from 8 kg per year in 2014/2015 to 7.4 kg per year in 2015/2016. Likewise, the average per capita consumption of maize flour decreased from 16.35 kg per year in 2014/2015 to 10.1 kg per year in 2015/2016. To fulfill protein requirement in the diet, people are consuming more meat products. The average per capita consumption of chicken, egg, buff and milk was 6.9, 2, 2 and 37.1 kg per year respectively in 2015/2016 (AHS, 2016).

The annual growth rate of maize for food to feed from 1991 to 1999 was 2.2% and 45.10% respectively (Gerpacio, 2001). KC et al. (2015) reported that total maize production in 2014 was 2.28 million mt and the quantity of maize requirement for food per year was around 2.9 million mt, which indicates a deficit of only 0.67 million mt. However, there is a need of about 6.46 million mt feed to smoothly run the existing poultry industries in Nepal. Thus, the demand for maize in Nepal is shifting from food to feed for poultry and livestock.

Growing livestock feed demand in Nepal

High demand of feed for poultry and livestock is due to the implementation of many livestock and poultry development projects (Table 3) in the recent past, where the public and private sectors intensified related development activities. This led to high populations of cattle, buffalo, poultry, sheep/goat and swine. Upadhyay et al. (2017) reported that over 20 years (from 1995-2014) annual growth rate of cattle, buffalo, chicken, pigs and goats were 0.26, 2.47, 5.73, 3.61 and 2.07 respectively.

Table 3. Recent Livestock and Poultry development programs in Nepal.

Name of project/program	**Activities**
Community livestock development project (CLDP) in 43 districts (2003-2011); Third livestock development project (TLDP) in 19 districts (1997 –2004).	Supply of breeding buffalo bulls and AI services, and extension of annual and perennial forage production services have been provided in 43 districts.
Second livestock development project (SLDP) (1987- 1988).	Provision of vaccines, drugs, disease control measures, distribution of improved planting materials for animal nutrition and fodder development, provision of genetic materials and stock, and extension and support services in animal husbandry.
High mountain agribusiness and livelihood improvement projects (HIMALI) in 10 districts (2012-18).	Forage production demonstration executed 26 times, purchase of 30 yak/nak and distribution to farmers.
Avian influenza control program in 75 districts (2006/07-2010/11).	Conducted surveillance, quarantine measures, controlled outbreak of bird flu in Kaski and Sunsari district in 2006/2007.
Livestock development program in Karnali zone (ongoing since 2011/2012).	Massive distribution of bucks of goats and sheep, bulls of cattle and buffalo, poultry. Animal health services also provided.
National livestock breed improvement program in 45 districts (ongoing since 2011/2012).	235 artificial insemination Kendra established, technicians trained, large number of animals artificially inseminated.
National FMD control program in 15 districts (2011/2012).	12 lakh livestock were vaccinated for free. Publication of awareness materials and distribution was done.
National HS & BQ control program in 26 districts.	Four lakh 50 thousand cattle and buffaloes were vaccinated for free. Publication of awareness materials and distribution was done.
National ranikhet disease control program in 26 districts.	In rural areas, six lakhs chickens were vaccinated for free. Publication of awareness materials and distribution was done.
National PPR control programs (ongoing since 2001).	Free vaccination, quarantine measures, vaccine bank management, PPR Test Elisa Test Kit procurement. Publication of awareness materials and distribution was done.
PACT in 25 districts (2009-2018).	Herd improvement support works.

These development programs have scaled up high yielding animal breeds (mainly cattle, buffalo, goats, pig and poultry) and associated technologies (like animal health improvement) in the country. Demand for livestock and poultry feed was followed by establishment of feed industries.

Poultry feed production, maize requirement and import in Nepal

Focus group discussions were carried out to assess issues related to feed production in Nepal. This industry demands dent type, yellow color and bold size grains. There is a huge demand of poultry feed for a rapidly growing poultry industry in Nepal. The NFIA has 114 member businesses, about 93% of which produce and supply poultry feed in the country, while the rest produce and supply livestock feed.

Total of 724,192 metric tons of poultry feed were produced in FY 2013/2014 (Bhattarai, 2016). At present, about 2200 Mt of poultry feed are produced and supplied everyday by these feed industries (personal interview NFIA). An estimated 0.8 million metric tons of poultry feed are produced annually in Nepal. Maize is the key ingredient in poultry feed, with major attributes for selection being; grain color (yellow), bold grain, moisture content of less than 16%, free from disease, insects and inert materials, and uniform in size. Other major ingredients used in poultry feed include soyabean cake, sesame cake, sunflower cake, de-oiled rice bran, broken rice and wheat, fish meal, bone meal, mineral mixtures, vitamins and feed additives. Poultry feed industries use available domestic maize for feed production, but since local supply is not enough, imported maize from India and other countries make up the difference (FAO, 2014).

While analyzing the import trend of maize grains only, it was found that about 0.35 Million Mt of maize grain in FY 2015/2016 was imported from India, Brazil and Argentina. The compounded annual growth rate of maize imports was 30.5% over the last eight years in Nepal (Figure 2).

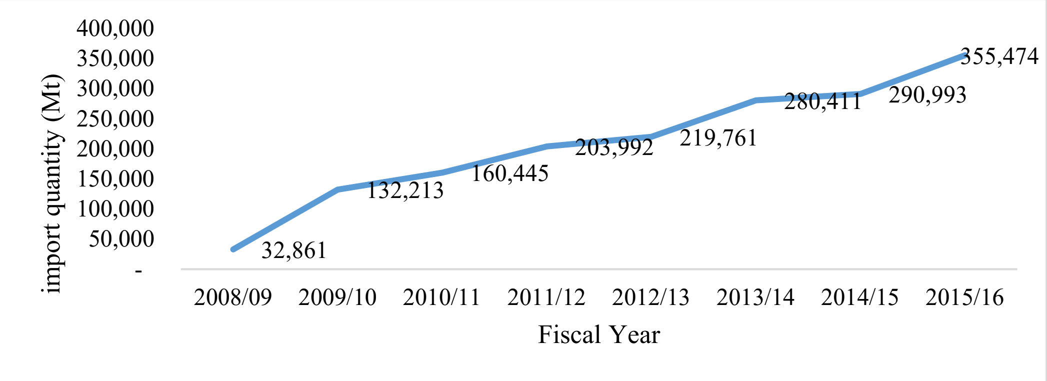

Figure 2. Trend of maize import in Nepal; Source: Source: MOAD, 2017 (Statistical Information on Nepalese Agriculture, 2008/09-2015/16).

Conclusion and way forward

With the surge of new high yielding breeds of cattle and buffalo in the hills through big livestock development projects in the past, maize is now more widely used as concentrate feed rather than as food. Poultry businesses have further contributed to this trend. Rice is now socially preferable to maize and protein consumption also gone up due to a rise in per capita income. Maize variety development in Nepal has so far been targeting food security. This study aims to realign the maize variety development strategy to increasing feed demand for livestock and poultry.

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Specialty Maize Industry in Thailand – Status and Prospects

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Introduction

Sweet corn (*Zea mays* L. *saccharata*) production is mostly in temperate zone, especially for the three world's largest exporters (the United States, France, and Hungary). However, the success of tropical sweet corn production occurs in Thailand where it has been continuously developing super sweet corn (shrunken-2 gene, *sh2*) more than 40 years. At beginning, sweet corn was produced for the fresh market and processing in the last two decades. Presently, it is the most economically important vegetable crop in Thailand. Sweet corn is used in two ways, fresh and processed consumption (whole kernel canning, cream-style canning, and whole ear freezing, etc.). The sweet corn breeding programs in Thailand have been developed by public and private sectors which was reviewed by Aekatasanawan and Aekatasanawan (2007).

Baby corn (*Zea mays* L.) production was initiated in Taiwan. However, the success of baby corn production occurs in Thailand (Chutkaew and Paroda, 1994; Aekatasanawan, 2001). Baby corn breeding programs in Thailand has been continuously developed by public and private sectors for 40 years. Baby corn is used in two ways, fresh or processed consumption. It's ears are popular as canned ears or with stir-fried vegetables in Chinese-American and European restaurants. Nearly two decades, a market for fresh baby corn ears in trays has emerged in Europe, mainly for use as a decorative, crisp vegetable in salads (Aekatasanawan, 2001). Baby corn is popular vegetable because of its high nutritive value and freedom from pesticides compared with other vegetables. Generally, there is no need to apply pesticides. The young cob is wrapped tightly in its husk.

Baby corn is the young and unfertilized ear of the corn (Zea mays L.) plant harvested when the silks have either not emerged or just emerged (1 to 3 cm). The husked young ear in canned or fresh ear style is more popular vegetable because of its sweetness, flavor, and crispness. Generally, the requirements of baby corn for the fresh market or processing are 1) ear size of 4 to 9 cm length and 1.0 to 1.5 cm diameter and 2) good quality; i.e., yellow color, straight ovary row arrangement, unfertilized and unbroken ear, and size within factory specifications.) For baby corn production, varieties are grown under high plant densities (120,000 to 160,000 plants ha^{-1}) and irrigation and high nitrogen application (Aekatasanawan, 2001). Someone might use low plant density for picking 3-4 ears per plant.

Sweet Corn Industry in Thailand

Thailand is the world's largest exporter of sweet corns. Thai sweet corn market has dramatically increased in the past 20 years and sweet corn industry has been continuously developing more than three decades. In 2017, Thailand exported canned sweet corn 208,860 tons, worth 189.56 million dollars and frozen sweet corn 22,782 tons, worth 27.03 million dollars. In the global trade, Japan, is the largest customer for canned and frozen sweet corn, followed by S. Korea and Taiwan for canned sweet corn and Iran, Taiwan and China for frozen sweet corn. In 2016, Thailand is the largest exporter for the quantity of canned sweet corn accounting for 27.83% of the world's export volume (Table 1) and 22.39% for its value (Table 2). The following exporters for canned sweet corn were Hungary, France and United States of America (Tables 1-2).

Baby Corn Industry in Thailand

Thailand is the world's largest exporter of baby corn. In 1973, it exported only 90 tons of canned baby corn, worth 0.03 million dollars. By1998, the volume and value had increased to 54,643 tons, worth 42.89 million dollars. Thailand also exported 2,220 tons of fresh baby corn in 1988, worth 1.54 million dollars, but exports increased dramatically to 11,924 tons in 1998, worth 1.87 million dollars. The greatest consumers of canned baby corn besides Thailand are USA, Netherlands, Japan, Germany, Canada, Australia, Hong Kong, UK, France, Singapore, South Korea, etc. During 2002-2004, Thailand exported preserved baby corn products to almost 100 countries and fresh baby corn to approximately 30 countries. worth nearly 1,700 m baht in each year. Thailand has continuously dominated for the world leading exporter. In 2017, it exported canned baby corn 25,504 tons, worth 26.64 million dollars (Table 3).

Table 1. Quantity (in tons) of canned sweet corn of exporting countries during 2010-2016.

Country	2010	2011	2012	2013	2014	2015	2016
World	**687,910**	**704,015**	**709,307**	**743,918**	**756,666**	**707,542**	**751,809**
Thailand	**173,170**	**184,178**	**172,188**	**167,025**	**200,044**	**186,060**	**209,251**
Hungary	140,643	128,545	140,950	179,041	176,329	163,455	179,779
France	112,926	120,915	122,233	113,563	115,011	102,334	97,264
United States of America	117,885	106,140	111,411	102,992	94,688	92,543	95,813
China	36,853	44,916	36,578	46,653	44,858	39,537	45,133
Other	106,433	119,321	125,947	134,644	125,736	123,613	124,569

Source: Thailand Ministry of Commerce.

Table 2. Value (in US Dollars) of canned sweet corn of exporting countries during 2010-2016.

Country	2010	2011	2012	2013	2014	2015	2016
World	**831.1**	**915.2**	**954.5**	**1,005.6**	**1,023.9**	**850.4**	**875.4**
Thailand	**161.4**	**189.2**	**183.9**	**178.7**	**206.3**	**180.0**	**196.0**
Hungary	176.5	174.4	186.5	220.7	228.7	179.3	185.0
France	194.2	218.1	215.3	207.6	210.4	149.8	140.1
United States of America	119.4	106.9	125.2	129.3	118.3	114.1	107.8
China	36.3	48.8	42.7	57.4	58.9	54.9	75.5
Other	143.4	177.6	200.9	211.8	201.3	172.3	171.0

Table 3. Exports of canned baby corn (quantity in tons) from Thailand during 2015-2017.

Country	2015		2016		2017	
	Quantity[1]	Value[2]	Quantity	Value	Quantity	Value
World	**25,797,455.0**	**934.3**	**26,607,144.0**	**976.8**	**25,504,125.0**	**932.5**
U.S.A.	10,498,162.0	309.8	11,470,710.0	342.8	11,276,048.0	334.7
Japan	2,897,215.0	164.2	2,957,522.0	179.6	2,763,737.0	167.1
Australia	1,583,612.0	63.9	1,493,442.0	57.8	1,714,932.0	65.1
Canada	1,948,914.0	70.6	1,882,149.0	67.5	1,636,498.0	57.0
Israel	566,784.0	23.2	1,018,560.0	36.2	1,088,748.0	39.4
Turkey	349,567.0	12.7	239,937.0	9.1	393,317.0	14.9
Germany	1,048,772.0	33.3	607,547.0	19.9	476,757.0	16.8
Philippines	332,520.0	12.9	428,298.0	17.9	473,076.0	18.7
United Arab Emirates	409,472.0	15.8	568,577.0	19.9	385,475.0	16.9
Netherlands	362,722.0	12.9	455,492.0	15.5	454,566.0	15.9
Chile	562,314.0	24.5	624,240.0	28.5	597,372.0	27.5
Taiwan	568,616.0	17.0	368,602.0	11.1	365,966.0	11.2
Singapore	264,133.0	10.5	301,743.0	12.0	188,125.0	7.8
Sweden	671,942.0	22.9	437,057.0	15.5	468,041.0	13.8
New Zealand	256,294.0	8.5	343,967.0	12.2	289,558.0	10.5
Denmark	173,224.0	6.9	199,403.0	8.7	223,085.0	9.2
Norway	311,507.0	12.5	345,262.0	14.0	280,420.0	11.6
Mexico	42,090.0	2.1	94,242.0	5.3	23,760.0	1.7
United Kingdom	438,163.0	15.9	500,494.0	19.0	572,523.0	24.2
Russian Federation	120,650.0	3.2	180,438.0	4.4	126,098.0	3.3
France	266,938.0	13.6	398,215.0	18.2	221,691.0	9.7
Kuwait	50,565.0	2.2	39,948.0	1.9	57,184.0	2.4
	23,724,176.0	**859.1**	**24,955,845.0**	**916.9**	**24,076,977.0**	**879.3**

[2] million baht; 32-36 baht = 1 USD.
Source: Thailand Ministry of Commerce.

Summary

The success story of sweet corn and baby corn industries in Thailand is composed of 1) high-yielding hybrids with good eating quality, 2) good production management of fresh corn, 3) good processing plants and 4) good cooperation between public and private sectors. The prospect of specialty maize industry in Thailand is frozen sweet corn and fresh baby corn for export.

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Marker-assisted Introgression of *Waxy1* Gene into Elite Inbreds for Enhancement of Amylopectin in Maize Hybrids

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Introduction

Maize (*Zea mays* L.) has emerged as one of the most important cereal crops all over the world (Hossain et al. 2018). It is consumed as a staple crop by millions of people in countries of Africa, Asia and Latin America (Shiferaw et al. 2011). Maize assumes global significance because of its versatility in utilization, such as human food, livestock feed and raw materials for several agro-based industries (Prasanna et al. 2010, Choudhary et al. 2014). Among the varied types of specialty corns, waxy corn - also known as sticky maize or glutinous maize - is a popular choice in South Asia (Xiaoyang et al. 2017) and is a prospective raw material for industry (Devi et al. 2017). Waxy corn contains 95-100% amylopectin (a branched-chain starch) whereas normal maize contains 70-75% amylopectin (Zhou et al. 2016). In countries like Thailand, Vietnam, Laos, Myanmar, China, Taiwan, Philippines and Korea, waxy maize is an important component of diet. Due to high amylopectin content, waxy corn is highly viscous and easily digested in the human gut (Lu and Lu 2012). These characteristics make waxy maize widely popular in frozen food processing and livestock feeding industries (Yang et al. 2013). In addition, amylopectin is a major ingredient in paper, textile, corrugating, adhesive and food industries (Bao et al. 2012). Due to its starch composition and economic value, waxy corn holds considerable promise as an economically viable crop worldwide (Tian et al. 2009).

Waxy maize originated from the cultivated flint maize through mutation (Fan et al. 2008, Zheng et al. 2013). *Waxy1* (*Wx1)* gene consists of 4.8 kb which contains 14 exons and 13 introns and is mapped on the short arm of chromosome-9 (Klosgen et al. 1986, Mason et al.1998). *Wx1* codes granule-bound starch synthase (GBSS-I) enzyme which catalyzes amylose synthesis from ADP-glucose in amyloplasts of maize endosperm. Different types of mutation such as insertion of transposon, retroposon and fragments of few nucleotides and deletion of nucleotides result in mutant allele (*wx1*). These mutations create the synthesis of altered transcript - with premature stop codon, change in amino acids in key domain, splicing or translational errors - that in turn partially block the activity of wild-type *Wx1* allele or inhibit the activity of GBSS-I which results in lower amylose and higher amylopectin in grains (Bao et al. 2012, Zhang et al. 2013). Generally, GBSS-I coded by dominant *Wx1* is highly active in non-waxy maize; however, GBSS-I coded by recessive *wx1* possesses reduced activity (Wessler et al. 1986, Liu et al. 2007).

People in north-eastern states of India prefer waxy maize as food over traditional maize. *Mimban* landrace, among other waxy landraces, is very popular due to its high amylopectin content. The green cobs from waxy maize are a popular breakfast item in urban areas and can be an important source of livelihood to farming communities. However, the grain yield potential of these waxy landraces is low compared to single cross hybrids, and they possess very narrow adaptation. So far, no waxy maize hybrid has been developed and commercialized in India (Devi et al. 2017). Few countries such as China (Yu et al. 2012, Zheng et al. 2013, Hao et al. 2015), Vietnam (Liet and Tinh 2009) and Korea (Park et al. 2008) have reports on waxy maize germplasm. ICAR-Indian Agricultural Research Institute (IARI), New Delhi, has developed a set of waxy inbreds selected from diverse source populations and targeted introgression breeding strategies (Devi et al. 2017). These waxy inbreds can be effectively used as donor lines for improvement of amylopectin in maize. Marker-assisted selection (MAS) provides the easiest way to improve already existing maize hybrids for amylopectin in a short time (Zunjare et al. 2018, Sarika et al. 2018). Use of inexpensive DNA markers that are tightly linked or present within the gene helps in introgression of target gene(s) into a genetic background without any progeny testing (Gupta et al. 2013). It also significantly reduces number of breeding cycles required to reconstitute the recurrent parent genome. The present investigation was undertaken to introgress the favorable allele of *wx1* in elite inbred parents of agronomically superior commercial maize hybrids through marker-assisted backcross breeding (MABB).

Materials and Methods

Plant Materials

Seven parental inbreds viz., HKI323, HKI1105, HKI1128, HKI193-1, HKI193-2, HKI161 and HKI163 of nine released hybrids [HM4 (HKI1105× HKI323), HM8 (HKI1105× HKI161), HM9 (HKI1105× HKI1128), HM10 (HKI193-2× HKI1128), HM11 (HKI1128× HKI163), HQPM1 (HKI193-1 × HKI163), HQPM4 (HKI193-2 × HKI161), HQPM5 (HKI163 × HKI161) and HQPM7 (HKI193-1 × HKI161)] low in amylopectin were targeted for marker-assisted introgression of *wx1* allele. The popular and commercial maize hybrids are adapted to diverse agro-ecologies of India (Table 1). Recurrent parents were crossed with donor lines. MGU1-*wx1* developed at IARI, New Delhi was used as a donor line for high amylopectin.

Table 1. Details of popular commercial hybrids targeted for enhancement of amylopectin.

S. No.	Hybrids	Parental lines	Maturity	Area of adaptation
1	HM4	HKI1105 × HKI323	Medium	Across the India
2	HM8	HKI1105 × HKI161	Medium	Andhra Pradesh, Telengana, Tamil Nadu, Maharashtra & Karnataka
3	HM9	HKI1105 × HKI1128	Medium	West Bengal, Bihar, Jharkhand & Orissa
4	HM10	HKI193-2 × HKI1128	Medium	Delhi, Punjab, Haryana, Western Uttar Pradesh, Rajasthan, Madhya Pradesh, Gujarat, Andhra Pradesh, Telengana, Tamil Nadu, Maharashtra and Karnataka
5	HM11	HKI1128 × HKI163	Late	Across the India except Himalayan belt
6	HQPM1	HKI193-1 × HKI163	Late	Across the India
7	HQPM4	HKI193-2 × HKI161	Late	Across the India except Himalayan belt
8	HQPM5	HKI163 × HKI161	Late	Across the India
9	HQPM7	HKI193-1 × HKI161	Late	Karnataka, Andhra Pradesh, Telengana, Tamil Nadu & Maharashtra

Marker-assisted backcross breeding scheme (MABB)

Two backcross generation-based MABB schemes were followed in the present study. Crop of rainy season (July-November) was grown at IARI Experimental Farm, New Delhi (29°41'52.13"N and 77°0'24.95"E), while the winter season (December-April) was grown at Winter Nursery Centre (WNC), Hyderabad (17°21'50.39"N and 78°29'42.31"E). Plant × plant crosses were made between recurrent parents (as females) and donors (as males) in 2016 during rainy season. The F_1s of the seven crosses were grown during winter season in 2016/ 2017. Heterozygosity of the F_1s was tested using gene-specific marker; the true F_1s were used as males and backcrossed to their respective recurrent parents. BC_1F_1 progenies were grown during rainy season in 2017, and foreground positive plants were backcrossed to recurrent parents. BC_2F_1 populations were raised during winter season in 2017/ 2018. BC_2F_2 populations being raised during rainy season in 2018 will be selfed to generate BC_2F_3 progenies. The progenies possess high degree of similarity for plant, ear and grain characteristics.

Genotyping of populations

SSR marker *phi027* and *InDel* based *wx2507* markers present within *waxy1* gene (Table 2 and Figure 1) were used to distinguish the parental lines, and foreground selection in BC_1F_1 and BC_2F_1 populations was undertaken (Yang et al. 2013). P*hi027* was used in HKI323, HKI1105 and HKI1128 based populations, while *wx2507* was used in HKI161, HKI163, HKI193-1 and HKI193-2 based populations. The chi-square test was performed using the standard procedure for testing the goodness of fit of the observed segregation pattern at the *wx1* locus in each of the generations. Polymerase chain reaction (PCR) was performed using protocol standardized at Maize Genetics Unit, IARI (Devi et al. 2017). Agarose of 4% concentration (Lonza, Rockland, ME USA) was used for separating the amplicon at 120 V for 2-3 hours along with 100bp DNA ladder (MBA-Fermentas). The amplified products were visualized using a gel documentation system (Alpha-Innotech, California, USA) and scored for the presence and absence of designated allele.

Table 2. Details of gene-based markers used in foreground selection of *wx1* allele.

Marker	**Type**	**Primer sequence (5$^{/}$-3$^{/}$)**	**Reference**
phi027	SSR	F: CACAGCACGTTGCGGATTTCTCT R: GCGTACGTACGACGAAGACAC	www.maizegdb.org
wx-2507F/RG	*InDel*	F: ACCTCAAGAGCAACTACCAGTC R: AAGGACGACTTGAATCTCTCC	Shin et al. 2006

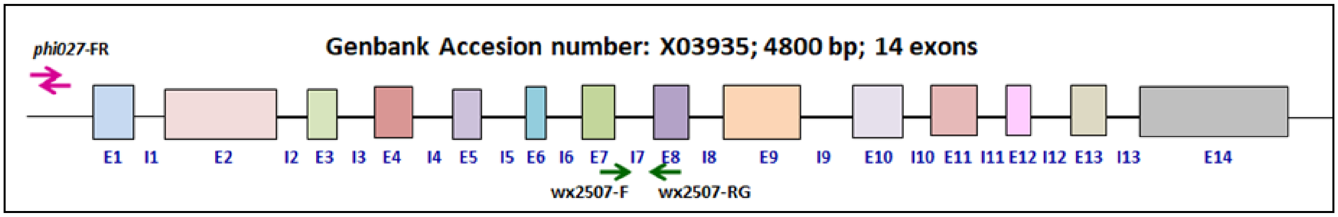

Figure 1. *Waxy1* gene structure depicting locations of *phi057* and *wx2507*

Results and Discussion

Marker polymorphism among parents

SSR marker *phi027* amplified a fragment 158bp in recurrent parents and approximately 145bp in donor parent (Figure 2). The search for recurrent and donor parent polymorphism for *wx2507* showed a 260bp amplicon (favorable allele) in the donor parent, and a distinct 280bp amplicon was generated in the recurrent parent (Figure 3).

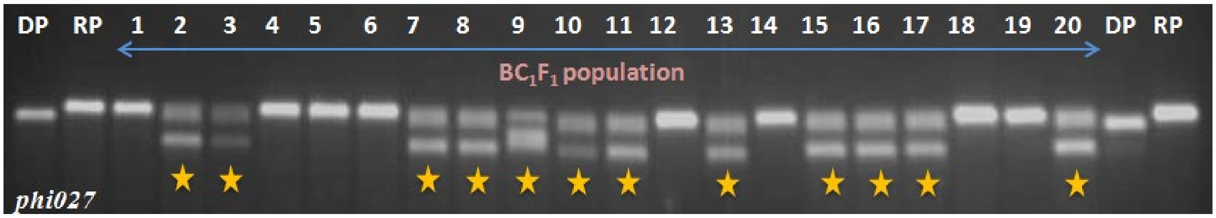

Figure 2. Segregation of *phi057* in HKI1105 × MGU1-*wx1*. DP: donor parent, RP: recurrent parent, Star indicates heterozygote.

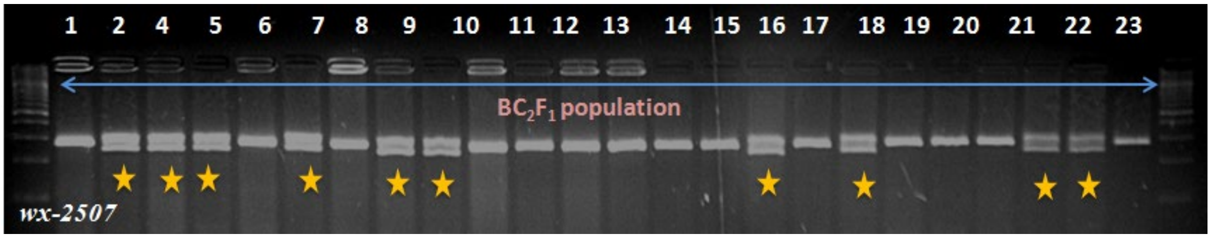

Figure 3. Segregation of *wx2507* in HKI193-1 × MGU1-*wx1*. DP: donor parent, RP: recurrent parent, Star indicates heterozygote.

Marker-assisted foreground selection for wx1

BC_1F_1 populations

In BC_1F_1 populations, 104-115 plants across seven crosses were genotyped (Table 3). Markers clearly differentiated the homozygotes from the heterozygotes. Zhang et al. (2013) and Yang et al. (2013) have successfully used gene-based markers for the selection of *wx1* allele in maize. An average of 55 plants with *Wx1/Wx1* and 53 plants with *Wx1/wx1* were identified (Figure 2). Although the average number of segregants were in congruence with the Mendelian ratio of 1:1, there were few cases in specific crosses where segregation distortion (SD) was observed.

Table 3. Average segregation pattern of *wx1* in different backcross populations.

Generation	Population size		*Wx1/Wx1*		*Wx1/wx1*		Chi-square	P-value
	Mean	Range	Mean	Range	Mean	Range		
BC_1F_1	108	104-115	55	43-64	53	42-62	0.037	0.85^{NS}
BC_2F_1	108	102-111	57	36-100	51	10-72	0.333	0.56^{NS}

BC_2F_1 populations

In BC_2F_1 populations, 102-111 plants across seven crosses were genotyped (Table 3). An average of 57 segregants were of *Wx1/wx1* while 51 possessed the genotype of *Wx1/wx1*, suggesting the segregation ratio of 1:1 (Figure 3). However, the range for *Wx1/Wx1* was 36-100, while the same for *Wx1/wx1* was 10-72. This indicated that in specific crosses SD was observed. The reason for occurrence of SD may be due to the presence of many segregation distortion regions (SDRs) throughout the maize genome (Lu et al. 2002). The reasons of SD could be the presence of genes such as gametophytic factors (*ga*) (Mangelsdorf and Jones 1926) or naturally occurring gene mutants like *dek* (defective kernel) and *emb* (embryo-specific mutation) (Neuffer et al. 1997). The genetic background of the target allele also influenced SD in different generations (Babu et al. 2013). For example, HKI163 based progenies showed SD in all the backcross generations, whereas HK1193-2 based progenies did not show SD in any of the generations (Table 3). Segregation distortion was found among most of the generations, which were evaluated in winter season, suggesting that SD could have been influenced by the environment. This observation is consistent with the results of Vancetovic (2008).

The BC_2F_2 kernels on heterozygous (*Wx1/wx1)* BC_2F_1 ears segregated for normal and waxy kernels (Figure 4). This suggested the efficiency of *phi027* and *wx2507* in selecting *wx1* allele.

Figure 4. Segregation of BC_2F_2-based normal and waxy kernels on BC_2F_1 ears

Marker-assisted background selection

More than 100 polymorphic SSRs distributed throughout the 10 chromosomes in each of the crosses were identified. These SSRs have been used for background selection. Segregants with high recovery of recurrent parent genome (RPG) were selected in BC_1F_1 and BC_2F_1 populations. The selection of progenies with high recovery of RPG helped in achieving high degree of phenotypic similarity for plant, ear and grain characteristics (Muthusamy et al. 2014, Hossain et al. 2018, Sarika et al. 2018, Zunjare et al. 2018).

Conclusion

The present study was aimed at enhancing the amylopectin in the maize kernel. Marker-assisted selection for *wx1* allele successfully identified the heterozygotes (*Wx1/wx1*) in the BC_1F_1 and BC_2F_1 populations. The phenotypic segregation of normal and waxy BC_2F_2 kernels on BC_2F_1 ears suggested the efficiency of markers in selecting the *wx1* allele. Background selection has led to high degree of phenotypic similarity in just two generations of backcrosses. The waxy inbreds and hybrids being developed here would possess higher amylopectin compared to normal maize, which is significant for food and industrial processing. This is the first report of targeted improvement of amylopectin in maize in India.

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Development of Two Sweet Corn Populations with Resistance to Northern Corn Leaf Blight Disease

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Introduction

Sweet corn is an important economic crop in Thailand. Most of the yield is processed as a canned sweet corn. In 2018, the export value had amount $196 million (Thai Food Processors Association, 2017). The most of planted area is in the northern region of the country (Office of agricultural Economics, 2017) which has low temperature. This characteristic causes the problem of an important leaf disease, Northern corn leaf blight (NCLB). It is caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs, which would be a serious outbreak when low temperature at night is ranging from 18-27 degrees Celsius and with high humidity ranging from 90-100% (Juliana *et al*., 2005). This disease can reduce yield of sweet corn 20%-90% which depends on variety environment and management. (Cox, 1956; Raid, 1990; Juliatti *et al*., 2007). So, growing resistance variety is the most effective way to control this disease. (Lipps and Mills, 2002)

The resistance to NCLB is controlled by qualitative and quantitative genetics. Qualitative resistance is control race-specific and inherited by single gene whereas quantitative resistance is race-non-specific and oligogenic or polygenic (Geiger and Heum, 1989). Depending on the environment, qualitative resistance of maize NCLB may have a partial effect while quantitative resistance may have a complete effect (Welz and Geiger, 2000). In addition, as most of the gene action is additive and the level of resistance is related to the number of lesions (Hooker, 1978; Ribeiro *et. al*., 2016) Thus, the germplasm should improve resistance to NCLB for breeding program in the future. The population improvement can use recurrent selection which effective to improve it. Recurrent selection is an excellent method which increase the frequency of favorable alleles in each cycle. Likewise, S_2 reciprocal recurrent selection, which is an effective selection for quantitative genetic and additive gene effect. Furthermore, it can improve two populations with heterosis along the way. (Hallauer, Carena and Miranda., 2010)

The improvement of two sweet corn populations resistance to NCLB disease at the Department of Agriculture (DOA) by S_2 reciprocal recurrent selection was initiated in 2016. This research aims to select S_2 lines with resistance to NCLB disease from CN-NLBCH66C_0S_2 and CN-NLBHX75C_0S_2 populations and to evaluate yield of the progenies between these populations.

Materials and Methods

In 2018, S_2 lines from each population were evaluated for NCLB and yield trial of topcross hybrids between two populations were done. (Figure 1).

Inoculation and NCLB disease evaluation

In dry season, the artificial NCLB disease field was conducted at Chiang Mai Field Crops Research Center, Chiang Mai province, Thailand. Hibrix 3, susceptible variety to NCLB disease, were planted for spreader rows. At the age of three weeks, V3-V4 stage, fungi were inoculated using the colonized sorghum kernels into leaf whorls at the evening and then left the disease spread naturally.

After 2 weeks of growing the spreader rows, 175 S_2 lines of CN-NLBCH66-RRSC$_0$S$_2$, 167 S_2 lines of CN-NLBHX75-RRSC$_0$S$_2$ and Hibrix 3, susceptible check, were planted within spreader field. Data were collected for leaf area infected by NCLB disease. The measurement method was modified by Vincelli and Hershman (2011); 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) at 28 days and 55 days after planting. Based on the percentage of the total leaf area, S_2 lines infected not exceeded 40% of total leaf area from both populations were selected.

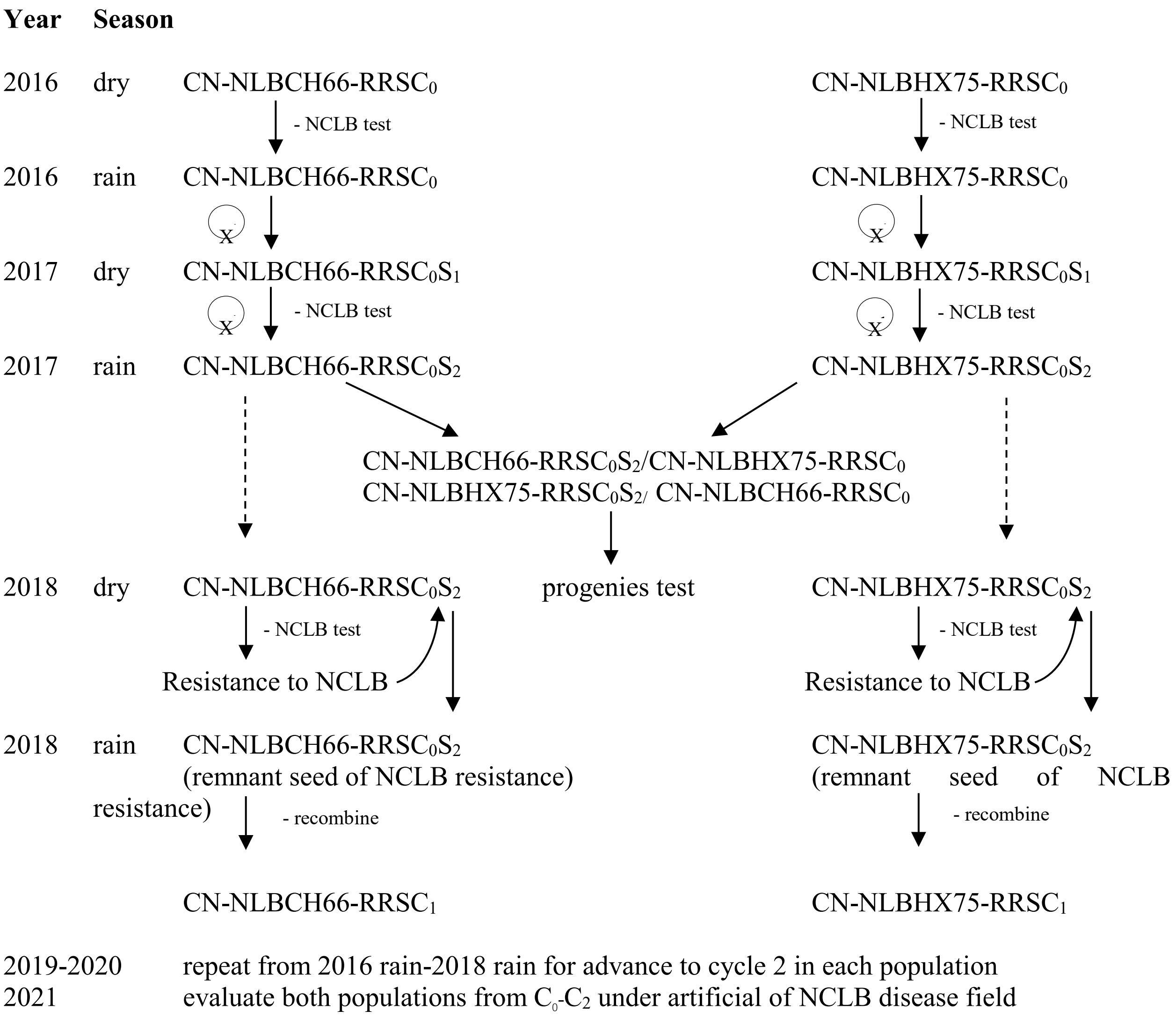

Figure 1. Breeding scheme of development of sweet corn populations for northern corn leaf blight resistance by S_2 Reciprocal Recurrent Selection in 2016-2021.

Progeny evolution

For an evaluation of topcross hybrids between populations, S_2 lines from both populations were crossed by C_0 cycle of opposite population, CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$ at Chai Nat Field Crops Research Center, Chai Nat province, Thailand. (Figure 1). 157 hybrids from CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and 196 hybrids from CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$ were planted in augmented design compared with four hybrid sweet corn commercial varieties, Chai Nat 2, Songkhla 84-1, Hibrix3 and Wan 1351. Data were collected for yield of the best fifteen ears with husk and without husk and degree brix (°brix).

Results and Discussion

The evaluation for NCLB disease resistance showed that CN-NLBCH66-RRSC$_0$S$_2$ and CN-NLBHX75-RRSC$_0$S$_2$ lines had percentage of leaf area disease infected ranging from 3-40% and 5-38% of total leaf area, respectively (data not shown) while Hibrix 3 had percentage of leaf area disease infected average 40% of total leaf area at 28 days after planting (Table 1). On the other hand, at 55 days after planting, CN-NLBCH66-RRSC$_0$S$_2$ and CN-NLBHX75-RRSC$_0$S$_2$ lines had percentage of leaf area disease infected ranging from 15-75% and 10-48% of total leaf area, respectively (data not shown) while Hibrix 3 had percentage of leaf area disease infected average 68% of total leaf area (Table 1). From the result, 146 of S$_2$ lines of CN-NLBCH66-RRSC$_0$S$_2$ and 105 of S$_2$ lines of NLBHX75-RRSC$_0$S$_2$ were selected with disease infected not exceeded 40% of total leaf area at 55 days after planting (data not shown).

Table 1. Percentage of disease infected, the best fifteen ears with husk and without husk weight and sweetness of S$_2$ lines selected from CN-NLBCH66-RRSC$_0$S$_2$ and CN-NLBHX75-RRSC$_0$S$_2$ populations at Chai Nat and Chiang Mai province of Thailand in dry season, 2018.

S. No.	Pedigree	% Disease infected		With husk[1/]	Without husk[1/]	° Brix[2/]
		28d	55d	(mean.adj)	(mean.adj)	
1	CN-NLBCH66-RRSC0)-1-2	10	25	5.43	3.74	13.56
2	CN-NLBCH66-RRSC0)-1-3	12	25	5.73	4.02	15.05
3	CN-NLBCH66-RRSC0)-2-1	6	20	6.25	3.84	12.01
4	CN-NLBCH66-RRSC0)-2-2	10	28	5.63	3.82	13.51
5	CN-NLBCH66-RRSC0)-9-1	5	25	5.35	3.54	14.16
6	CN-NLBCH66-RRSC0)-13-1	5	20	6.81	4.21	14.05
7	CN-NLBCH66-RRSC0)-18-1	6	25	6.45	3.94	14.48
8	CN-NLBCH66-RRSC0)-19-1	3	32	5.65	4.17	14.28
9	CN-NLBCH66-RRSC0)-21-3	8	18	5.45	3.57	14.78
10	CN-NLBCH66-RRSC0)-30-3	20	40	6.35	4.14	15.00
11	CN-NLBCH66-RRSC0)-34-2	20	38	5.61	4.01	12.02
12	CN-NLBCH66-RRSC0)-34-5	20	35	5.33	3.82	13.40
13	CN-NLBCH66-RRSC0)-35-2	8	20	5.51	4.26	14.37
14	CN-NLBCH66-RRSC0)-40-2	5	20	6.55	4.04	14.76
15	CN-NLBCH66-RRSC0)-41-1	18	25	6.35	4.37	14.18
16	CN-NLBCH66-RRSC0)-41-3	15	28	5.83	4.22	15.22
17	CN-NLBCH66-RRSC0)-43-1	18	30	5.53	3.92	13.51
18	CN-NLBCH66-RRSC0)-44-1	20	20	5.33	3.92	13.93
19	CN-NLBCH66-RRSC0)-44-2	28	38	6.85	4.77	14.20
20	CN-NLBCH66-RRSC0)-62-1	10	30	5.81	3.91	14.03
21	CN-NLBCH66-RRSC0)-69-2	18	28	5.33	3.82	14.23
22	CN-NLBCH66-RRSC0)-80-1	22	32	6.10	4.11	15.58
23	CN-NLBCH66-RRSC0)-84-1	15	35	5.71	4.21	13.81
24	CN-NLBCH66-RRSC0)-84-2	12	35	7.15	5.07	14.32
25	CN-NLBCH66-RRSC0)-85-2	10	25	6.23	4.42	15.14

26	CN-NLBCH66-RRSC0)-88-1	40	28	5.65	4.04	15.08
27	CN-NLBCH66-RRSC0)-90-2	20	38	6.10	4.17	13.58
28	CN-NLBCH66-RRSC0)-92-2	18	30	5.65	3.74	13.70
29	CN-NLBCH66-RRSC0)-94-1	17	30	6.33	4.52	13.00
30	CN-NLBCH66-RRSC0)-94-3	18	30	5.43	3.82	14.91
31	CN-NLBCH66-RRSC0)-96-1	28	40	5.53	3.72	16.03
32	CN-NLBCH66-RRSC0)-96-3	20	40	5.43	3.34	14.23
33	CN-NLBCH66-RRSC0)-112-3	30	35	5.35	3.74	14.20
34	CN-NLBCH66-RRSC0)-113-1	30	35	5.33	3.44	14.86
35	CN-NLBCH66-RRSC0)-114-1	18	25	5.35	3.64	14.66
36	CN-NLBCH66-RRSC0)-115-3	15	30	5.65	3.87	14.36
37	CN-NLBCH66-RRSC0)-119-1	20	33	6.05	4.14	12.79
38	CN-NLBCH66-RRSC0)-126-2	30	38	5.95	3.74	12.97
39	CN-NLBCH66-RRSC0)-127-1	20	35	6.15	3.74	14.26
40	CN-NLBCH66-RRSC0)-131-2	10	20	5.85	4.24	13.57
41	CN-NLBCH66-RRSC0)-131-3	10	22	6.35	4.47	14.07
42	CN-NLBCH66-RRSC0)-133-2	38	40	5.83	3.74	15.51
43	CN-NLBCH66-RRSC0)-134-1	18	40	5.91	4.01	13.23
44	CN-NLBCH66-RRSC0)-134-4	20	40	6.55	4.77	13.23
45	CN-NLBCH66-RRSC0)-135-1	30	38	5.43	4.02	14.17
46	CN-NLBCH66-RRSC0)-138-2	16	30	5.53	3.42	13.51
47	CN-NLBCH66-RRSC0)-140-3	23	40	5.43	3.62	13.72
48	CN-NLBCH66-RRSC0)-141-1	32	35	5.43	3.52	13.05
49	CN-NLBCH66-RRSC0)-141-2	30	40	5.51	3.51	13.72
50	CN-NLBCH66-RRSC0)-141-3	20	40	6.35	4.07	14.09
51	CN-NLBCH66-RRSC0)-141-4	20	40	5.61	3.81	14.75
52	CN-NLBCH66-RRSC0)-145-1	32	40	5.85	4.17	13.60
53	CN-NLBCH66-RRSC0)-151-2	20	35	6.23	4.07	13.93
54	CN-NLBCH66-RRSC0)-152-2	30	38	5.41	3.71	15.24
55	CN-NLBCH66-RRSC0)-152-3	18	38	5.61	3.36	13.41
56	CN-NLBCH66-RRSC0)-153-1	18	25	7.05	4.57	13.88
57	CN-NLBCH66-RRSC0)-154-3	15	35	6.35	4.47	13.72
58	CN-NLBCH66-RRSC0)-158-1	22	35	6.55	4.17	14.44
59	CN-NLBCH66-RRSC0)-158-3	20	36	5.33	3.72	14.06
60	CN-NLBCH66-RRSC0)-159-3	20	36	5.42	4.11	13.04
61	CN-NLBCH66-RRSC0)-165-2	25	25	5.43	3.64	14.98
62	CN-NLBCH66-RRSC0)-166-1	15	20	5.63	3.84	14.10
63	CN-NLBCH66-RRSC0)-166-2	16	25	5.55	3.64	15.12
64	CN-NLBCH66-RRSC0) 167-1	20	30	5.43	3.92	14.32
65	CN-NLBCH66-RRSC0)-167-2	23	38	5.63	3.82	13.09
66	CN-NLBCH66-RRSC0)-168-2	22	32	6.05	4.14	14.59

67	CN-NLBCH66-RRSC0)-169-1	18	30	5.95	3.84	13.88
68	CN-NLBCH66-RRSC0)-169-2	28	40	5.33	3.52	13.63
69	CN-NLBHX75-RRSC0)-1-2	20	40	5.93	3.64	14.81
70	CN-NLBHX75-RRSC0)-1-3	30	40	5.73	3.64	14.34
71	CN-NLBHX75-RRSC0)-2-2	27	30	6.45	4.34	12.71
72	CN-NLBHX75-RRSC0)-4-2	25	40	5.53	3.62	14.11
73	CN-NLBHX75-RRSC0)-6-2	20	35	5.63	3.84	14.80
74	CN-NLBHX75-RRSC0)-7-2	17	25	6.13	4.04	15.19
75	CN-NLBHX75-RRSC0)-7-4	24	30	6.75	4.24	13.02
76	CN-NLBHX75-RRSC0)-14-1	22	40	5.43	3.82	13.92
77	CN-NLBHX75-RRSC0)-17-1	14	30	5.61	4.21	15.88
78	CN-NLBHX75-RRSC0)-19-1	22	40	6.86	4.32	12.99
79	CN-NLBHX75-RRSC0)-19-5	28	40	6.28	4.32	13.25
80	CN-NLBHX75-RRSC0)-23-3	23	28	7.25	4.77	13.84
81	CN-NLBHX75-RRSC0)-27-3	24	40	5.95	3.94	13.85
82	CN-NLBHX75-RRSC0)-30-3	16	30	5.81	3.91	14.47
83	CN-NLBHX75-RRSC0)-34-3	22	40	6.01	4.21	16.56
84	CN-NLBHX75-RRSC0)-39-1	27	33	6.80	4.21	13.77
85	CN-NLBHX75-RRSC0)-41-1	20	36	5.63	3.82	14.14
86	CN-NLBHX75-RRSC0)-43-1	22	40	5.45	3.74	13.58
87	CN-NLBHX75-RRSC0)-43-2	25	40	6.95	6.47	13.60
88	CN-NLBHX75-RRSC0)-44-1	25	40	6.03	3.94	14.21
89	CN-NLBHX75-RRSC0)-44-2	25	40	5.95	4.14	14.26
90	CN-NLBHX75-RRSC0)-45-2	25	35	5.83	3.64	13.96
91	CN-NLBHX75-RRSC0)-45-3	16	35	7.55	4.87	13.84
92	CN-NLBHX75-RRSC0)-45-4	13	35	6.51	3.91	14.35
93	CN-NLBHX75-RRSC0)-52-1	28	38	5.51	3.41	13.80
94	CN-NLBHX75-RRSC0)-53-1	20	30	5.95	4.47	14.09
95	CN-NLBHX75-RRSC0)-59-1	26	38	6.15	4.67	14.01
96	CN-NLBHX75-RRSC0)-59-2	23	36	5.61	3.79	14.75
97	CN-NLBHX75-RRSC0)-60-2	16	28	7.45	4.97	14.18
98	CN-NLBHX75-RRSC0)-64-3	16	35	5.95	4.04	13.98
99	CN-NLBHX75-RRSC0)-65-1	17	22	5.33	4.12	13.59
100	CN-NLBHX75-RRSC0)-74-1	15	28	5.33	3.62	12.17
101	CN-NLBHX75-RRSC0)-75-2	14	38	7.65	5.27	11.97
102	CN-NLBHX75-RRSC0)-75-3	16	38	5.93	4.04	13.53
103	CN-NLBHX75-RRSC0)-79-2	25	36	5.61	3.81	14.29
104	CN-NLBHX75-RRSC0)-79-3	23	40	6.95	4.47	13.92
105	CN-NLBHX75-RRSC0)-106-1	22	40	5.51	3.71	12.91
106	CN-NLBHX75-RRSC0)-106-2	25	40	5.95	4.14	15.33
107	CN-NLBHX75-RRSC0)-106-3	23	40	5.71	3.81	13.90

108	CN-NLBHX75-RRSC0)-115-2	32	30	6.05	4.24	15.63
109	CN-NLBHX75-RRSC0)-117-1	18	38	6.55	4.57	13.99
110	CN-NLBHX75-RRSC0)-117-3	18	35	6.15	4.27	13.99
111	CN-NLBHX75-RRSC0)-118-2	12	27	6.15	4.47	14.46
112	CN-NLBHX75-RRSC0)-126-2	25	34	6.01	3.91	13.76
113	CN-NLBHX75-RRSC0)-129-2	28	35	5.91	3.91	14.64
114	CN-NLBHX75-RRSC0)-129-3	13	38	6.01	4.01	15.26
115	CN-NLBHX75-RRSC0)-130-1	25	20	5.95	3.94	13.43
116	CN-NLBHX75-RRSC0)-130-2	20	25	7.05	4.87	14.15
117	CN-NLBHX75-RRSC0)-130-3	12	25	5.31	3.51	13.94
118	CN-NLBHX75-RRSC0)-131-1	18	15	5.85	3.97	14.31
119	CN-NLBHX75-RRSC0)-131-2	21	12	6.21	4.01	14.70
120	CN-NLBHX75-RRSC0)-150-2	32	22	5.85	3.87	14.17
121	CN-NLBHX75-RRSC0)-152-1	22	38	5.33	3.92	14.84
122	CN-NLBHX75-RRSC0)-153-2	21	18	5.75	4.37	15.40
123	CN-NLBHX75-RRSC0)-158-1	19	15	6.45	4.47	14.19
124	CN-NLBHX75-RRSC0)-158-2	36	38	6.01	3.69	13.10
125	CN-NLBHX75-RRSC0)-158-3	14	30	6.25	4.14	13.60
126	CN-NLBHX75-RRSC0)-159-1	18	32	5.83	3.94	13.62
127	CN-NLBHX75-RRSC0)-159-2	17	30	5.73	4.04	14.31
128	CN-NLBHX75-RRSC0)-159-3	16	30	5.71	3.91	14.53
129	CN-NLBHX75-RRSC0)-164-1	37	22	5.55	3.97	15.01
130	CN-NLBHX75-RRSC0)-172-1	25	35	5.33	3.54	15.78
131	CN-NLBHX75-RRSC0)-173-2	18	18	5.32	3.71	15.05
132	CN-NLBHX75-RRSC0)-176-2	20	22	5.35	3.84	14.05
	Average[3/]	24	38	5.40	3.75	14.28
133	Cha iNat2[4/]	-	-	7.15	4.68	13.29
134	Hibrix 3	40	68	7.03	4.68	15.03
135	Wan 1351[4/]	-	-	6.83	4.70	15.09
136	Song Khla 84-1[4/]	-	-	5.29	3.71	14.37
	Average	-	-	6.70	4.53	14.44
	C.V. (%)[3/]	-	-	11.70	9.70	-
	SE	-	-	0.64	0.37	-

[1/] The best 15 ears with husk and without husk of CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$

[2/] Sweetness (°Brix) of CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$

[3/] Average from 353 hybrid from CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$

[4/] No result NCLB disease evaluation

The progenies evaluation of CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ and CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$ showed that averaged the best fifteen ears with husk and without husk weight were about 5.40 and 3.75 kg, respectively and average sweetness both of them was about 14.28°bix

(Table 1). While four commercial hybrid variety had averaged the best fifteen ears with husk and without husk weight about 6.70 and 4.53 kg, respectively and they had sweetness about 14.44°bix. 84 hybrids from CN-NLBCH66-RRSC$_0$S$_2$/CN-NLBHX75-RRSC$_0$ were selected which had averaged the best fifteen ears with husk weight ranging from 5.33-7.55 and without husk weight ranging from 3.34-5.37 kg (data not shown). Moreover, 105 hybrids from CN-NLBHX75-RRSC$_0$S$_2$/CN-NLBCH66-RRSC$_0$ were selected which had averaged the best fifteen ears with husk and without husk weight ranging from 5.32-7.65 and 3.41-6.47 kg, respectively (data not shown). According to the results, 84 of S$_2$ line from CN-NLBCH66-RRSC$_0$S$_2$ and 105 of S$_2$ lines from CN-NLBHX75-RRSC$_0$S$_2$ were selected.

From those results together, sixty-eight S$_2$ lines from CN-NLBCH66-RRSC$_0$S$_2$ were selected with disease infected ranging from 3-40% and 18-40% of total leaf area at 28 and 55 days after planting, respectively (Table 1). Moreover, topcross hybrids had ears with husk weight ranging from 5.33-7.15 kg and without husk weight ranging from 3.34-5.07 kg and average sweetness ranging from 12.01-16.03°bix. Similarly, sixty-four S$_2$ lines from CN-NLBHX75-RRSC$_0$S$_2$ were selected with disease infected ranging from 12-37% and 12-40% of total leaf area at 28 and 55 days after planting, respectively. In addition, the topcross hybrid had ears with husk and without husk weight ranging from 5.32-7.65 and 3.41-6.47 kg, respectively and average sweetness ranging from 11.97-16.56°brix.

Conclusion

Sixty-eight S$_2$ lines from CN-NLBCH66-RRSC$_0$ and sixty-four S$_2$ lines from CN-NLBHX75-RRSC$_0$ were selected for the percentage of leaf area disease infected not exceeded 40% of total leaf area and high ear yield. The new cycle of two improved populations will be developed from that selected lines in the rainy season, 2018.

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Development of Synthetic Maize Source Material According to Silage Quality Values

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Introduction

Maize is the most widely produced cereal in the world, thanks to its versatile use, adaptation capability and yield. Based on August 2018 data, the global maize production the world reached 1061.05 million tons (www.fas.usda.gov). In Turkey, maize has the third highest cultivation area, following wheat and barley. It is successfully grown both as a main crop and as a second crop. Approximately 78% of grain maize produced in the country is used in the feed industry and 20% in the starch industry. Maize planting for silage, especially as second crop, has become very popular. In 2016, silage production reached 20.139 million metric tons and the average yield was 47.3 t/ha.

Maize breeders have become increasingly aware of the importance of the genetic diversity of germplasm. In future, genetic gains in maize will depend on effective use of genetic diversity. It is necessary to combine functionally useful genetic diversity, and develop germplasm that meet the client needs. Priority characters in germplasm selection include abiotic stress tolerance (drought, low and high temperature, salinity), resistance to major diseases (leaf blight, ear and stem rot, anthracnose), resistance to insects (maize borer), yield and yield components, and grain and silage quality (protein, oil, starch, NDF, ADF, cellulose ratio, etc.). Breeders use different methods for developing germplasm. In choosing the methods to be used in the breeding program for the development of germplasm, many factors such as infrastructure, personnel and ecology influence the selection of the method. Although the population breeding studies are still continuing in different maize breeding programs in Turkey, the elite inbred lines are also being used in creating synthetic resource materials after intensive evaluation for different characteristics.

Maize silage is a high-quality food for ruminants. It is the most economical and most commonly produced coarse fodder in the world, and is used extensively in cattle fattening by being enriched with proteins, especially in countries like the United States, Netherlands, Germany and France (Alçiçek and Karaayvaz, 2003). Maize is a preferred plant for silage in Turkey because it is easy to produce as a main and second crop, amenable for fermentation, and for economical formulation of coarse feeds. Maize silage (80-90 tons per hectare) is equivalent to about 25 tons of barley in terms of feed value. As a result, maize silage can provide a daily gain of 600-700 g without any additional feed (Yaylak and Alçiçek, 2003).

In Turkey, there are breeding programs for silage separate from grain maize. Unlike grain maize, plant characteristics are at the forefront in case of silage breeding. After silage is done, digestibility and feed quality are important factors. For the development of quality silage varieties, parents should also come from the same breeding program. It is possible to develop quality silage hybrid maize varieties with inbred lines which are developed by selection according to the silage quality values. Breeders have selected for fiber structure, acid silicon salt and lignin concentration of stalk using three cycles of S_1 recurrent selection method in Wisconsin Quality Synthetic (WQS) material. At the end of this study they divided the material into low and high. These two materials were hybridized with Mo17 and H99. Material was developed by using S2 top-cross hybrid selection method considering all plant yield, neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), crude protein and starch values (Frey et al., 2004).

Materials and Methods

In this study, synthetic source material development method was used. S_1 Recurrent Selection Method was applied to obtain the starting material. Hybridizations were made according to the half-diallel technique. Yield experiments were set up in randomized block design. Progeny Control Yield Test was conducted based on Simple Balanced Lattice Test pattern. In these experiments, 95,240 plants/ha plant density was used. Silage quality parameters (SQPs) were determined by classical methods.

Table 1. Standard values for silage quality parameters
NDF and ADL

Quality Standard	ADF %	NDF %
The best	<31	<40
1	31-35	40-46
2	36-40	47-53
3	41-42	54-60
4	43-45	61-65
5	>45	>65

Results and Discussion

The silage yield experiment was initially established in 2009, with 17 lines selected according to features such as plant height, number of leaves and greenness. NDF, ADF, crude fiber, crude protein and crude oil values were evaluated in the present experiment in addition to leaf/stem ratio, stalk/plant ratio, stem/plant ratio, and green plant yield (Table 2). Based on this, 9 lines were selected considering green plant yield and silage quality parameters (SQPs).

Table 2. Results of the silage yield trial of maize inbred lines.

Lines	NDF %	ADF %	Crude cellulose %	Protein %	Oil %	Green Plant Yield (GPY) (t/ha)
ADK 433	45.9	45.1	20.9	8.3	1.42	**61.77**
ADK 434	45.2	45.6	23.2	**10.7**	1.04	40.56
ADK 438	43.5	45.5	23.2	9.4	1.45	51.13
ADK 451	42.4	46.6	20.3	9.2	1.72	57.69
ADK 455	46.9	45.1	23.5	8.7	1.29	59.12
ADK 514-1	**31.3**	**41.7**	**15.8**	9.5	2.23	30.90
ADK 533	40.7	46.1	22.2	9.1	2.07	41.87
ADK 604	44.7	44.8	21.9	10.3	1.95	39.64
ADK 651	43.2	46.3	21.1	9.4	2.15	36.02
ADK 689	37.3	44.2	19.2	8.5	**2.98**	50.46
ADK 694	42.3	45.2	20.2	9.9	1.60	50.54
ADK 719	43.3	46.0	21.5	9.1	2.56	40.87
ADK 720	45.3	44.5	20.5	10.6	1.32	46.27
ADK 726	44.7	46.3	21.9	10.4	2.14	42.88
ADK 728	45.3	46.7	21.9	9.9	1.86	53.78
ADK 733	41.0	46.2	21.5	9.6	2.19	40.03
MAE 9301	45.6	47.9	25.8	8.4	1.42	**61.76**
CV %	6.9	2.4	8.8	11.2	31.6	6.6
Significant	**	**	**	Ns	**	**

** – significant at $P \leq 0.01$ * – significant at $P \leq 0.05$

Using the selected lines, a half-diallel with 36 crosses were made. In 2011, these 36 hybrids were tested along with check varieties for GPY and SQPs. Silage quality values and GPY were evaluated together and 15 combinations were identified as most promising (Table 3).

Table 3. GPY and SQPs of half-diallel hybrids and selected number of 15 combinations (with lower and upper values)

Parameters	GPY (t/h)	Dry Matter %	ADF %	NDF %	ADL %	Crude cellulose %
All half-diallel hybrids	28.9-80.3	27.7-34.1	32.5-39.7	39.2-49.0	6.9-13.3	18.8-27.8
Selected half-diallel hybrids	28.9-65,0	28.6-34.1	32.5-36.6	39.2-43.7	6.9-11.5	18.8-23.8

An equal amount of seeds were mixed from the selected hybrid combinations to provide a physical mixture. For the homogenous distribution of the genetic structure, these materials were planted in a recombination block and crossed to obtain the starting material for population breeding in 2012.

Using the S_1 recurrent selection method, the obtained starting population was planted in 2013. In starting the population, 562 self-pollinations were made, and 142 families were selected at harvest. The selected S_1 families were subjected to "progeny silage yield test" in 2014. The experiment was based on 12x12 lattice trial design. Four check hybrids were also included in the experiment. Green plant yield varied between 48.14-102.7 t/ha in progeny control silage yield trial (Table 4).

Table 4. GPY and SQPs of S_1 families and check hybrids lower and upper values

Parameters	GPY (t/h)	ADF %	NDF %	ADL %	Crude cellulose %
S_1 families	48.14-102.4	26.4-43.5	37.4-65.4	0.8-9.8	9.3-25.7
Check Hybrids	80.14-102.7	28.5-33.5	45.6-55.6	2.8-5.4	15.2-15.6

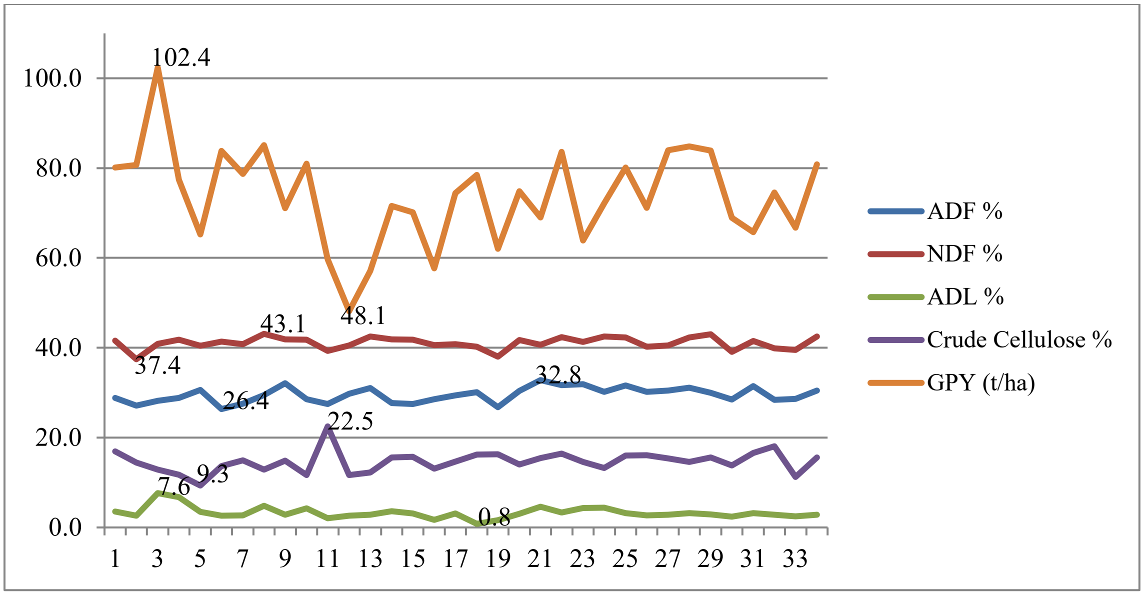

Figure 1. GPY and SQPs of selected S_1 families (with lower and upper values)

Progeny control silage yield test results and silage quality values were evaluated together and 34 families were selected from the population (Figure 1). Recombination block was created with selected families and crosses were made between the blocks. A cycle of population breeding was completed by creating a recombination block and ADASLJSYN S_1 (C_1) synthetic population was obtained at harvest in 2016. The obtained population was used as a source material for the development of new inbred lines. These new inbred lines were used as source germplasm for derivation of doubled haploid (DH) lines by the maize breeders of the National Maize Program in 2017.

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Prospect of Specialty Maize as Functional Food to Support Food Diversification in Indonesia

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Introduction

The variety of maize kernel colors (yellow, red, black and purple) hint at their richness in functional food components. This paper discusses purple maize, because its rarity is a cause for curiosity. Purple maize contains various nutrients, particularly anthocyanin compounds. Local variety purple maize (from Gorontalo province) produces a deep purple color but has low productivity (Suarni and Subagio, 2013). The Indonesian Cereals Research Institute (ICERI) has been developing superior varieties of purple maize by maintaining the purple pigment grains, and breeding for higher yield. The specific advantage of this variety is its high anthocyanin component.

Anthocyanin antioxidant activity is influenced by the system used as a substrate, and conditions used to catalyze the oxidation reaction (Pokorny *et al* . 2001). The presence of these compounds in the growth phase of maize plants depends on the variety and its physiological state. Information about the percentage of purple maize anthocyanin components is still difficult to obtain, therefore, research on the effect of harvest age on nutritional components, characterization of physicochemical and functional properties, and diversification of superior products in necessary. Based on the results of the study, users can refer to the appropriate harvest phase according to the desired product both as functional and industrial food ingredients. In future, it is expected that superior varieties of purple maize can be used in the community to develop healthy food products.

Nutrition Content, Anthocyanin and Purple Maize Physicochemical Properties

Study on nutritional value of purple maize was conducted in 2015-2017. Proximate composition and young harvested purple maize anthocyanins are shown in Table 1.

Table 1. Proximate composition and young harvested purple maize anthocyanins.

Harvest age / Strain varieties	Water (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Anthocyanin (µg / g)
PMU (S1) Synth.F.C1						
70 days	59.52 b	0.59 bcd	3.48 d	0.39 d	36.02 d	1.55
75 days	50.23 d	0.64 abc	4.08 b	0.76 b	44,29 b	5.51
80 days	47.09 e	0.76 a	5.42 a	1.08 a	45.65 a	5.94
Maluku local purple maize						
70 days	62.05 a	0.47 d	2.79 f	0.32 d	34,37 e	1.02
75 days	59.12 b	0.52 dc	3.13 e	0.59 c	36,64 d	1.25
80 days	52.94 c	0.69 ab	3.75 c	0.77 b	41.85 c	1.46

Note: The same row number is not significantly different from the 0.05 DMRT test difference.

Results of the analysis showed that water, ash, protein, fat and carbohydrate content varied at different harvesting ages. Maize harvested at mild stage showed a decrease in water content with increasing age of dap (day after harvest). On the contrary, ash, protein and fat content went up with increase in age of the plant. Likewise, carbohydrate levels increased, with decrease in water content of maize kernels. Candidates for purple maize varieties from various germplasm origins were evaluated for proximate components (water, ash, fat, protein, crude fiber and carbohydrates) (Table 2).

Table 2. Proximate composition of purple maize harvest, 2015.

Variety /lines	Water (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Rough fiber (%)
PMU (S1) Synth.F.C1	10.86	1.59	9, 28	3.98	74,56	2.73
PTU (S1) F.CO	11.05	1.49	8.81	4.05	74.60	2.99
PVU.FS. CO	10.11	1.69	8,28	4.34	75.58	3.02
PPU. (S1) .C1	11.12	1.57	7.78	3.79	75.74	2.28

Maize kernel water content is influenced by kernel size and hardness in the drying stage. The highest protein levels in the PMU (S1) Synth.F.C1 (9.28%) and the lowest in the PPU (S1). C1 line (7.78%) were observed. Fat content of the four purple maize kernel samples was not much different. The fat content of purple maize varied within a range of 3.79% - 4.34%, the lowest being in PPU. (S1) .C1

Table 3. Components of anthocyanin, amylose, maize fiber, and maize meal.

Variety of varieties	Anthocyanin (µg / g)	Amylose (%)	Food fiber (%)
PMU (S1) Synth.F.C1	51.36	5.77	9.16
PTU (S1) F.CO	37.15	8.02	6.01
PVU (S1) CO	20.86	7.02	8.25
PPU. (S1) .C1	12.10	6.04	11.27

The main types of anthocyanin in purple maize are cyaniding-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside (Moreno et al. 2005). Anthocyanin analysis in the sample above was calculated as TAC (µg /g cyanidine). Anthocyanin content of PMU (S1) strain. Synth.F.C1 is higher than other strains. Amylose and amylopectin ingredients have a role in determining the properties of foods processed from starch, such as maize flour (Suarni, 2010). The results of the analysis of the amylose content of the four purple maize flour ingredients were within a range of 5.77-8.02%, the lowest in PMU (S1) Synth.F.C1, and the highest in PTU (S1) F.CO. The amylose content observed was included in the low amylose category, so it entered the group of pulverized maize. The content of food fiber in the four samples of purple maize flour was high, ranging from 6.01% to 11.27%; the highest in PPU (S1) .C1 and the lowest in PTU (S1) F.CO. Maize flour is advantageous over wheat flour as a food ingredient because it contains relatively higher food fiber.

Results of amylographic analysis on purple maize flour showed several measured parameters including gelatinization time and peak viscosity; the level of softness and crispness of the processed product can also be seen from back viscosity (set back viscosity). The viscosity to four samples of purple maize flour is very low and PMU (S1) Synth.F.C1 is higher than the others. The decrease in viscosity during heating shows the stability of the paste during heating where the lower the breakdown, the more stable the paste that is formed will be against heat.

Table 4: Amylographic properties of purple maize flour

Amilography	PMU (S1 Synth.F.C1	PPU. (S1) .C1	PTU (S1) D.CO	PVU (S1) CO
Gel Time (minutes)	-	-	-	-

Gel temp (° C)	87.0	85.5	82.5	87.0
Peak Time (minutes)	-	-	-	-
Peak Temp (° C)	-	-	-	-
Peak Viscosity (BU)	-	-	-	-
Viscositas 93 ° C (BU)	50	130	150	80
Viscosity 93 ° C / 120 '(BU)	80	170	190	110
Viscosity 50 ° C (BU)	300	320	380	210
Set Back Visc. (BU)	220	150	190	100

Among effects on the amylographic properties of flour, is the composition of amylopectin and amylose. The magnitude of the *breakdown viscosity* indicates that swollen flour granules are fragile and cannot stand the heating process. Amylose greatly affects the hardness of the product because of its ability to form strong hydrogen bonds between amylose, or between amylose and amylopectin, after products are baked and cooled (Yu, *et al.,* 2009). Singh *et al.,* (2005) stated that maize, especially waxy type, is suitable for use in various food preparations such as baking to improve the typical texture characteristics of the product. This is related to the physical and rheological characteristics (amylographic properties) of maize starch. Furthermore, it appears that dietary fiber content of maize flour is relatively high (9.16%) compared to wheat (2.13%). High food fiber can also increase violence (Lee and Lin, 2008) and reduce elasticity (Singh *et al.,* 2012).

Water absorption capacity is related to the composition of the granules, and the physical properties of the starch after water is added. According to Elliason (2004), starch granules can be wet and spontaneously dispersed in water. Flour oil absorption capacity is influenced by the presence of protein on the surface of starch granules, which form complexes with starch, then provide a place for the oil to bind. In connection with this, flour KPM (0.870) is higher than purple maize flour (0.796).

Purple Maize Processed Products

Young harvested maize can be processed into purple maize juice and purple maize ice cream. Another product is *dodol* (sugar = 155g, 30 minutes cooking) local purple maize flour, the treatment most received and preferred by panelists. The product still maintains anthocyanin content due to relatively short cooking time. Physiologically matured purple maize can be processed into semi-finished ingredients in the form of flour. Purple maize flour can substitute flour up to 80% for processed brownies, although the panelists scored the highest on 40% and 70% substitution by steamed cooking methods. Anthocyanin levels can still be maintained compared to roasting methods. Maize cake substitution products for flour (90:110) are most suitable for basic ingredients of purple maize flour and still contain anthocyanins.

Specialty Maize Development Strategy in Indonesia

The diversification of special maize-based functional food is still limited, but there are good prospects for the development of anti-cholesterol, polysaccharide-based functional food from maize. Special maize superior varieties, including high productivity and potential purple maize as functional food, can be explored in ready-to-consume products (Suarni and Subagio 2013).

The need for maize centers, specifically on special maize cultivation, can be carried out in the framework of technology transfer to farmers and agricultural extension workers. Activities may include application of organic fertilizers, biological fertilizers and biological pesticides, as well as processing maize crop products to be functional food ingredients.

Special maize development still faces various problems, especially related to market creation and price guarantee, as well as institutional aspects for sustainable maize development.

Conclusion

Purple maize can be processed into various preparations ranging from harvesting, to cooking milk, to physiological cooking. The anthocyanin content can still be maintained, even though it decreases. Just like other specialty maize (yellow and red maize), black maize contains antioxidants, dietary fiber and high minerals. The prospect of developing maize in Indonesia to have a market has increased, because people have begun to change their eating patterns (consuming healthy food).

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Impact and Use of Biofortified Maize in Southern China

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Introduction

The major nutritional components of maize grain are starch, protein, and lipids, but the bioavailability of the protein in human and animal nutrition is limited by low levels of the essential amino acids, lysine and tryptophan. Maize has between 3.5-5% oil content, while the micronutrients zinc and iron content are found at an average at 22.1 and 27.1 ug/g, respectively (USDA Natl. Nutrient Database). Maize is the grain of choice in the mountainous areas of southwestern China due to its' high yields under rainfed conditions often grown on less productive, sloping land. Maize also provides the potential for increased income through animal husbandry. An increase in the nutritional and energy value of the grain, provides to these small farmers, a chance to enhance their diet and household income, delivered by way of improved seed (Bouis, 2003; Ortiz-Monasterio et al., 2007)

Breeding Nutritionally Enhanced, Locally Adapted Germplasm

A major challenge for introducing any improved nutritional trait to southwestern China, is to add stress resilience to the many abiotic and biotic stresses present. Yunnan province, possesses climatic conditions to exploit both tropical and temperate maize germplasm, but is a challenging environment with periodic drought stress, and poor soil fertility. Biotic stresses including pre and postharvest insect pests and the diseases: turcicum leaf blight and gray leaf spot; leaf rusts; and *Fusarium* and *Gibberella* ear rots limit the direct use of temperate germplasm. Temperate introgression lines made with local germplasm has been found to provide valuable germplasm that adds yield stability, improved agronomic traits, and high heterosis. Many of the nutritional donor traits are being worked in backcrossing programs with the best locally adapted inbreds in the Yunnan Academy of Agricultural Sciences (YAAS), breeding program. These introgression lines are seen as the key to developing commercially competitive hybrids for use in the region.

Targeted Traits for Nutritional Enhancement of Maize Grain

High oil

Early efforts for improving the nutritional and energy value of maize began over 100 years ago, with the initial recurrent selection for improving oil content (Hopkins et al., 1903; Smith, 1908). Besides providing industry with high quality vegetable oil, animal feed can be enhanced with maize possessing higher levels of oil. Animal feed with an oil content of 7%, improved the feed efficiency over normal maize in swine production (Nodstrom et al, 1972), where oil in the grain provides more than twice the metabolizable energy than the equivalent weight of starch (Lambert, 1994). A positive correlation was found also between the percentage of germ protein and the concentration of tryptophan in the kernel, which may also lead to an enhanced feed value of the grain (Miller and Brimhall, 1951). Inheritance of maize oil content is controlled mostly by additive effects (Miller et al, 1981), and a basic understanding on the genetic control of maize oil content was carried out using lines derived from the Illinois long term high oil, low oil selection, which identified > 50 quantitative trait loci (QTL) affecting oil content (Laurie et al., 2004). To counter the lower yields found with high oil maize hybrids, commercial high oil maize (HOM) production fields utilize the xenia effect on oil content in the female parent, a male sterile female parent, and heterosis to enhance both oil content and yield (Hammes, 1997; Bulant and Gallais, 1998).

In China, initial work on high oil maize (HOM), began in the 1980's with the introduction of temperate germplasm, which had a narrow genetic base, low yield potential, lack of broad adaptation, and was susceptible to many key diseases found in China maize growing environments. Much progress has been made in HOM in China including broader adaptation, improvements in yield potential, and the use of three genetic effects (TEU) of pollen xenia, heterosis and male-sterile cytoplasm for hybrid production, to develop high-yielding breeding programs (Song & Cong, 1998; Chen *et al.* 2001; Duan *et al.* 2000; Chen *et al.* 2003), and a commercial hybrid, Lingaoyou 1 (Gao & Wang, 2002). To understand the genetic basis of maize oil content and quality, 1.03 million SNPs were used to characterize 368 maize inbred lines and found 74 loci significantly associated with maize oil concentration and fatty acid composition (Li et al.,2013). Through lipid metabolism studies and transcriptomics, 50 gene candidates were associated with modulating acyl-lipid classes using Chinese high oil germplasm (Abreu e Lima et al., 2018). This basic research opens the possibility to potentially modify the oil composition in maize, for further improving nutritional quality

Quality Protein Maize (QPM)

Improved protein quality was identified in a maize endosperm mutation. Mertz et al. (1964) found that the doubly recessive *opaque-2* gene (o_2) provides nearly twice the content of lysine and higher tryptophan in comparison with normal maize, where both amino acids are limiting for the utilization of total maize grain protein. Poor grain quality traits related to a chalky endosperm linked with opaque-2 led to increased insect and ear rot susceptibility in comparison to normal maize, and limited its' direct use. Studies at the same, identified that nitrogen absorption and retention were improved in human feeding trials with o_2 versus normal maize (Bressani, 1966) while overall digestibility was unchanged. Paez et al. (1969) identified o_2 endosperm modifiers genes, for improving endosperm hardness and grain quality. Both the International Maize and Wheat Improvement Center (CIMMYT), and the University of Natal, South Africa, developed maize o_2 breeding programs that combined improved protein and grain quality, with selection for resistance to biotic pests and diseases, that led to the development and use of "Quality Protein Maize" (QPM) (Vasal et al., 1980; Brown et al., 1988; Prasanna et al., 2001). Additional nutritional studies found the prolamin levels in QPM maize decrease while there is an increase in glutelin levels, which are higher in lysine content and have a higher digestibility (Villegas et al., 1980). This change leads to lower levels of the amino acid leucine versus isoleucine, which may increase niacin availability (Geetha et al., 1991). QPM maize was also found to double the biological value of maize protein (Bressani, 1992). A thorough review of QPM development is presented by Vasal (2001).

In China, QPM work has been carried out in both temperate and subtropical environments, where CIMMYT and South African germplasm, have been effectively introduced and used in the development and commercialization of QPM germplasm in southwestern China. In YAAS, both white and yellow commercial hybrids have been released including the white hybrid, Yunrui 21, and the yellow hybrid Yunrui 1. Two new promising yellow QPM hybrids Y102 and Y105 are currently in two years of multi-locational provincial and national testing respectively.

The exploitation of the locally adapted QPM parents, in the development of high oil, improved protein quality hybrids, have led to the development and release of commercial hybrids. Table 1 presents key parents in the formation of these multi nutritional trait hybrids. The high oil hybrid Yunrui 8 has a yield potential up to 9848 kg/ha. identified in multi-location registration trials, is highly resistant to *Fusarium* and *Gibberella* ear rots and head smut, and is resistant to turcicum and maydis leaf blights based on national evaluations. Yunrui 8 is a leading recommended nationally registered hybrid, which since its' release in 2005 has occupied a cumulative area of more than 0.5 million ha. in southwestern China with a yield increase of more than 0.43 million tons, and an increased value of greater than 118 million US dollars. Yunrui 8 provides added yield stability, improved food safety, with enhanced nutritional traits for use as food and for animal husbandry, which is especially valuable in the less-developed mountainous areas of the region.

Table 1. Germplasm resources and characters of six temperate high-oil inbred lines and four tropical inbred lines.

Inbred	Original	Oil content (%)	Ecology type
Y46	Suwan1	---	Tropical
CML161	Pool25QPM	---	Tropical
CML171	Pool25QPM	7.72	Tropical
CMl166	Pop66QPM	---	Tropical
GY276	BHO	---	Temperate
GY717	BHO	---	Temperate
GY923	ALEXHO	10.66	Temperate
GY220	ALEXHO	10.13	Temperate
GY237	ALEXHO	13.21	Temperate
GY798	ALEXHO	9.13	Temperate

Micronutrients

Potential for improving the micronutrient content in crops through breeding can reduce global micronutrient deficiencies in the human diet (Bouis, 2003). Vitamin A, zinc and iron are the key micronutrients found limiting in the human diet, and deficiencies in one or more of these three micronutrients affect an estimated 2.5 billion people worldwide (Black et al., 2013). Vitamin A deficiency (VAD), is most severe in children and pregnant women (Rice et al., 2004) and an estimated 190 million children globally, are affected (WHO, 2009) leading to reduced growth and development, weakened immune response, and blindness. Biofortification of maize with increased levels of the carotenoids that contribute to the formation of provitamin-A(proVA), a precursor of vitamin A, was identified as a way to reduce VAD in humans depending on maize based diets (Bouis 2003; Ortiz-Monasterio et al., 2007) and the HarvestPlus set a proVA concentration of 15 ug/g as the breeding target goal for proVA maize. Genetic variation for carotenoid content and proVA exists in maize (Maziya-Dixon et al., 2000, Harjes et al., 2008; Babu et al., 2013), and basic pathways leading to enhanced proVA germplasm has been elucidated for provitamin-A biofortification. The key genes *lcyE* and *crtRB1* (Harjes et al. 2008; Yan et al. 2010) and *zep1* and *lut1* (Owens et al., 2014) were identified for enhancing proVA. Marker assisted selection for the favorable alleles of the *crtRB1* gene have led to the development and deployment of improved germplasm (Li et al. , 2015 and Pixley et al., 2013), respectively.

Improved mineral content of maize, especially zinc and iron can be significantly influenced by soil factors and growing conditions, and both minerals are essential in the diet. More than 900 million persons globally are estimated to have inadequate or deficiencies in zinc and iron (Saltzman et al., 2017). Zinc and iron are normally found in low concentrations in maize, but genetic variation has been identified for both zinc and iron in QPM inbreds (Pandey et al., 2015) and elevated zinc concentrations were found at a higher frequency in QPM versus normal germplasm (Hindu et al., 2018). The genetic variation for iron in maize was found at levels too low to meet daily nutritional requirements and the HarvestPlus target of 52 ug/g , but zinc levels in several genotypes were found above the target of 33 ug/g (Bouis and Welch, 2010). In Zambia, zinc biofortified maize was found to meet the dietary needs of young children (Chomba et al., 2015), and a zinc biofortified maize variety BIO-MZN01 with a zinc concentration of 36% over normal maize was released in Colombia in 2018 through CIMMYT/ HarvestPlus/Agriculture for Nutrition Health (A4NH)/ and International Center for Tropical Agriculture (CIAT) collaborative efforts (http://www.HarvestPlus.org).

Under HarvestPlus/China, which involves several Chinese institutions, the major focus has been on biofortification of maize for proVA. Two temperate donors maize inbred line, Hp321-1 (provided by Prof. Jianbing Yan, Huazhong Agricultural University) and A619 (provided by Prof. Torbert Rocheford, Purdue University) have been utilized in breeding efforts for improved proVA concentration. In YAAS biofortification of QPM inbreds using MAS and the Hp321-1 donor has been performed (Li et al., 2015). Collaborative research with China Agricultural University utilizing the A619 donor, led to the development of the high proVA hybrid YR 506, with a mean proVA content above >15 ug/g for use in Southwestern China. In multi-locational trials the hybrid had a 8% yield increase compared to local check hybrid. Further temperate introgression activities have developed locally adapted A619 backcross four lines, for use in the southwestern China growing environment.

The QPM inbred parent CML166, which is adapted to Southwestern China, is utilized in the development of both QPM and HOM, improved protein quality hybrids (Table 1), and Hindu et al. (2018) reported this inbred is also a high zinc donor with 36 ug/g Zn concentration.

Future Breeding Efforts

Research will continue to develop multi- trait, nutritionally enhanced hybrids for use in the region, Targeting the rural poor communities remains a top priority of the Chinese government, and the hybrids will continue to play a role in enhancing human and animal nutrition, and economic livelihoods in these communities, as well as in neighboring countries sharing similar maize production environments.

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Molecular Breeding for Development of Biofortified Maize Hybrids in India

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Introduction

Micronutrient malnutrition resulting from consumption of unbalanced diet has emerged as one of the major health concerns, particularly in the developing and under-developed world (Bouis 2018). Globally, around two billion people suffer from malnutrition, while 815 million people are undernourished (Global Nutrition Report 2017). It is so widespread that 88% of the countries experience two or three forms of malnutrition. It causes loss of annual GDP by up to 11% in Asia and Africa. Southern Asia is most affected by malnutrition, with 34.1% and 15.4% of children under the age of five being stunted and wasted, compared to the global average of 22.9% and 7.7% respectively. In India, where 21.9% of the population lives in extreme poverty, it is estimated that 15.2% of Indians are undernourished (IFPRI 2016), 38.4% of Indian children aged five and below are stunted, 21.0% are wasted, and 7.5% are severely wasted due to consumption of foods low in nutrition. Further, 58.4% of Indian children (6-59 months), and 22.7% and 53.0% of adult men and women (15-49 years) suffer from anemia. Thus, malnutrition poses serious socioeconomic consequences to the country (National Family Health Survey-4 2015-16).

The United Nations' Sustainable Development Goals (SDGs) places great importance on nutrition, and by extension alleviating malnutrition. Every $1 invested in a proven nutrition program is akin to benefits worth $16 (IFPRI 2016). Thus, a balanced and nutritious diet assumes great significance to mitigate malnutrition (Gupta et al. 2015).

Various approaches *viz*., (i) food-fortification (ii) medical-supplementation and (iii) dietary-diversification are generally used for alleviating micronutrient malnutrition. However, these avenues have not been successful in the long run. Lack of purchasing power, poor infrastructure, crop seasonality, expense and lower bioavailability are some of the reasons that affect their successful implementation (Lieshout and Pee 2005). Biofortification, a strategy of increasing micronutrient density in edible parts of plant through plant breeding, is a viable, sustainable and cost-effective means for enhancing required levels of micronutrients in food (Bouis et al. 2011). Maize serves as an important source of energy, proteins and an array of essential nutrients, and is an integral part of diet among millions of people worldwide (Yadav et al 2015; Neeraja et al. 2017). Micronutrients such as lysine, tryptophan, provitamin-A, vitamin E, iron (Fe) and zinc (Zn) are lacking in normal maize endosperm. Favorable alleles of key genes imparting higher micronutrients in endosperm, and associated markers, provide opportunity to develop biofortified maize hybrids through molecular breeding (Table 1). Here we present the status and research efforts being undertaken on molecular breeding for development of biofortified maize hybrids in India.

Table 1. Details of genes and markers being used in marker-assisted selection of nutritional traits in maize.

S. No.	Trait	Genes	Chr.	Marker	Type	Reference
1.	Lysine and tryptophan	*opaque2*	7	*umc1066* & *phi057*	Gene-based SSR	Gupta et al. 2013
2.	Lysine and tryptophan	*opaque16*	8	*umc1141* & *umc1149*	Linked-SSR	Yang et al. 2005
3.	Provitamin-A	*crtRB1*	10	3'TE-based marker	Gene-based *InDel*	Yan et al. 2010

4.	Provitamin-A	*lcyE*	8	5'TE-based marker	Gene-based *InDel*	Harjes et al. 2008
5.	α-tocopherol	*VTE4*	5	Promoter/ 5'UTR-based marker	Gene-based *InDels*	Li et al. 2012
6.	Low phytate	*lpa1-1*	1	Allele-specific dominant marker	Gene-based	Abhijith 2018
7.	Low phytate	*lpa2-1*	1	CAPS	Gene-based	Abhijith 2018
				umc2230	Linked-SSR	Tamilkumar et al. 2014

Genetic Improvement of Essential Amino Acids

Proper growth and development of the human body requires 0.66 grams of protein per kilo of body weight per day (WHO/FAO/UNU 2007). The daily requirement of lysine is 30 mg/kg and 35 mg/kg body weight for adults and children respectively. Daily requirement for tryptophan is 4 mg/kg and 4.8 mg/kg of body weight in adults and children respectively. The deficiency of these amino acids leads to susceptibility to various diseases, and retarded mental and physical development (Galili and Amir 2013). Protein-energy malnutrition (PEM) now known as protein energy undernutrition (PEU), caused the highest number of deaths worldwide in 2016 (Nyakurwa et al. 2017). Pregnant women, the elderly and children are most vulnerable to PEU (Mpofu et al. 2014), thus urgent action is required. Food with balanced protein - especially with higher lysine and tryptophan - helps to combat the disease.

Introgression of opaque2

The discovery of the *opaque2* (*o2*) mutant in maize by Jones and Singleton in the 1920s was significant, as it enhances accumulation of lysine and tryptophan in the endosperm of normal maize (Mertz et al. 1964). Quality Protein Maize (QPM) results from the combination of recessive allele of *o2* (chromosome-7) and endosperm modifiers that increase the kernel hardness in the endosperm (Hossain et al. 2007; 2008a,b; Pandey et al. 2015a). Lysine concentration in *o2* maize is about 4% compared to 2% in normal maize, while tryptophan concentration is 0.8% compared to 0.4% in the wild types (Hossain et al. 2018). In India, Shakti, Rattan and Protina, the *o2*-specific soft endosperm-based maize composites were released in 1971 by All India Coordinated Research Project (AICRP) on Maize (Prasanna et al. 2001), and these are perhaps the first set of biofortified varieties developed through targeted breeding approaches across crops in the country. Hard endosperm-based *o2* composite, Shakti1 was released in 1997. Later, a series of QPM hybrids *viz*., Shaktiman1 (2001), Shaktiman2 (2004), HQPM1 (2005), Shaktiman3 (2006), Shaktiman4 (2006), HQPM5 (2007), HQPM7 (2008), HQPM4 (2010), Pratap QPM Hybrid1 (2013), and Shaktiman5 (2013) were released in India (Gupta et al. 2015). These biofortified hybrids were developed through conventional breeding approaches.

The cloning and characterization of the *o2* gene, followed by detection of three gene specific SSRs *viz*., *phi057*, *phi112* and *umc1066*, offer advantages in molecular marker-assisted conversion of non-QPM lines into their QPM versions (Prasanna et al. 2010; Pandey et al. 2018). Marker-assisted selection (MAS) derived QPM hybrid, Vivek QPM9, was released in 2008 by the ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora (Gupta et al. 2013). Vivek QPM9 is the *'first MAS-based maize cultivar'* released for commercial cultivation in India (Table 2). Molecular breeding efforts at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, have led the development of QPM versions of five normal commercial hybrids, *viz*., HM4, HM8, HM9, HM10, and HM11 using marker-assisted backcross breeding (MABB) approach (Hossain et al. 2014, 2018). Three of these QPM varieties *viz*., Pusa HM4 Improved, Pusa HM8 Improved and Pusa HM9 Improved were released in 2017 (Table 2) (Yadava et al. 2017). To develop this QPM version of hybrids, parental inbreds *viz*., HKI323, HKI1105 and HKI1128 were targeted for two generation-based backcrossing and assisted by marker-aided introgression of *o2* allele from three QPM donor inbreds *viz*., HKI161, CML161 and HKI193-1. *O2*-based simple sequence repeat (SSR) markers (*umc1066* and *phi057*) were successfully deployed for selection of *o2* allele. The introgressed inbreds possessed higher phenotypic resemblance to the respective recipient lines, including grain yield and modified kernels. Endosperm protein quality across inbreds was significantly improved by 52-95% and 47-118% for lysine and tryptophan respectively. The reconstituted QPM hybrids also possessed significantly higher lysine (48-74%) and tryptophan (55-100%) over original hybrids (Hossain et al. 2018). Considering the potential of MABB, several institutions *viz*., ICAR-Indian Institute of Maize Research (IIMR), Ludhiana; Acharya NG Ranga Agricultural University (ANGRAU), Hyderabad; CSK-Himachal Pradesh Krishi Vishvavidyalaya (CSK-HPKV), Palampur and Punjab Agricultural University (PAU), Ludhiana, are using MAS for the development of QPM hybrids.

Table 2. List of biofortified maize hybrids developed through molecular breeding and released in India

S. No.	Name of the hybrid	Nutritional trait(s)	Year of release	Average grain yield	Zone for which released
1.	Vivek QPM9	Tryptophan (0.83%) and lysine (4.19%)	2008	5.8 t/ha (NHZ) and 5.4 t/ha (PZ)	Northern Hill Zone (NHZ) & Peninsular Zone
2.	Pusa HM4 Improved	Tryptophan (0.91%) and lysine (3.62%)	2017	6.4 t/ha	Northern Western Plain Zone (NWPZ)
3.	Pusa HM8 Improved	Tryptophan (1.06%) and lysine (4.18%)	2017	6.3 t/ha.	Peninsular Zone (PZ)
4.	Pusa HM9 Improved	Tryptophan (0.68%) and lysine (2.97%)	2017	5.2 t/ha	North Eastern Plain Zone (NEPZ)
5.	Pusa Vivek QPM9 Improved	Provitamin-A (8.15 µg/g), tryptophan (0.74%) and lysine (2.67%)	2017	5.6 t/ha (NHZ) and 5.9 t/ha (PZ)	Northern Hill Zone (NHZ) & Peninsular Zone (PZ)

Introgression of opaque16

A recessive *opaque16* (*o16*) (on chromosome-8) isolated from Robertson's Mutator (Mu) stock was discovered by Yang et al. (2005). Research efforts at IARI, New Delhi, revealed that genotype with *o16o16* possessed nearly two-fold more lysine (0.247%) and tryptophan (0.072%) in mutants, than *O16O16*-based wild type (0.125% lysine and 0.035% tryptophan (Sarika et al. 2017). Sarika et al. (2018a) reported that *o16* does not influence the endosperm attributes such as grain hardness and vitreousness. The study of starch and protein complexes in endosperm through scanning electron microscope also revealed the compact packaging and hard vitreous endosperm of *o16* lines as observed in normal endosperm. Zein synthesis is not affected in the mutant as well. The mechanism of *o16* on nutritional improvement is thus completely different from the *o2*. Genotype with *o16o16* therefore offers great advantage to the breeders over *o2o2* as accumulation of endosperm modifiers is not required in QPM breeding (Sarika et al. 2018a). The newly developed *o16o16*-based progenies developed here would serve as a valuable genetic resource in the QPM breeding program in India (Sarika et al. 2017). Further, marker-assisted pyramiding *o2* and *o16* in four *o2*-based QPM hybrids *viz*., HQPM1, HQPM4, HQPM5 and HQPM7 have been undertaken at IARI, New Delhi (Sarika et al. 2018b). The linked SSRs *viz*., *umc1141* and *umc1149* were used to pyramid *o16* in *o2* genetic background, and MAS-derived inbreds possessed as high as 76% and 91% more lysine and tryptophan, respectively, over the recurrent parents. Hybrids with *o2o2/o16o16* also showed an average increase of 49% and 60% in lysine and tryptophan over the original hybrids, with the highest enhancement at about 64% and 86% respectively. This is the first report of enhancement of lysine and tryptophan by *o16* in maize genotypes adaptable to sub-tropics. Multi-location evaluation of the reconstituted hybrids revealed similar grain yield and attributing traits to their original versions (Sarika et al. 2018b). In some areas of the country, white maize is a popular choice as food over yellow maize, thus two normal white maize hybrids viz., HM5 and HM12 have now been targeted for marker-assisted introgression of *o2* and *o16*.

Genetic Improvement for Provitamin-A

Vitamin A is vital for vision and healthy reproductive and immune systems in humans (Sommer and West 1996). Around 4.4 million preschool-age children and 20 million pregnant women (one third are clinically night blind) suffer from visible eye damage and night blindness due to vitamin A deficiency (VAD). Although maize possesses the highest levels of carotenoids among cereals (Tiwari et al. 2012; Sivaranjani et al. 2013, 2014), the concentration of provitamin-A is very low (0.02 to1.75 µg/g) (Vignesh et al. 2012). Considering various factors of processing and absorption, the target level of 15 µg/g of provitamin-A in maize has been set by HarvestPlus program (Ortiz-Monasterio et al. 2007).

In carotenoid biosynthetic pathway of maize, *lycopene-ε-cyclase* (*lcyE*) and *β-carotene hydroxyalse1* (*crtRB1*) genes have been identified to significantly regulate the accumulation of provitamin-A (Babu et al. 2013; Vignesh et al. 2012, 2013; Muthusamy et al. 2015a,b,c, 2016; Zunjare et al. 2017, 2018 a,b,c). Harjes et al. (2008) showed that a variation at the *lcyE* gene (bin 8.05) alters flux down α-carotene versus β-carotene branches and causes a three-fold difference in provitamin-A compounds. Mutant *crtRB1* gene (bin 10.05) blocks the conversion of β-carotene into β-cryptoxanthin, and further to zeaxanthin, thereby enhancing the provitamin-A concentration (Yan et al. 2010). The strong effect (2-10 fold) of favorable allele of *crtRB1* for enhanced provitamin-A in maize is now well established and has been used to develop provitamin-A rich maize lines/ hybrids worldwide (Babu et al. 2013; Choudhary et al. 2014, 2015; Muthusamy et al. 2014; Zunjare et al. 2017; 2018a).

Introgression of crtRB1

At IARI, New Delhi, the favorable allele of *crtRB1* gene from CIMMYT-HarvestPlus genotypes was introgressed in the parental inbreds of three popular maize hybrids *viz*., HM4, HM8 and Vivek Hybrid27 using MABB approach (Muthusamy et al. 2014). The parental inbreds *viz*., V335, V345, HKI1105, HKI161 and HKI323 were used as recurrent parents, while HP465-30, HP465-35, HP467-6, HP467-13 and HP467-4 were used as donors for *crtRB1*-favorable allele. The introgressed progenies possessed 8.6 to 16.4 µg/g of β-carotene, while the reconstituted hybrids recorded 10.5-21.7 ug/g of β-carotene (Muthusamy et al. 2014). The improved version of Vivek Hybrid27, and two independently derived provitamin-A rich hybrids, APH1 and APH2, are currently under various stages of national testing. To further diversify the provitamin-A rich inbreds, marker-assisted pedigree program was followed; several elite normal inbreds were crossed with HP704-22 as *crtRB1* donor parent. F_2 populations were genotyped and plants homozygous for *crtRB1*were selected. These newly developed inbreds possessing higher level (>15 µg/g) of provitamin-A carotenoids would be used in the provitamin-A rich hybrid breeding program. Considering the success of MAS for *crtRB1*, IIMR, Ludhiana; ANGRAU, Hyderabad; CSK-HPKV, Palampur and VPKAS, Almora have now initiated the development of provitamin-A hybrids through MABB.

Genetic Improvement for both QPM and Provitamin-A

Improvement in normal maize

We at IARI have attempted to combine QPM and provitamin-A by marker-assisted stacking of *crtRB1* and *o2*. Muthusamy et al. (2014) targeted VQL1 and VQL2 as parental inbreds for marker-assisted introgression of *crtRB1* allele. Pusa Vivek QPM9 Improved is the first variety released in the country that possesses higher provitamin-A (8.15 µg/g), tryptophan (0.74%) and lysine (2.67%). This is also the country's first multi-nutrient rich maize hybrid. Several researchers have demonstrated the cumulative and positive effects of *crtRB1* and *lcyE* genes for provitamin-A accumulation (Babu et al. 2013; Zunjare et al. 2017). Zunjare et al. (2018a) in India stacked the favorable alleles of *crtRB1*, *lcyE* and *o2* for biofortifying four hybrids for provitamin-A, lysine and tryptophan. Four elite QPM parental lines (HKI161, HKI163, HKI193-1 and HKI193-2) which are the parents for four commercial QPM hybrids *viz*., HQPM1, HQPM4, HQPM5 and HQPM7 with wide popularity in India, were targeted. The mean provitamin-A content of introgressed lines of HKI161, HKI163, HKI193-1 and HKI193-2 was 12.93µg/g, 8.23µg/g, 10.69µg/g and 11.54µg/g respectively. The mean provitamin-A in HQPM1-, HQPM4-, HQPM5- and HQPM7-based reconstituted hybrids was 9.95µg/g, 10.47µg/g, 9.63µg/g and 12.27µg/g respectively. Original hybrids *viz*., HQPM1, HQPM4, HQPM5 and HQPM7 had lysine content of 0.298%, 0.337%, 0.352% and 0.374%, while the same for tryptophan was 0.078%, 0.084%, 0.082% and 0.086% respectively. These provitamin-A rich hybrids are in various stages of national testing. Besides provitamin-A rich versions of recently released QPM hybrid, Pusa HM8 Improved has been developed and is also being evaluated under national trials.

Similarly, QPM version of HKI1128, an elite parental inbred of popular maize hybrids [HM9 (HKI1105 × HKI1128), HM10 (HKI193-2 × HKI1128) and HM11 (HKI1128 × HKI163)] was targeted for introgression of *crtRB1* (Goswami et al. 2016). HKI1128 was earlier converted into QPM through marker-assisted selection of *o2* allele (Hossain et al. 2018), and other parental lines *viz*., HKI1105, HKI193-1 and HKI163 have been improved for protein quality and provitamin-A in an earlier program (Hossain et al. 2018; Zunjare et al. 2018a). The *crtRB1*-based progenies of HKI1128Q possessed higher mean provitamin-A 10.75µg/g compared to HKI1128Q (3.38µg/g). Essential amino acids *viz*., lysine (mean: 0.303%) and tryptophan (0.080%) were high among the introgressed progenies (Goswami et al. 2016). This newly derived provitamin-A rich HKI1128Q is being used for hybrid development.

IARI-bred provitamin-A rich hybrids were analyzed using a simulated *in vitro* digestion/Caco-2 cell model at ICMR-National Institute of Nutrition (NIN), Hyderabad, and it was observed that the consumption of 200 g/day biofortified maize grains would contribute to 52-64% of recommended dietary allowance (RDA) for adult Indian men, after adjusting for cooking losses and conversion factors (Dubey et al. 2018). Several institutions *viz*., PAU, Ludhiana and Tamil Nadu Agricultural University (TNAU), Coimbatore, have now initiated the development of provitamin-A rich QPM hybrids using molecular breeding.

Improvement in sweet corn:

Sweet corn, consumed in fresh and processed form, is an important source of energy and nutrients (Hossain et al. 2013; Mehta et al. 2017a,b,c). Sweet corn kernels and soups are popular (Khanduri et al. 2010, 2011), but to date, no sweet corn hybrid in India has been improved for nutritional quality. Availability of *crtRB1* and *o2* genotypes and associated markers provide opportunity to improve nutritional quality of sweet corn. Three *shrunken2* (*sh2*)-based sweet corn inbreds *viz*., SWT016, SWT017 and SWT018 were targeted for enrichment of provitamin-A, lysine and tryptophan. These are parents of two sweet corn hybrids; ASKH1 (SWT016 × SWT017) and ASKH2 (SWT016 × SWT018) developed at IARI, New Delhi. HKI193-2 and HKI161 introgressed with *crtRB1* and *o2* were used as donor parents (Zunjare et al. 2018a). Similarly, parental lines (SWT019 and SWT020) of ASKH4 (*sh2*-based sweet corn hybrid) were also targeted for enhancement of essential amino acids and vitamin A by marker-assisted introgression of *o2* and *crtRB1* genes. ASKH4 hybrid was recently released and notified for commercial cultivation in 2018. Parental lines of provitamin-A rich versions of HQPM1, HQPM4, HQPM5 and HQPM7 have been converted to *sh2*-based sweet corn versions. Consequently, nutritionally enriched genotype being developed here would increase the acceptability of sweet corn.

Genetic Improvement for Vitamin E

Vitamin E, or tocopherol, plays essential biological roles by protecting the human body from reactive oxygen species and free radicals (Bramley et al. 2000). Recommended dietary allowance for vitamin E is 4 mg/day for a 0-6 months old child, 15 mg/day for both males and females and 19 mg/day for lactating mothers (Institute of Medicine 2000). Vitamin E deficiency symptoms include progressive damage to nervous and cardiovascular systems (Traber et al. 2008). Vitamin E is composed of four isoforms (α, β, δ, γ), while γ-tocopherol constitutes ~80% of the total tocopherol, and α-tocopherol accounts for ~20% of the total pool. However, γ-tocopherol is less absorbed in the body due to lack of affinity of receptors in the body. On the contrary, α-tocopherol is the most favored fraction and is well absorbed in the body. Li et al. (2012) has reported two insertion/deletions (*InDel7* and *InDel118*) within *ZmVTE4* (*γ-tocopherol methyl transferase*) gene significantly affect the accumulations of α-tocopherol.

Improvement in normal maize

Efforts to enhance vitamin E levels in maize was initiated at IARI, New Delhi (Das et al. 2018). Fifty-four maize inbreds representing four haplotypes of *ZmVTE4* selected out of >450 diverse inbreds were evaluated. Wide variation in α- (3.2-28.6μg/g), γ- (3.5-52.4μg/g), δ- (1.3-9.6μg/g) and total-tocopherol (16.4-87.7μg/g) was observed. The mean α-tocopherol was 16.2μg/g in the most and 7.6μg/g in the least favorable haplotypes. HKI-1378, DQL-784-5-1 and CML-218 were identified as the most promising stable inbreds. Novel SNP and *InDels* in the desired haplotype of *ZmVTE4* were also identified. The most favorable allele of *ZmVTE4* was introgressed into provitamin-A rich versions of four QPM hybrids by MABB. Original inbreds *viz.,* HKI161, HKI163, HKI193-1 and HKI193-2 possessed a mean of 8.0μg/g of α-tocopherol, compared to 15.2μg/g in the introgressed progenies. These newly derived inbreds also possessed high lysine, tryptophan and provitamin-A. The multi-nutrient rich maize inbreds developed are important in alleviating malnutrition through sustainable and cost-effective approvitamin-A ch.

Improvement in sweet corn

Two promising *sh2*-based sweet corn hybrids, ASKH-1 (SWT16 × SWT17) and ASKH-2 (SWT16 × SWT18) developed at IARI were targeted for enrichment of vitamin A and E. MABB was followed to introgress favorable alleles of *crtRB1* and *VTE4* for enhancing provitamin-A and vitamin E respectively. A HarvestPlus derived line was used as donor. Promising BC_2F_2 segregants having homozygosity at *sh2*, *crtRB1* and *VTE4* were selected. The newly derived progenies resembled their recurrent parents for plant, ear and grain characteristics. These introgressed progenies would be used for reconstitution of hybrids, besides serving as valuable donors. The improved sweet corn genotypes with high vitamin A and E would further increase their acceptability. This is the first effort in the country to simultaneously enrich sweet corn with both vitamin A and vitamin E.

Genetic Improvement for Bioavailability of Kernel Iron and Zinc

Among micronutrients, deficiency of iron (Fe) and zinc (Zn) poses serious health constraints worldwide (Bouis 2018). Fe deficiency adversely affects cognitive development, resistance to infection, work capacity, productivity and pregnancy (Scrimshaw 1984). Zn is involved in cellular growth and differentiation, and deficiency causes impaired growth, immune dysfunction, increased morbidity and mortality, adverse pregnancy outcomes and abnormal neurobehavioral development (Prasad 1996). Breeding efforts to develop crop varieties with target level of kernel Fe (60 μg/g) and Zn (38 μg/g) were undertaken worldwide including in India (Prasanna et al. 2011; Chakraborti et al. 2011a,b; Pandey et al. 2015b; Mallikarjuna et al. 2014, 2015). However, not much success was achieved primarily due to polygenic nature and high genotype × environment interactions (Gupta et al. 2015). One of the alternative ways to effectively enhance Fe and Zn in maize is to increase their bioavailability through manipulation of anti-nutritional factor such as phytic acid (PA).

PA is composed of myoinositol 1,2,3,4,5,6-hexakisphosphate, and represents approximately 75-80% of the total phosphorous present in the maize grain (Raboy 2009). PA possesses strong negative charges due to presence of phosphate groups and binds with positively charged mineral ions *viz*., Fe and Zn, thereby reducing their bioavailability in the human body to 5% and 25% respectively (Bouis et al. 2011). Moreover, monogastric animals - including humans, poultry and swine - cannot digest PA in their gut, since they lack phytic acid hydrolyzing enzyme phytase. As a result, the phytate is expelled directly to the environment along with excreta, posing a serious concern in piggery and poultry farming where the continuous expulsion of high phosphorous load causes pollution in nearby water bodies (Jorquera et al. 2008). Extensive research in seed PA has led to the isolation of three *lpa* mutations in maize namely *lpa-1*, *lpa-2* and *lpa-3.* Compared to the wild-type kernels, they contain 66%, 50% and 50% less phytic acid respectively (Shi et al. 2005). These *lpa* mutants can be effectively introgressed to enhance the bioavailability of Fe and Zn.

Development of markers for lpa1 and lpa2

Though *lpa* mutants are available, quantification of phytic acid is destructive in nature. Non-availability of gene-based markers for selection of *lpa1* and *lpa2* genes poses limitations in breeding programs. Here, we developed and validated gene-based markers for *lpa1-1* and *lpa2-1* genes. The *lpa1-1* mutation is due to a C to T transition. Based on this sequence information, mutant and wild specific markers were developed and validated across eight F_2 populations segregating for *lpa1-1* allele (Dosad et al. 2016 & 2017). The *lpa2-1* gene was sequenced in mutant and wild type using seven overlapping primers. Nucleotide polymorphisms that distinguished mutant from wild type allele were selected and used for designing CAPS marker. This co-dominant CAPS marker has been validated across five F_2 populations segregating for *lpa2-1* allele (Abhijith 2018).

Introgression of lpa1 and lpa2

In India, novel inbreds possessing *lpa-1-1* and *lpa-2-1* alleles were developed on crossing with elite maize genotypes (Abhijith 2018). Two mutants were crossed with each of the seven recurrent parents *viz*., HKI323, HKI1105, HKI1128, HKI161, HKI163, HKI193-1, and HKI193-2. These are the parents of nine hybrids *viz*., HM4, HM8, HM9, HM10, HM11, HQPM1, HQPM4, HQPM5 and HQPM7. QPM and/or provitamin-A versions of these hybrids developed earlier at IARI were targeted for reduction of PA through MABB approach. Markers thus developed at IARI are being used for selection of *lpa* genes. Earlier, *lpa2* was successfully introgressed into UMI395 and UMI285 using linked SSR at TNAU, Coimbatore (Sureshkumar et al. 2014; Tamilkumar et al. 2014). Several institutions *viz*., IIMR, Ludhiana and VPKAS, Almora are now developing low PA-based hybrids through molecular breeding. The inbreds thus developed would be used for development of low PA-based hybrids that would possess higher bioavailability of Fe and Zn.

Future Prospects

Effective collaborations among various national and international research institutions are important for the development of biofortified maize hybrids adapted to diverse agro-ecologies of the country. Considering the importance of alleviating malnutrition, the Indian Council of Agricultural Research (ICAR), has initiated Consortia Research Platform (CRP) on Crop Biofortification to further strengthen breeding programs on nutritional quality. Several crops like maize, wheat, rice, pearl millet, sorghum and small millets have been targeted for enrichment of micronutrients. ICAR funded CRP on Molecular Breeding has also been initiated to emphasize the need for molecular breeding in accelerated development of biofortified maize hybrids. Several ICAR institutions and State Agricultural Universities (SAU) are part of these networks for effective coordination and collaboration. Department of Biotechnology (DBT), Department of Science and Technology (DST), and the Government of India have also funded several projects on maize biofortification to develop hybrids rich in nutritional quality. Integration of doubled haploid technology would further accelerate the breeding cycle and development of biofortified maize hybrids. However, research collaborations among various national partners under National Agricultural Research System (NARS) and international research organizations like CIMMYT and HarvestPlus would further help in sharing novel germplasm and expertise for the development of biofortified maize.

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Adaptation Pattern of Introduced Biofortified Maize Varieties in Nepal

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Introduction

Maize is Nepal's second most important cereal following rice in area, production and productivity and it covers almost 0.9 million ha of the cultivated area. It is estimated that 74% of Nepal's maize area is located in the hills and its area in the hills is higher than that of rice and wheat combined. The maize area, production and productivity has increased by 3%, 18% and 15% respectively from its level a decade ago and the national average yield of maize in Nepal is 2.55 t ha^{-1}. It is the major staple food in the hills and accounts for 43% of the area of food crops in the hills and a quarter of Nepal's cereal production comes from maize (MOARD, 2018; Koirala, 2017). Nepal also has one of the highest per capita consumption of maize in South Asia with 98gm/person/day (Ranum et al; 2014). Apart from human consumption, maize is the main source of feed for the poultry industry in Nepal and its annual demand is increasing at the rate of 11% (CDD, 2011; KC et al; 2015).

Despite the reducing global trend of under nutrition, South Asia is still has the highest rate of children malnutrition. According to the Global Nutrition Report (2017), two of every five of the world's stunted children and more than half of all wasted children lived in South Asia. Nepal has significantly reduced children malnutrition rates from its levels in the mid-1990s. The various health and nutrition interventions, maternal education, increased public investments are among the reasons for the noticeable decline of malnutrition in Nepal (Headey and Hoddinott, 2015). However, the current rates are still among the highest compared to global and regional standards. According to Nepal Demographic and Health Survey (NDHS) 2016, the stunting (height for age) rate is 36%, which means almost one out of three children is stunted and one out of four is wasted (weight for height) and a tenth of them are underweight (weight for age), Fig 1. However, the rate of malnutrition within Nepal also varies from province to province and between mountains and terai. The rate of stunting is highest in the mountains (47%) while proportion of wasting and underweight is highest in the terai (12% and 33% respectively). Province 6 has the highest number of stunted children (55%) while provinces 3 and 4 have the lowest (29%). In addition, nearly one-third of pre-school children in Nepal are affected by subclinical vitamin A deficiency.

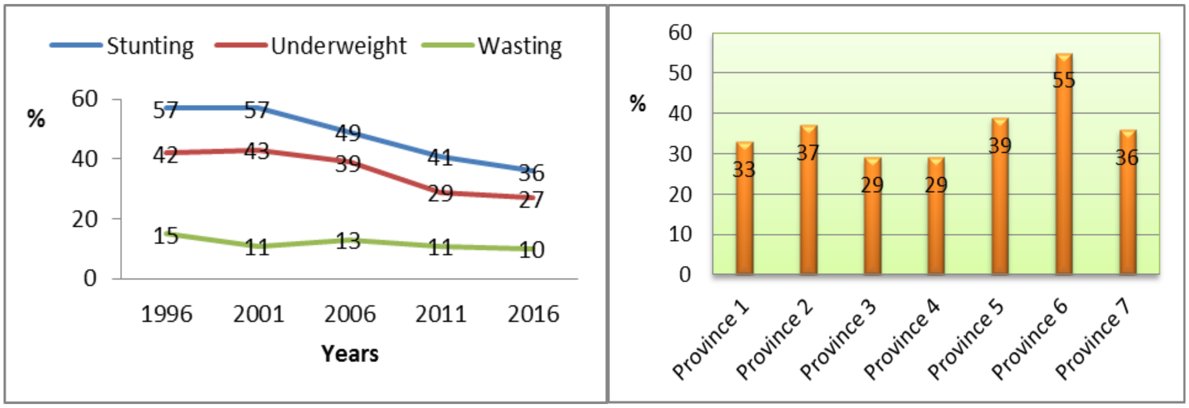

Figure 1. (A) Trends of children malnutrition in Nepal (%); **(B)** Province wise rate of stunting among preschool children (<5 years) (Redrawn by the authors from the data of NDHS, 2016).

Attaining food and nutritional security is among the top priorities of government of Nepal to increase availability and access to more nutritious foods. Biofortification or the breeding of staple food crops to increase their micronutrient density is widely viewed as a valuable, cost effective, accessible and affordable option, as compared to nutritional diversification, industrial fortification and pharmaceutical supplementation, to sustainably improve the nutritional status of malnourished populations. Several studies show that maize varieties enriched with provitamin-A, kernel Zn and QPM contributes to reduce malnutrition in communities where maize is a dietary staple and often a sole source of energy (Bjarnason and Vasal, 1992; Bressani, 1991, Elwyn et al. 2015; Maqbool et al., 2018; Pfeiffer& Clafferty, 2007; Prasanna et al., 2001; Pixley et al., 2013)

The evaluation of nutritious maize in Nepal started with the introduction and validation of various Quality Protein Maize germplasm from CIMMYT. The first QPM variety was released in 2008 by the name Poshillo Mekkai-1(simply means nutritious maize-1). Field research conducted during 2013-14 in Nepal show the significant performance of this and other open pollinated varieties of QPM compared to normal check varieties and farmers also reported the good milling quality of QPM grains as compared to normal maize (Jiban et al. 2015, Koirala, 2017). During 2017-18 cropping season Nepal under the Nepal Seed and Fertilizer Project introduced biofortified maize products enriched with kernel zinc and provitamin-A for the first time. The NSAF project also tested the first QPM hybrids introduced from CIMMYT- Latin America breeding hub (Colombia). However, the agronomic performance and adaptation pattern of these newly introduced biofortified maize products in Nepal is not documented so far. Hence, the objectives of this study are: preliminary identify good performing entries and further recommend them for wider scale testing in Nepal; share performance information of biofortified maize products to major stakeholders including but not limited to researchers, seed companies, nutrition and health workers so that they can plan and implement nutritional interventions in Nepal and beyond.

Materials and Methods

Germplasm

Thirty-two yellow QPM and eight white kernel Zn enriched hybrids were introduced from CIMMYT's Latin America breeding hub in Colombia. The QPM hybrids were compared with two and the Zn trials with one normal check making the total number of entries for QPM and Zn to be 34 and nine respectively. In addition, 18 provitamin-A enriched hybrids introduced from International Institute of Tropical Agriculture (IITA), Ibadan-Nigeria were compared with two normal maize hybrids. The trials were conducted during winter 2017 (QPM and Zn) and spring 2018 (PVA). Generally, the biofortified maize products were adapted to tropical environments and had a high level of lysine and tryptophan, kernel Zn and provitamin-A carotenoids in the endosperm. The lists of the tested germplasms are presented in Table 1.

Description of the trial sites and field management

The QPM and Zn trials were evaluated in three locations during winter 2017 and the PVA trials were conducted during spring 2018 cropping seasons. The QPM trials were conducted at Lumbini Seed Company at Bahirawaha, National Maize Research Program (NMRP) at Rampur and at Unique Seed Company in Danghadhi. The Zn trials were conducted at GATE Nepal Seed Company and at the Regional Agricultural Research Station both in Banke district and at NMRP research site in Jhapa, Eastern Nepal. The two PVA trials were conducted in Kialali district at the trial site of Unique and Panchashakti seed companies. All the trials were managed under optimum conditions and the QPM and Zn trials were conducted under irrigations and the PVA trials received only supplementary irrigation. Details on the description of the trials sites is presented in Table 2.

Table 1. List of biofortified maize products evaluated during 2017/18

QPM (Yellow kernel)				Provitamin-A (Orange kernel)		Zn enriched (White kernel)	
Entry no	Entry code	Entry no	Entry code	Entry no	Entry code	Entry no	Entry code
1	SA2282-1	21	SA2283-8	1	EEPVAH-2	1	SA2299-5
2	SA2282-2	22	SA2283-9	2	EEPVAH-3	2	SA2300-1
3	SA2282-3	23	SA2283-10	3	EEPVAH-4	3	SA2299-4
4	SA2282-4	24	SA2283-11	4	EEPVAH-5	4	SA2272-1
5	SA2282-5	25	SA2283-12	5	EEPVAH-7	5	SA2272-2
6	SA2282-6	26	SA2283-13	6	EEPVAH-8	6	SA2291-1
7	SA2282-7	27	SA2283-14	7	EEPVAH-9	7	SA2291-2
8	SA2282-8	28	SA2283-15	8	EEPVAH-10	8	SA2291-3
9	SA2282-9	29	SA2283-16	9	EEPVAH-11	9	Local Check
10	SA2282-10	30	SA2283-17	10	EEPVAH-12		
11	SA2282-11	31	SA2283-18	11	EEPVAH-13		
12	SA2282-12	32	SA2283-20	12	EEPVAH-14		
13	SA2282-13	33	SA2286-1	13	EEPVAH-15		
14	SA2282-14	34	Local Check	14	EEPVAH-24		
15	SA2282-16			15	EEPVAH-25		
16	SA2223-1			16	EEPVAH-26		
17	Local check			17	EEPVAH-27		
18	SA2283-5			18	EEPVAH-28		
19	SA2283-6			19	Local Check		
20	SA2283-7			20	Local Check		

Table 2. Description of the trial sites used for the evaluation of biofortified maize products

Location	District	Latitude	Longitude	Altitude (m)	Code	Trial type	Plot size (m x m)	Density (plants ha^{-1})
Bankatti-6	Banke	28^{0} 13' 28" N	81^{0} 35' 18" E	189	GATE	Zn enriched	4.0 x 0.60	83,333
Khajura	Banke	28^{0} 06' 34" N	81^{0} 35' 46" E	148	KAJU	Zn enriched	4.0 x 0.60	83,333
Dhangadhi-13	Kialali	28^{0} 44' 16" N	80^{0} 35' 15" E	180	UNIQ	QPM/Provitamin-A	4.0 x 0.60	83,333
Dhangadhi-6	Kailali	28^{0} 42' 27 " N	80^{0} 36' 25 " E	194	PNCH	Provitamin-A	4.0 x 0.60	66,666
Rampur	Chitwan	27^{0} 39’ 16” N	84^{0} 21’ 02” E	188	RAMP	QPM	4.0 x 0.60	83,333
Bhalwari	Rupendehi	27^{0} 36' 07" N	83^{0} 28' 58" E	123	LUMB	QPM	4.0 x 0.60	83,333
Maharanijhoda	Jhapa	26° 33′ 36″ N	87° 40′ 48″ E	100	JHAP	Zn enriched	4.0 x 0.60	83,333

Experimental design and statistical analysis

The QPM and Zn trials were planted in alpha-lattice design (Patterson and Williams, 1976) with two replicates and the PVA trials in RCBD. Grain yield at each locations was statistically analysed with META-R software (Alverado et al., 2016). Grain yield (GY) was calculated by using following conversions;

$$\text{GY (t/h)} = \left(\frac{FW\,(g)}{1000}\right) \times (\mathbf{100} - \boldsymbol{Moi}\%)\ /(\mathbf{100} - \mathbf{12.5}) \times \left(\frac{\mathbf{10}}{\boldsymbol{PlotSize}}\right) \times \boldsymbol{Shelling}\ \%$$

Where, FW is field weight, Moi% is moisture percentage of grains at harvest, 12.5% moisture level and 80% shelling percentage was considered to estimate the grain yield. Boxplot was generated for grain yield for individual locations using STAD-R (Descriptive Statistics for Experimental Designs) software (Pacheco et al., 2017).

Results and Discussion

High Zn trials

The introduced entries performed higher than the normal check at all the three locations. The highest yield was recorded at GATE where entry 4 (SA2272-1) yielded 10.95 t ha^{-1}. All the introduced zinc varieties were not showing statistical difference from the local hybrid check (entry 9). At JAHP and KAJU sites, entries 3 (6.80 t ha^{-1}) and 1 (7. 28 t ha^{-1}) performed significantly above the checks (entry 9), respectively. Based on the mean performance of the entries, the highest yield was recorded at GATE (entry 4 with 10.90 t ha^{-1}) and the lowest yield at KAJU (entry 9, 3.06 t ha^{-1}) (Table 3).

Table 3: Grain yield performance (t ha^{-1}) of Zn enriched maize varieties.

Ranks	**GATE**		**JAHP**		**KAJU**	
	Entry	**Yield**	**Entry**	**Yield**	**Entry**	**Yield**
1	4	10.90	3	6.80	1	7.28
2	9	10.24	1	5.95	2	5.56
3	1	9.98	2	4.45	3	5.37
4	6	9.88	5	4.35	8	5.03
5	3	9.23	7	4.18	6	4.25
6	2	8.93	9	4.12	7	4.10
7	8	8.71	8	4.10	4	3.69
8	5	8.33	6	3.92	5	3.39
9	7	7.51	4	3.85	9	3.06
Mean	**9.30**		**4.63**		**4.63**	
LSD$_{0.05}$	**3.77**		**1.38**		**2.61**	
CV %	**16.00**		**13.00**		**22.00**	
P	**Ns**		*****		*****	

QPM trials

The result of the top ten performing QPM entries out of the 34 is listed under Table 4. The QPM entry 18 was among the top performing entries at LUMB by yielding 12.56 t ha^{-1}, while entry 29 and 31 was the number one hybrids by yielding 8.29 and 11.03 t ha-^{1} at RAMP and UNIQ, respectively. The check variety entry 34 ranked number one at LUMB and number three at RAMP and was not among the top ten at the UNIQ site. The performance of the QPM entries was statistically different at RAMP and UNIQ while the entries performance was statistically insignificant at LUMB. The result from this trial can be an indication for a better or comparative performance of QPM as compared to normal maize hybrids based on the grain yield (Table 4).

Table 4. Grain yield performance (t ha^{-1}) of top ten QPM hybrids.

Rank	**Locations**					
	LUMB		**RAMP**		**UNIQ**	
	Entry	**Yield**	**Entry**	**Yield**	**Entry**	**Yield**
1	34	14.00	29	8.29	31	11.03
2	18	12.56	16	8.11	27	10.94
3	24	12.51	34	8.04	21	10.48
4	16	12.24	22	8.02	16	10.07
5	6	12.17	31	7.88	32	9.44
6	3	12.14	17	7.27	23	8.81
7	28	11.81	28	7.03	3	8.78
8	30	11.77	5	6.99	28	8.75
9	23	11.72	27	6.91	29	8.58
10	20	11.68	21	6.77	22	8.49
Mean	**10.72**		**5.55**		**7.25**	
$LSD_{0.05}$	**3.32**		**2.47**		**3.97**	
CV %	**15.22**		**21.83**		**26.96**	
P	**ns**		*******		*****	

Table 5. Grain yield performance (t ha^{-1}) of top ten PVA hybrids (total number of entries).

Ranks	**Locations**			
	PNCH		**UNIQ**	
	Entry	**Yield**	**Entry**	**Yield**
1	14	6.55	14	9.28
2	20	6.34	7	9.09
3	16	5.57	17	7.17
4	18	5.17	4	6.73
5	13	5.14	9	6.56
6	6	5.13	2	6.48
7	19	5.07	10	6.47
8	10	5.04	16	6.39
9	17	4.96	8	6.18
10	4	4.60	11	6.18
Mean	**4.72**		**6.32**	
$LSD_{0.05}$	**1.93**		**4.23**	
CV%	**19.50**		**31.97**	
p	**ns**		**ns**	

PVA trials

Out of the total 20 entries, the PVA hybrid (entry 14) was the highest at both the locations. However, it yielded higher at UNIQ by giving 9.28 t ha^{-1}. The check variety was the second highest at PNCH while it was not among the top ten at UNIQ. Although the entries were not statistically different, the result, however, show the comparable performance of the PVA hybrids with the normal maize counterparts.

A boxplot was constructed for each location and for each replication. Each box-whisker plot is depicting the one replication at one location; hence, there are two different box-whisker plots for each location separately. For the Zn trials the maximum GY was recorded 10.62 t ha^{-1} at GATE and the lowest is 2.23 t ha^{-1} at KAJU (Figure 2). Highest mean for GY was 16.24 t ha^{-1} at LUMB and lowest was 1.45 t ha^{-1}at UNIQ for QPM hybrids (Figure 2). For the PVA trials the highest GY was 12.78 t ha^{-1} and the lowest is 2.71 t ha^{-1} both at UNIQ (Figure 3). The boxplots also show the mean and other descriptive statistics.

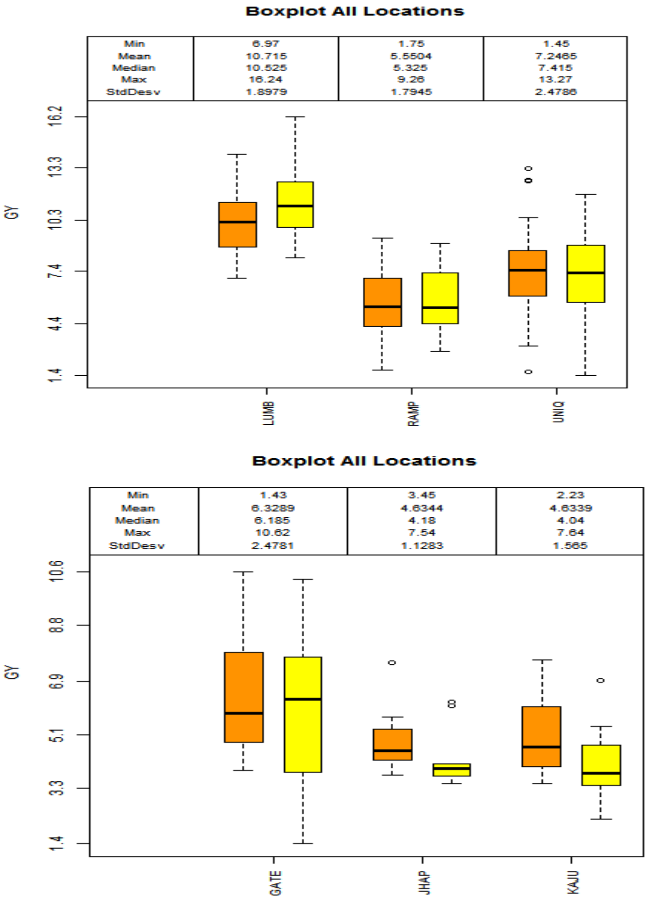

Figure 2 (A). Boxplot depicting GY of 34 QPM hybrids (including check) tested in Nepal (top);
(B) Boxplot depicting GY performance of nine Zn fortified maize in Nepal (bottom).

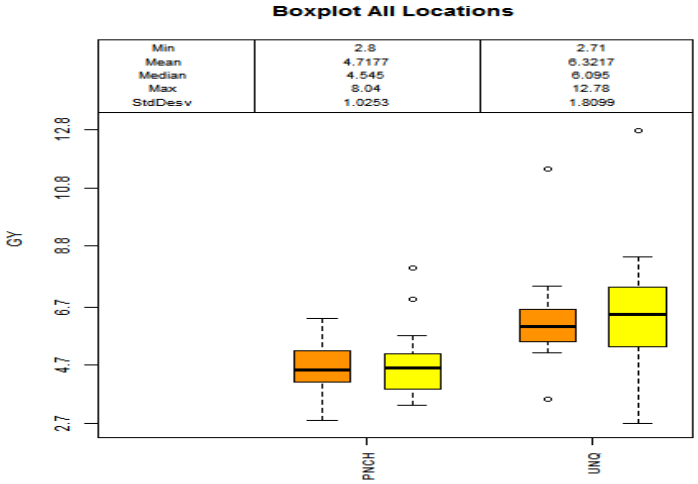

Figure 3. Boxplot depicting GY performance of 20 PVA hybrids tested in Nepal during spring 2018.

Conclusion

Three different types of biofortified maize trials viz QPM, Kernel Zn and PVA enriched varieties were tested in Nepal during the 2017-18 production seasons. All the trials were introduced to Nepal for the first time which necessitated studying their performance and adaptation pattern in the diverse maize growing ecology of Nepal. Entries 4, 3 and 1 performed better or at par with the locally adapted checks. Similarly, entries 18, 29 and 31 are among the best performing entries for the QPM hybrids and entry 14 was the best performing among the PVA hybrids. The result of this study showed the existence of a good selection potential among the introduced biofortified maize products. It is also a good indication to national programs to devise a product development and deployment plan for the biofortified maize products. However, these trials need to be replicated across seasons and locations to identify more stable and well adapted entries for variety release and further seed scale up.

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Genetic Mapping of Kernel-Zinc and Iron in Maize (*Zea mays* L.)

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Introduction

The ominous hunger and micronutrient malnutrition or hidden hunger have increased in recent decades both in developed and developing countries (Welch and Graham 1999; Graham et al. 2001). Zinc (Zn) and Iron (Fe) deficiencies have been reported as primary food-related health problems among populations (Lu et al. 2008; White and Broadley 2009) that are majorly dependent on cereal and legume-based diets (Gibson 1994). Both Zn and Fe are important in growth and development of humans, therefore deficiency in both nutrients results in serious diseases such as low immunity, decreased cognitive development in children and iron-deficiency or anemia (IDA) (Broadley et al. 2007). Options available for eliminating deficiency are to increase dietary supply of these nutrients through supplementation, fortification or bio-fortification (Bouis et al. 2003). Many people in developing countries rely on a staple diet of cereals such as rice (*Oryza sativa*) and maize (*Zea mays* L.), which are low in Zn and Fe content (Bouis 2000). Bio-fortification, which relies on both conventional breeding and modern biotechnology to improve the concentration of essential nutrients in major staple crops, has emerged as an alternative approach to tackle malnutrition in the developing world (Pfeiffer and McClafferty 2007; Bouis and Welch 2010).

Cereals constitute the dominant portion of human diets, particularly in developing nations (Bohra et al. 2014). Maize is a major cereal crop worldwide and is considered a valuable source of essential nutrients for human and animal nutrition. Exploration of potential genetic resources with variations of grain micronutrient densities, and in-depth understanding of the genetic basis of nutrient accumulation in crops, helps to improve levels of mineral nutrients and vitamins in staple food crops (Bohra et al. 2015). Finding genes and understanding the genetic mechanisms which control accumulation of Zn and Fe in grains of major cereals is the precondition for biofortification breeding program. Previous studies have shown that the Zn and Fe metabolism – which involves mobilization, uptaking, translocation and accumulation - is a complex process regulated by many genes (Bashir et al. 2012).

Micronutrients concentration is a complex quantitative trait greatly influenced by environmental conditions (Xu et al. 2011). Determining the genetic factors controlling micronutrient concentration is essential for Marker assisted selection (MAS) and map-based cloning. The application of molecular markers for quantitative trait loci (QTL) analysis has provided an effective approach to determining these genetic factors. QTL mapping provides information on the chromosomal locations contributing to the quantitative variation of complex traits (Zhang et al. 2010). Over the past few years, some loci that are responsible for Zn and Fe concentration-related traits have been detected through QTL mapping in various kinds of crops, particularly in grains of major staple foods such as rice (*Oryza sativa L.*), wheat (*Triticum aestivum*) and maize (*Zea mays* L.), which have been shown to contain low levels of micronutrients. However, previous results that pertained to the genomic location, confidence intervals or total variance explained by QTL were inconsistent because of different genetic backgrounds, environments, and/or mapping methods. An integrated genomic approach involving association mapping and traditional QTL mapping is currently the most efficient strategy for rapid dissection of quantitative traits.

In this aspect, the current study has tried to integrate genome-wide association study (GWAS) and QTL mapping using association mapping panel and Double Haploid (DH) populations to delineate markers, genes, QTLs regulating kernel Zn and Fe concentrations, with the ultimate objective of genetic enhancement in maize.

Materials & Methods

Plant material, micronutrient analysis & genotyping

A set of 923 inbred lines representing CIMMYT and partners' germplasm was used as an association mapping panel and was grown in three different environments at CIMMYT research stations in Mexico. Three DH populations (DHP1, DHP2 and DHP3) with population sizes of 96, 112 and 143 respectively were derived from the crosses between high Zn lines with low or moderate Zn lines identified from the AM panel. These populations were planted in replicated trials in two environments at Celaya in 2014 and in Tlatizapan in 2015 and 2017.

Random samples of 6g were ground into fine powder (< 0.5 μm), using a Retsch™ miller (model MM400). Flour was collected in 15 ml plastic tubes and analyzed by X-ray fluorescence using X-ray fluorometer (XRF) Oxford instruments™, model X-Supreme 8000® and readings were recorded. DNA was extracted from leaf samples of 3-4 weeks old seedlings using the standard CIMMYT laboratory protocol (CIMMYT 2005). The association mapping panel and three DH populations under study were genotyped for single nucleotide polymorphism (SNP) using genotyping-by-sequencing (GBS) method at the Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA.

GWAS for kernel-Zn and Fe

A smaller dataset of 347,765 SNPs which met the filtering criteria of call rate (CR) ≥ 0.7 and minor allele frequency (MAF) ≥ 0.03 was used for GWAS. MLM (mixed linear model) corrected for population structure and kinship (Q+K) using SVS V_8.6.0. Manhattan plots were plotted using the −log10 P values of all SNPs used in analysis. The appropriateness of the model was evaluated through Q–Q plots. Significant associations were declared at p values less than 5.03×10^{-05}, based on a modified Bonferroni correction considering LD decay in the panel (Hindu et al. 2018).

Map construction and QTL mapping

Based on uniform coverage on all 10 maize chromosomes, a total of 132, 130, 148 polymorphic markers in DHP1, DHP2, DHP3 populations, respectively, were used for linkage map construction and QTL mapping. The recombination frequency between linked loci was transformed into genetic distance (centimorgan cM) using Kosambi's function. The genetic linkage map was built using software QTL IciMapping (v4.1) with an overall length of 9707.18 cM and an average interval of 73.53 cM for DHP1, 6668.72 cM with average interval of 51.29 cM in DHP2 and 7534.09 cM with average interval of 50.90 cM in DHP3. Linkage groups were inferred at a log of the odds (LOD) threshold of 3.0. QTL analysis was conducted using inclusive composite interval mapping (ICIM) analysis by the QTL IciMapping software (v4.1) with the following parameters: 1 cM walk speed and 1,000 permutations of the phenotypic data at 1% level to determine the significance threshold for QTL detection.

Results

GWAS for kernel-Zn and Fe

The average kernel Zn in the panel was 27.04 μg/g DW, with a range of 17.11–43.69 μg/gDW. The average Fe concentration was 14.65 μg/g DW with a range of 8.19–25.65 μg/g DW. Moderate positive correlation was found between the two traits across the environments ($r = 0.49$, P value ≤ 0.001).

GWAS was carried out with MLM model correcting for both population structure and kinship matrix. A total of 20 SNPs were found to have a significant association with kernel Zn. Among the 20 SNPs identified for kernel Zn, 14 were located within predicted gene models, five of which were within models with functional domains generally related to metal ion binding or transport, or specifically to Zn ion binding. 26 SNPs were found to be significantly associated with kernel Fe. The proportion of variance explained by individual SNPs ranged from 1.8 to 2.41%. Among the 26 SNPs, 20 were located within predicted gene models.

QTL mapping

The kernel Zn ranged from 15.6 and 48.0 μg/g DW across the two environments and three populations, similarly kernel Fe ranged between 6.3 and 24.5 μg/g DW. DHP2 showed wider range of concentrations for both kernel Zn and Fe.

A total of eight significant QTLs controlling kernel- Zn and six significant QTLs for kernel Fe content were detected in the three DH populations independently (Table 1). For Zn content, three QTLs were identified on chromosome 7 and the rest were on 1, 3, 8, 9 and 10 respectively, whereas for Fe content, QTLs were found on 2, 4, 5, 7 and 8 Chromosomes. The detected QTLs for Zn content explained 5.63–20.07% of phenotypic variation, of which QTL located on chromosome 7 exhibited the largest proportion of variance. The detected QTL for Fe content on chromosome 4 accounted for 18.07% of phenotypic variation with LOD score of 4.94. Noteworthy in this case was the co-localization of QTL for both Zn and Fe content on chromosome 7 (Figure 1).

Table 1. QTLs identified by ICIM analysis for kernel-Zn and Fe concentrations.

Popn.	QTL	Marker Interval	Chr	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	GWAS hits in QTL region
DHP1	qZn7-1	--	7	6	S7_173448589	S7_172932713	3.10	17.14	-0.56	S7_173181689, S7_173181689
	qZn7-2	--	7	5	S7_173837564	S7_173448589	3.80	17.03	-0.57	
DHP2	qZn1	--	1	40	S1_273696896	S1_246409151	2.89	12.0	-0.82	S1_253905760
DHP3	qZn3	--	3	23	S3_220702957	S3_216594389	5.69	10.35	0.62	S3_216414851 S3_217239429 S3_220850649
	qZn7-3	--	7	105	S7_167859391	S7_171537807	10.75	20.07	-0.87	S7_169938048
	qZn8	--	8	76	S8_132202657	S8_119267967	3.42	5.63	-0.46	S8_125472630 S8_12787643 S8_131170051 S8_131517511
	qZn9	umc1310 -bnlg128	9	34	S9_152192799	S9_151306650	2.99	4.83	0.43	S9_151265550 S9_151631020
	qZn10	--	10	107	S10_3191244	S10_2633682	3.50	5.73	0.46	--
DHP1	qFe2	bnlg1690 - umc1890	2	47	S2_196303840	S2_214666474	2.89	16.20	0.28	S2_202159020 S2_202178029 S2_205926644 S2_209921472
DHP2	qFe4	--	4	69	S4_148426633	S4_169618780	5.13	17.94	0.48	--
	qFe8	--	8	46	S8_149918171	S8_161744799	3.36	11.35	0.38	--
DHP3	qFe5	--	5	35	S5_14653506	S5_8626904	4.30	11.69	0.32	S5_14802921
	qFe7	--	7	104	S7_167859391	S7_171537807	4.93	12.44	-0.33	S7_167221014 S7_168921087 S7_168921933 S7_171036361
	qFe8	--	8	32	S8_170035480	S8_169253471	2.74	7.15	0.25	--

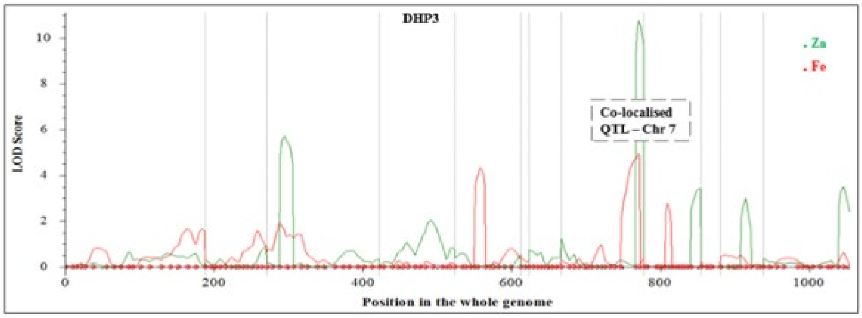

Figure 1. Co-localized QTL identified by ICIM analysis for kernel Zn and Fe in CML 465/CML451 population (DHP3)

Discussion

The objective of this study was to estimate the genetic loci underlying kernel Zn and Fe concentrations. We were able to identify multiple loci that influence kernel Zn and Fe concentrations using GWAS and QTL study. Significant positive correlations between kernel Zn and Fe concentrations were found in our study, which was consistent with previous studies (Lung'aho et al. 2011; Baxter et al. 2013). This suggests that these traits might have some common genetic mechanisms leading to their accumulation in grains.

The panel with 923 inbred lines showed moderate population structure within it, based on the principal component analysis. The moderate structure that was observed in the present study panel may be due to the inclusion of multiple sources of germplasm, whether from the temperate breeding pools from South Africa or the drought tolerant donor lines from CIMMYT. Unlike linkage mapping, association mapping can explore all the recombination events and mutations in a given population with a higher resolution (Yu and Buckler 2006). GWAS was performed using multiple statistical models, and the MLM correction for population structure and kinship was found to control genomic inflation to the minimum level. In total, 46 (Zn-26, Fe-20) marker-trait associations were declared significant based on significance threshold corrected for multiple testing corrections and taking average extent of genome-wide LD into consideration.

QTL analysis showed a total of 14 QTLs for kernel Zn (8 QTLs) and Fe (6 QTLs) in the populations (Table 1). The number of QTLs detected in each study depends on the genetic diversity among parents, population size and the number of markers tested (Brondani et al. 2002). It was difficult to compare the QTLs identified in different populations. Previous studies have reported QTL mapping and meta-QTL analysis for kernel Zn and Fe in maize (Lung'aho et al. 2011; Qin et al. 2012; Ŝimić et al. 2012; Baxter et al. 2013; Jin et al. 2013). We found an interval i.e. the QTL on chromosome 9 for Zn reported by Jin et al. (2013). The same study identified an interval that controlled Kernel Fe on chromosome 2. We also found a QTL for both kernel Zn and Fe co-localized on chromosome 7. This co-localization of QTLs could explain the positive correlations between concentrations of these two micronutrients. Co-localization of QTLs affecting different traits suggests either a single pleiotropic locus is involved in controlling multiple traits, or that several separate loci affecting independent traits are in close proximity (Ding et al. 2010).

We compared the genomic positions of these QTLs against the ones detected in this study to determine if any of GWAS identified SNPs fall within reported QTL intervals. Reported chromosomal bins 3.04 (Qin et al. 2012), 4.06, 5.04, (Jin et al. 2013) and 9.06–07 (Qin et al. 2012; Jin et al. 2013) were found to have significant SNPs for kernel Zn in this study. All the findings indicated some commonality in the genetic basis for kernel Zn and Fe, suggesting these traits could be improved simultaneously (Welch and Graham 2004).

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Marker-Assisted Introgression of *opaque2* and *crtRB1* for Enhancement of Amino-Acids and Provitamin-A in Sweet Corn

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Introduction

Of the various specialty corns, sweet corn (*Zea mays* ssp. *mays* var. *saccharata*) has emerged as the popular choice worldwide (Hossain et al., 2013). Global import of frozen sweet corn was valued at over US $423 million, while the same for preserved sweet corn was estimated to be US $1034 million (FAOSTAT 2017). The demand for sweet corn has increased tremendously in the last few years primarily due to urbanization, increased consumption and availability of organized food processing industries.

Recessive *shrunken2* (*sh2*) mutation that alters starch composition and increases the accumulation of sugars has been abundantly used in development of sweet corn cultivars (Feng et al., 2015). Sweet corn ears are harvested at immature stages of endosperm development (generally 20-24 days after pollination) and hold a significant share in both domestic and international markets (Lertrat and Pulam, 2007). Sweet corn is used as both a fresh and processed vegetable, and is an important source of fiber, minerals and vitamins (Mehta et al., 2017a). Fresh sweet corn products like sweet corn milk and soups are gaining popularity in many countries, while sweet corn ears are eaten green as highly prized fresh products (Sa et al., 2016). Further, after the harvest of sweet corn cobs, green plants serve as fodder for cattle, therefore providing extra income to farmers (Bian et al., 2015, Mehta et al., 2017 b, c).

Micronutrient malnutrition, caused by inadequate intake of essential micronutrients in the daily diet, is a serious health problem worldwide, especially in under-developed and developing countries (Bouis and Saltzman, 2017). Micronutrient deficiencies afflict more than two billion individuals worldwide, 38% of them pregnant women and 43% being pre-school children (Garg et al., 2018). Lysine and tryptophan are required for protein synthesis, besides serving as precursors to several neuro-transmitters and metabolic regulators. Deficiency in lysine and tryptophan leads to fatigue, delayed growth, loss of appetite, depression, and anxiety in children (Nuss and Tanumihardjo, 2010). Moreover, unbalanced protein in the diet leads to protein energy malnutrition (PEM) that affects more than a billion people across the world (Hossain et al., 2018, Sarika et al., 2018). Vitamin-A is required for metabolism in humans, normal vision, and maintenance of epithelial cell integrity, immune system and reproduction. Vitamin-A deficiency (VAD) results in color blindness, growth retardation, xerophthalmia and increased the susceptibility to epidemic diseases (Zunjare et al., 2018). VAD affects nearly 190 million preschool-age children and nearly 20 million pregnant women, a third of whom are clinically night-blind (WHO, 2009). India alone reports about one-third of 120 million pre-school children (Akhtar et al., 2013), and nearly 10 million pregnant women are vitamin-A deficient (www.harvestplus.org).

Normal maize protein contains lower level of lysine (0.16-0.26%) and tryptophan (0.02-0.06%) which is less than half of the recommended dose specified for human nutrition (Vivek et al., 2008). Traditional yellow maize contains enough kernel carotenoids compared to other cereals. However, it is predominated by non-provitamin-A (provitamin-A) fractions and contains only 0.25-2.50µg/g of provitamin-A which is far below the nutritional requirement (15µg/g) for humans (Muthusamy et al., 2014, Gupta et al., 2015). Biofortification is the process of breeding staple food crops that are high yielding and dense in minerals and vitamins (Bouis and Saltzman, 2017). Biofortification is comparatively advantageous over food fortification, supplementation and dietary-diversification, as it offers a cost effective, long term and sustainable approach to fighting hidden hunger, and micronutrients reach the target group in their natural form (Hefferon KL 2016, Neeraja et al., 2017).

Recessive *opaque2* (*o2*) mutant was discovered in the 1960s and increases lysine and tryptophan nearly two-fold compared to normal maize (Mertz et al., 1964). Later, breeders combined desirable endosperm modifiers with *o2*, which led to the birth of nutritionally enriched vitreous maize endosperm popularly known as Quality Protein Maize or QPM (Vasal et al., 1980; Gupta et al., 2013; Pandey et al., 2016). Adoption of QPM based varieties with balanced amino acids profile (higher lysine and tryptophan) could potentially end of PEM across the world (Nyakurwa et al., 2017). Favorable allele of *β-carotene hydroxylase1* (*crtRB1*) leads to rapid doubling or more of total provitamin-A concentration by limiting the conversion of β-carotene into further components (Yan et al., 2010, Zunjare et al., 2018). So far, no sweet corn hybrid in India has been targeted for nutritional enhancement. With availability of *crtRB1*and *o2* mutants, and suitable gene-based markers, marker-assisted backcross breeding (MABB) is an effective approach for accelerated development of sweet corn genotypes with enhanced kernel quality.

Material and Methods

Plant materials

Experimental materials used in the present study comprised three *sh2*-based sweet corn inbreds *viz*. SWT-016, SWT-017 and SWT-018. These are the parents of two promising sweet corn hybrids; ASKH-1 (SWT-016 × SWT-017) and ASKH-2 (SWT-016 × SWT-018) developed earlier at ICAR-IARI, New Delhi. HKI193-2 and HKI161 earlier introgressed with *crtRB1* and *o2* were used as donor parents (Zunjare et al., 2018). Recurrent parents were crossed with donor parents and three crosses *viz*. SWT-016 × HKI193-2, SWT-017 × HKI161, and SWT-018 × HKI161 were attempted to stack *sh2*, *crtRB1* and *o2* in the genetic background of recurrent parents using marker-assisted selection (MAS).

Backcross breeding program

The backcross- and selfed- progenies were advanced at two places: Experimental Farm, ICAR-Indian Agricultural Research Institute (IARI), New Delhi (29°41'52.13"N and 77°0'24.95"E) and Winter Nursery Centre (WNC), ICAR-Indian Institute of Maize Research (IIMR), Hyderabad (17°21'50.39"N and 78°29'42.31"E). Recurrent parents (as females) and donors (as males) showing polymorphism for gene-based markers were crossed during rainy season (July to November), 2015, at New Delhi. The F_1s were raised at Hyderabad, India, during winter season (December to April) 2015/2016. Hybridity of these F_1s was tested using the *o2*-and *crtRB1* specific markers, and the true F_1s were backcrossed as male parents to their corresponding recurrent parents. BC_1F_1 progenies were grown at New Delhi during rainy season, 2016, and subjected for foreground selection. Plants heterozygous for both the genes (*o2* and *crtRB1*), along with high recovery of the recurrent parent genome (RPG), and maximum phenotypic similarity to recurrent parents were further backcrossed to respective recipient parents. BC_2F_1 populations were grown at Hyderabad during winter season (2016/ 2017), and foreground, background and phenotypic selections were carried out. Selected plants were selfed to generate BC_2F_2 seeds. BC_2F_2 families were raised during rainy season, 2017, at New Delhi and genotyped for *o2* and *crtRB1*. Plants homozygous for both genes were subjected to background and phenotypic selection. The selected plants were subsequently selfed to generate BC_2F_3 progenies. During each backcross and selfed generation, seeds with shrunken phenotype were selected, which ensured the presence of *sh2* allele in homozygous state. Crossing of parents and subsequent generations of SWT-016 × HKI193-2 and SWT-017 × HKI161 was followed as per the above procedure. However, for SWT-018 × HKI161, F_1 seeds could not be generated during rainy season, 2015, at New Delhi owing to non-synchrony of flowering. Hence, F_1s were generated at Hyderabad during winter season, 2016, and subsequent generations were eventually raised one generation later compared to other two crosses.

Genotyping

Genomic DNA was isolated from young seedlings using the standard CTAB procedure (Murray and Thompson, 1980). Polymerase chain reaction - PCR - (Bio-Rad, California, USA) was carried out for 20µl reaction mixture using Ready PCR Reaction Mix and a 'touch-down' procedure standardized at Maize Genetics Unit, ICAR-IARI, New Delhi (Sarika et al., 2018). The resulting PCR amplicons were resolved in 4% agarose gel for four hours at 120 volts. The resolved amplified products were visualized using a gel documentation system (Alpha Innotech, California, USA).

Marker-assisted foreground selection

Three gene-based SSR markers for *o2* gene *viz. phi057*, *phi112* and *umc1066* were screened for polymorphism to distinguish respective recipient and donor parents (Yang et al., 2004). Gene-based 3'TE *InDel* was used in foreground selection for *crtRB1* (Yan et al., 2010). The details of markers used in foreground selection are given in Table 1. The PCR amplicons were resolved in agarose gel and amplified fragments scored for presence of favorable *o2* and *crtRB1* alleles as per Sarika et al. (2018) and Zunjare et al. (2018) respectively. These polymorphic markers were employed in each of the backcross and selfed generations to select positive plants. Heterozygous plants were selected in the BC_1F_1 and BC_2F_1, and homozygotes were selected in BC_2F_2. Chi-square test was performed to test the goodness of fit of the observed segregation pattern at both *o2* and *crtRB1* locus in each of the generations.

Table 6. Details of gene-based markers used for foreground selection in MABB.

Gene	Bin location	Marker name	Marker type	Primer sequence (5'-3')	Primer	References
crtRB1	10.05	*3'TE InDel*	*InDel*	ACACCACATGGACAAGTTCG	Forward	Yan et al., 2010
				ACACTCTGGCCCATGAACAC	Reverse1	
				ACAGCAATACAGGGGACCAG	Reverse2	
opaque2	7.01	*umc1066*	*SSR*	ATGGAGCACGTCATCTCAATGG	Forward	Yang et al., 2004
				AGCAGCAGCAACGTCTATGACACT	Reverse	

Marker-assisted background selection

A set of 221 SSRs evenly distributed throughout the maize genome was used for polymorphic survey between respective recipient and donor parents. The sequences of the SSRs were retrieved from the maize genome database (www.maizegdb.org) and custom-synthesized (Sigma Tech., USA). The resultant polymorphic SSRs were employed in background selection of plants in BC_1F_1, BC_2F_1 and BC_2F_2 to recover RPG. The amplicons of markers used in background selection were scored as 'A' for the recipient allele, 'B' for the donor allele, and 'H' for the heterozygous genotype. Recovery percentage of RPG was estimated using formula, RPG (%) = $[A + (0.5H)/(A + B + H)] \times 100$ (Benchimol et al., 2005).

Results

Parental polymorphism for o2 and crtRB1

Among the three gene-based markers available for *o2*, *umc1066* showed a distinct polymorphism between all recurrent and donor parents. All the three recurrent parents amplified a 165bp (O_2) amplicon with *umc1066*, while donor parents amplified 159bp (*o2*) amplicon. For *crtRB1*, all the three recurrent parents revealed unfavorable allele of 296 bp, while donor parents possessed favorable allele of 543 bp.

Parental polymorphism for background selection

A total of 221 SSRs spanning all the bin locations in a maize genome map (www.maizegdb.org) were selected for the screening of polymorphisms between respective recipient and donor parents. Of the 221 markers, the number of markers screened per chromosome varied from 18 to 33 across the three crosses (Table 2). The number of observed polymorphic markers per chromosome across three crosses ranged from six (33.33%) to 22 (66.66%). These identified polymorphic markers were used in background selection for recovering the RPG.

Table 7. Chromosome wise SSRs screened and percentage polymorphism observed across crosses.

LG	No. of markers screened	RNP	Pol. (%)
1	22	8-11	36.36-50.00
2	19	7-10	36.84-52.63
3	33	14-22	42.42-66.66
4	23	7-14	30.43-60.86
5	24	8-13	33.33-54.16
6	20	9-12	45.00-60.00
7	20	9-16	45.00-80.00
8	18	7-9	38.88-50.00
9	18	6-9	33.33-50.00
10	24	7-14	29.16-58.33
Total	**221**	**102-113**	**46.15-51.13**

LG: Linkage group; RNP: Range of no. of observed polymorphic markers across crosses; Pol. (%): Range of polymorphism percentage across crosses.

Foreground and background selection

BC_1F_1 generation:

The number of progenies developed in BC_1F_1 was 256 including all the three crosses (Table 3). Foreground selection resulted in the identification of double heterozygotes (*O2o2*/C^+C) which were subsequently subjected to background selection. Significant segregation distortion (SD) was observed for *crtRB1* allele ($P<0.01$), while it was 1:1 for *o2* (Table 3). Recovery of RPG varied from 70.48 to 82.30%, with a mean of 77.49% over all the three crosses (Table 4). Plants with >78.00% RPG were selected for further advancement.

Table 8. Segregation pattern of *crtRB1*, and *opaque2* in different backcross- and self- generations across three crosses.

Generation	N	C^+C^+	C^+C	CC	χ^2	P-value	*o2o2*	*O2o2*	*O2O2*	χ^2	P-value
BC_1F_1	256	-	83	173	31.64	<0.0001**	-	124	132	0.25	0.6170ns
BC_2F_1	318	-	145	173	2.46	0.1167ns	-	160	158	0.01	0.9203ns
BC_2F_2	642	117	307	218	33	<0.0001**	114	330	198	22.48	<0.0001**

**Significant at P = 0.01; ns: Non-significant; N: No. of plants genotyped; df: degrees of freedom; *C+:* favorable allele of *crtRB1*; *C*: unfavorable allele of *crtRB1*; *o2*: favorable allele of *opaque2*; *O2*: unfavorable allele of *opaque2*.

BC_2F_1 generation:

From all three crosses, a total of 318 plants were raised in BC_2F_1 generation (Table 3). Foreground Selection was applied in these plants for both the targeted favorable alleles to identify the double heterozygotes. Chi square test showed that segregation ratios for both the targeted alleles fit into the 1:1 expected Mendelian ratio (Table 3). Background selection in the heterozygous plants led to the recovery of 83.19 to 92.86%, with an average of 87.20% for the three crosses (Table 4). Plants with >83% RPG were selected for advancing the generations.

Table 9. Recurrent parent genome recovery (RPG) (%) of introgressed lines across crosses.

Generation	Range of RPG (%) across crosses	Average RPG (%) across crosses
BC_1F_1	70.48 – 82.30	77.49
BC_2F_1	83.19 – 92.86	87.20
BC_2F_2	88.24 – 96.19	92.31

BC_2F_2 generation:

A total of 642 plants were grown in BC_2F_2, including all the three populations (Table 3). Genotyping using foreground markers identified homozygous plants carrying favorable allele of both *o2* and *crtRB1* in all the three crosses. The segregation pattern of both *o2* and *crtRB1* was significantly deviated from the expected segregation ratio of 1:2:1 (Table 3, Figure 1). The selected homozygous plants were genotyped for background selection for recovery of RPG. The average recovery of RPG for the three crosses was 92.31%, with a range of 88.24% to 96.19% (Table 4). Plants with >90% RPG and maximum phenotypic similarity with respective recurrent parents were selfed to generate BC_2F_3 progenies.

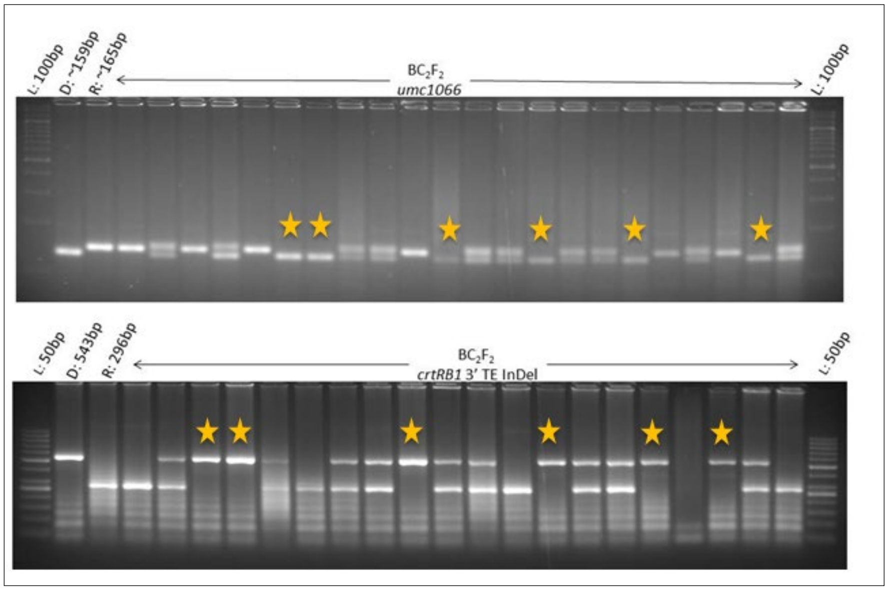

Figure 1. Segregation of *o2* and *crtRB1* in BC_2F_2 generation (SWT-016-based).
Star indicates segregants with donor allele in homozygous conditions.

Discussion

Lysine, tryptophan and provitamin-A are not synthesized in the human body or by other monogastric animals, thus should be provided through food sources (Pixley et al., 2013). Gene-based DNA markers for *o2* and *crtRB1* have been developed and well validated in maize germplasm (Babu et al., 2005; Yan et al., 2010). MABB has been utilized to introgress *o2* (Gupta et al., 2013; Hossain et al., 2018; Sarika et al., 2018) and *crtRB1* (Muthusamy et al., 2014; Liu et al., 2015; Zunjare et al., 2018) for biofortification of nutritional traits in maize. The present study employed MABB for enhancement of lysine, tryptophan and provitamin-A in three sweet corn inbreds by combining favorable alleles of *o2* and *crtRB1*. Due to the codominant nature of *umc1066* and *crtRB1*-3′TE *InDel*, heterozygotes were identified at the seedling stage prior to pollination. This allowed the rejection of unfavorable backcross progenies (dominant homozygotes), resulting in substantial time and labor savings (Gupta et al., 2013). In majority of the populations both *o2* and *crtRB1* segregated as per expected Mendelian ratio. However, SD was also observed for both *o2* (Gupta et al., 2013; Hossain et al., 2018; Sarika et al., 2018) and *crtRB1* (Babu et al., 2013; Muthusamy et al., 2014; Liu et al., 2015, Zunjare et al., 2018). This SD could be due to the presence of many SD regions throughout the maize genome (Lu et al., 2002), and *crtRB1* is known to be present in

SD region (Lu et al., 2002; Babu et al., 2013). Thus, it is necessary to grow a large population to get enough foreground positive plants in backcross and selfed generations.

SSR-based background selection was found to be effective in achieving >90% recovery of RPG within two generations of backcross (Gupta et al., 2013; Muthusamy et al., 2014; Liu et al., 2015; Hossain et al., 2018, Zunjare et al., 2018; Sarika et al., 2018). The present investigation revealed highest RPG recovery of >96% with an average of ~92% across the three populations. To achieve comparable results, conventional breeding would take many backcrosses. Since *o2* and *crtRB1* are recessive and endosperm specific traits, each of the backcrosses would require one generation of selfing after every generation of backcrosses. MABB approach significantly saved time, therefore accelerating the pace of breeding (Gupta et al., 2013; Muthusamy et al., 2014; Hossain et al., 2018; Zunjare et al., 2018; Sarika et al., 2018). In addition, conventional breeding for enhancement of lysine, tryptophan and provitamin-A is expensive, laborious and time consuming due to large scale phenotyping of the segregating populations.

Successful examples of application of MABB in development of nutritious maize hybrids in India have been the commercial release of MAS-derived QPM hybrids, Vivek QPM9 (Gupta et al., 2013), Pusa HM4 Improved, Pusa HM8 Improved and Pusa HM9 Improved (Hossain et al., 2018). Further, Pusa Vivek QPM9 Improved was recently released with high provitamin-A, lysine and tryptophan (Muthusamy et al., 2014). Sweet corn hybrids targeted for enhancement of provitamin-A, lysine and tryptophan would provide nutrition in a sustainable and cost-effective way. This is the first effort in India to improve nutritional content of sweet corn hybrids.

Conclusion

Sweet corn hybrids assume significance as food and fodder. The parental inbreds were crossed with provitamin-A and QPM donor inbreds. BC_1F_1, BC_2F_1 and BC_2F_2 populations were successfully genotyped using markers associated with *o2* and *crtRB1*. Heterozygotes in backcross generations, and homozygotes in selfed generations were identified. Background selection led to the high recovery of RPG in a shorter time. The selected progenies with *sh2*, *o2* and *crtRB1* possessed similar plant, ear and grain characteristics of recurrent parent. The introgressed inbreds hold great potential to develop provitamin-A rich QPM-based sweet corn hybrids. The biofortified sweet corn development presented here is the first such example in India.

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Introduction of Quality Protein Maize (QPM) Hybrids in Maize Cropping System of Pakistan

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Introduction

Maize is of great importance to countries like Pakistan, where rapidly increasing population, poultry and livestock already outstrip available food and feed supplies. Maize in Pakistan is cultivated as a multipurpose food and feed crop, mainly by poor farmers who have limited land and other resources. The poultry industry consumes more than 60% of Pakistan's maize, and availability of high-quality feed will help the industry continue to flourish. Multiple studies have proven that introduction of QPM has resulted in improved growth in mono-gastric animals like chickens. In Brazil and El-salvador, QPM in animal feed reduced use of soybean meal by about 50%, use of synthetic lysine (Lopez-Pereira, 1992), and saved 3-5% of costs in development of feed for poultry. Chinese QPM variety Zhong Dan-9409 which is used in animal feed, has an 8-15% yield advantage and about 80% more lysine and tryptophan.

According to the global hunger index of 2016 developed by the International Food Policy Research Institute (IFPRI), Pakistan is among the countries with alarmingly low food and nutritional security. According to reports from FAO (2016), more than 40 million Pakistanis (about 22% of the total population) are not getting proper nutrition. This figure is high compared to other Asian countries like India (15%), Bangladesh (16%), Nepal (8%) and China (9%). Pakistan Demographic and Health Survey 2012-2013 reports that 45% of children aged below five years show evidence of chronic malnutrition or stunting while 11% are acutely malnourished and require urgent treatment. Further, 30% of Pakistani children are reported to be underweight. This impaired development affects Pakistan's future generation both mentally and physically. Availability of low-cost and nutritionally enhanced food will help curb this trend significantly.

US farmers who fed *o2* maize silage to their dairy cattle saw increased milk production (Glover, 1992). QPM silage may hold distinct nutritional and economic advantages in the feeding of dairy animals (Gevers, 1995). Substituting normal maize with high-lysine maize on an equal weight basis can lessen the use of synthetic lysine in animal feeds and maintain proper amino acid balance (Asche et al., 1985; Burgoon et al., 1992; Knabe et al., 1992). The current supply of fodder in Pakistan is 40% less than actual demand, which negatively affects animal production of. To counter this deficiency farmers feed wheat straw to their animals, which not only lacks nutritional value but also increases costs. Environmental factors, unavailability of labor and decrease in agricultural land for fodder make availability of nutritive feed difficult. The simplest solution to this problem is corn silage, decreases total expenditure on feed and makes farming profitable by increasing milk and meat production. Thus, production and promotion of dual-purpose maize (stay green) will solve issues of silage shortage and human consumption, particularly with resource poor farmers.

Under the Agriculture Innovation Program (AIP)-Maize-CIMMYT, Pakistan introduced high yielding QPM maize hybrid comparable to existing normal maize hybrids and provided financial support for their evaluation and local seed multiplication. Replacement of normal maize with QPM will save about 50% in import expenditure for soybean seed which shot up significantly from 2013 to 2017.

Materials and Methods

Under AIP-CIMMYT, maize from spring and autumn 2014 - a total of 13 trials comprising 470 entries - was received and evaluated at field area of Maize Sorghum Millet and Fodder Program, Crop Sciences Institute (CSI), National Agriculture Research Center Islamabad Pakistan (Table 1). Of the 13 trials, one named PK14A898 13BEARHQPMY site 2 comprised 10 entries, including one local check with three replications using Randomized Complete Block Design (RCBD). The plant to plant distance was 15cm and 75cm between rows. The plot size was two rows and length of each row was four meters. After primary screening, two QPM maize hybrids were selected and tested further under National Uniform Yield Trial (NUYT) managed by the National Coordinator Maize, Pakistan Agriculture Research Council Islamabad.

Each year, more than 40 newly developed or introduced maize hybrids for both spring and autumn season, from over 10 to 15 locations, and representing four to five different maize growing areas are evaluated. At each location, a randomized complete block design with two replications is used. Planting date, planting density, and management practices are executed according to protocols outlined by the National Coordinator Maize. In addition to yield, several phenological traits like days to flowering (male & female), agronomic (plant & ear height, field weight, shelling %age, moisture% and finally calculated grain yield kg/ha) are recorded at most of the locations. Data for all trials are analyzed using MSTATC computer software.

Table 1. Under AIP-CIMMYT Maize, summary of trials received and evaluated at Maize sorghum millet & fodder program NARC, Islamabad Pakistan.

S. No.	Name of Trial	Entries	Reps	Outcome: each trial as Entry nos. (Grain Yield tons/ha)
				SPRING 2014
1	BEARHQPMY-2	10	3	Entry No.**1** (9.81), **4**(9.96)**, **6**(9.37) & **9** (7.36)*
2	TTWCLWQN-32	9	3	Entry No.**3** (7.35)
3	TTWCLY-3	9	3	Entry No. **2** (7.85)
4	EHYB-14149	60	3	Entry No. **6** (7.66), **20** (7.49), **46** (8.16), 49 (9.7) & **55** (7.57)
5	EPOP-1472	30	3	Entry No.**6** (9.1), **9**(7.47), **12** (7.34), 22 (7.29) & **28** (8.94)
6	IHYB-14146	60	3	Entry No. **5**(9.08), **7**(10.68), **10** (10.47) & **39** (9.2)
7	LHYB-14140	40	3	Entry No. **8** (12.1), **18** (10.6) & 21 (10.65)
				AUTUMN-2014
1	INBRED LINE	18	-	Pure seed increased (by hand selfing)
2	INBRED LINE	119	-	Pure seed increased by hand selfing
3	HPLET	75	2	Evaluation and 6 inbred lines were selected
4	CHTPROA	24	2	Entry No.**1** (9.3), **6** (9.44), **8** (9.55), **9** (8.88) & **22** (9.21).
5	ASA18HY	16	3	Entry No. **4** (9.54), **10** (8.99) & **12** (9.44).
6	BEARHQPMY	10	3	Entry No. **3** (9.11), 4 (8.08)** & 9 (7.89)*
7	TTWCWL	15	3	Entry No. **1** (9.52), **3** (9.01) & **12** (9.50).
8	TTWCYL	12	3	Entry No. **2** (10.77), **3** (9.92) & **5** (10.33).
***QPHM-200 and **QPHM-300**				

Based on two-year NUYT results + production technology and related studies viz; diseases, lodging, insect & maize plant descriptive, proposal for both hybrids viz; QPHM-200 & QPHM-300 was presented to the Variety Evaluation Committee (VEC), Pakistan Agriculture Research Council (PARC), which has recommended these hybrids for commercial cultivation.

Results and Discussion

Preliminary Yield Performance Trial

In spring 2014, the experiment comprised 10 entries including one check. Maximum grain yield (9.96 t/ha) was produced by hybrid SA-2146-75, followed by hybrid SA-2146-38 with a yield of 9.81 t/ha (Table 2). The check variety EV-7004 produced grain yield of 7.51 t/ha and ranked 7th. Three entries viz; No. 1 (SA2146-38), 4 (SA2146-75) and 6 (SA2125-23) were found top in yield with good plant and ear aspects having medium days to anthesis (82 to 84 days). Entry no. 4 (SA2146-75) and entry no. 9 (SA1988-5) - named QPHM200 and QPHM300 respectively - were selected as both their male and female parents were well adopted and produced good seed yield.

Table 2. Means data of different traits for BEARHQPMY Maize hybrids trial received from AIP-Maize CIMMYT and conducted at NARC Islamabad in spring and Kharif 2014.

	Traits	Name of Entries										**Mean**	LSD (0.05)	CV (%)
		SA 2146-38	SA 2146-39	SA 2146-40	SA 2146-75	SA 2125-21	SA 2125-23	SA 2125-24	SA 2125-25	SA 198-5	Check*			
Spring 2014	Grain Yield t/ha	9.81	7.77	8.97	9.96	5.31	9.37	7.02	7.89	7.36	7.51	**8.1**	2.24	14
	Yield Rank	2	6	4	1	10	3	9	5	8	7	**6**	3	-
	Cobs / plant	0.9	0.8	1	0.9	0.9	0.9	0.9	0.9	1	1	**0.9**	0.2	13
	Anthesis date	82.7	82.9	84.1	84.2	84.6	80.2	83.4	81.9	84.7	74.9	**82.4**	3.5	2
	ASI	4.1	4.1	4.9	2.8	5.5	3.1	3.2	3.9	5.1	2.1	**3.9**	2.3	31
	Plant Height (cm)	186	208	198	190	190	200	216	199	205	156	**195**	15.4	4
	Ear Height (cm)	87	103	99	92	92	96	102	105	104	62	**94**	8.8	5
	Ear /plt. ht Prop	0.46	0.48	0.49	0.48	0.48	0.47	0.46	0.53	0.5	0.39	**0.48**	0.04	4
	ASP Plant (1-5)	2.7	3	2.6	3	2.7	3	3.3	2.7	3.4	3.7	**3**	0.6	11
	ASP. Ear. (1-5)	2.8	3	2.5	2.8	2.5	2.1	3.5	2.6	2.8	2.5	**2.7**	1.2	24
	Moisture (%)	6.3	8	10.3	5.4	10.3	5.8	8.9	6.8	6.4	8	**7.6**	3.7	25
	Plants/plot	33.7	31.1	30.1	38.1	27.7	35.8	33.1	34.1	33.5	31.1	**32.8**	7.6	12
Autumn 2014	Grain Yield t/ha	7.85	7.46	9.11	8.08	6.99	7.44	7.22	7.37	7.89	6.67	**7.61**	1.34	10
	Yield Rank	4	5	1	2	9	6	8	7	3	10	**6**	3	
	Cobs / plant	0.9	1	1	1	1	1	1	1	1.1	1	**1**	0.1	3
	Grain Moisture (%)	31.3	29.7	29.8	31.8	29.4	28.7	32.7	29.5	31.4	28.2	**30.3**	2.7	5
	Anthesis date	50.3	51.2	49.9	52.9	54.3	51.3	52.2	49	51.6	50.2	**51.3**	2	2
	ASI	4	3.7	4	4	3.3	4.3	4.4	3.9	4.6	3.6	**4**	1.3	16
	Plant Height (cm)	240	248	246	243	231	248	256	225	235	218	**239**	17.7	4
	Ear Height (cm)	110	119	118	113	112	124	123	111	122	112	**117**	13.2	6
	Ear /plt. ht Prop	0.46	0.48	0.48	0.47	0.48	0.5	0.48	0.49	0.52	0.51	**0.49**	0.03	4
	Stem Lodging (%)	2.7	16.1	0.9	4.4	1.9	9.6	0.9	4.7	1.8	5	**4.8**	14	158
	Root Lodging (%)	4.98	4.36	0.66	-2.22	0.65	3.72	3.13	5.31	4.59	2.23	**2.74**	7.77	146
	ASP. Plt. (1-5)	2.5	2.5	2.5	2.5	2.5	2.7	2.3	2.5	2.7	3	**2.6**	0.5	10
	ASP. Ear. (1-5)	2.3	2	2	2.3	3	2.8	2.3	2.8	2.5	3	**2.5**	0.5	10
	Text (1-5)	1.5	1.8	1.8	1.8	1.8	1.7	1.3	1.2	1.3	1.3	**1.6**	0.5	18
	Plants/plot	38	29.7	37.3	38	37	34.3	32.7	34.3	39.3	35	**35.6**	5.4	8
	*Check name “EV-7004” for spring 2014 & “Haq Nawaz Gold” for autumn 2014													

The same trial was conducted in autumn 2014, and results revealed that maximum grain yield (9.11 t/ha) was produced by hybrid SA-2146-40 followed by hybrid SA-2146-75 with a yield of 8.08 t/ha (Table 2).

The check variety Islamabad Gold produced grain yield of 6.67 t/ha and was ranked 10th. The two top yielding hybrids (SA-2146-40 and SA-2146-75) had good plant and ear aspects with medium days to anthesis (49.9 to 52.9 days).

Results of National Uniform Yield Trial

The proposed hybrids - QPHM200 and QPHM300 - were included in National Uniform Maize hybrid (Yellow) Yield Trials conducted in spring 2015 and 2016. Data from 10 locations was compiled to observe adaptability performance and grain yield. Grain yield was compared to check hybrid Y. Wala as given in Table 3. QPHM200 produced maximum grain yield of 15534 kg/ha at Sahiwal and 14945 kg/ha at Manga Mandi, whereas check Y. Wala hybrid in the same locations yielded less grain; 12296 kg/ha and 11700 kg/ha respectively. At all 10 locations, the proposed hybrid QPHM200 averaged 1.2% higher yields than check Y. Wala hybrid. QPHM-300 out yielded the check at only four locations viz; Sahiwal 30.6%, Pakpattan 13.7%, Yousafwala 10.7% and Manga Mandi 6.2%.

Table 3. Means of grain yield (kg/ha) for Maize Hybrid NUYT (Yellow) conducted during spring 2015 and 2016

Year	Particulars	Locations (Grain Yield kg/ha)										**Ave. Hyb.**
		Manga Mandi	Dadu / Mardan	Yousf wala	Faisal abad	Sahi wal, (ICI)	Sahi wal	Arif wala	Pak pattan	Pir Sabak	Islam abad	
Spring 2015	**QPHM 200***	14945	1372	6475	7778	4917	15534	1279	4503	5514	6947	**6926**
	QPHM 300**	12422	1456	10508	6111	3153	16059	1187	4986	3024	6467	**6538**
	Y.WALA (Check)	11700	1511	9493	8111	3642	12296	2481	4387	6309	8521	**6845**
	Ave. Loc	**12534**	**1471**	**10629**	**7400**	**4835**	**16330**	**2243**	**5238**	**6187**	**8674**	**7554**
	LSD (0.05)	2319	212	3053	1523	2257	3752	972	435	1866	3120	**1951**
	C.V %	11.4	8.9	17.8	12	28.3	14.2	26.8	5.1	18.7	22.2	**16.5**
	% Increase / decrease*	**27.7**	**-9.2**	**-31.8**	**-4.1**	**35.0**	**26.3**	**-48.5**	**2.6**	**-12.6**	**-18.5**	**1.2**
	% Increase / decrease **	**6.2**	**-3.6**	**10.7**	**-24.7**	**-13.4**	**30.6**	**-52.2**	**13.7**	**-52.1**	**-24.1**	**-4.5**
Spring 2016	**QPHM 200***	9327	8874	8093	8634	7725	9131	9704	7923	8542	9037	**8699**
	QPHM 300**	8536	10124	10875	9841	8654	9515	11670	9772	8848	10644	**9848**
	CS-5800 (Check)	7687	10377	7545	9738	7246	8746	7088	7376	11198	8858	**8586**
	Ave. Loc	**8301**	**9228**	**10273**	**7369**	**8458**	**8622**	**8221**	**7699**	**7872**	**8643**	**8468**
	LSD (0.05)	764	754	486	615	1292	410	388	361	870	542	**220**
	C.V %	5.68	5.04	2.92	5.16	9.43	2.94	2.91	2.89	6.82	3.87	**5.12**
	% Increase / decrease*	**21.3**	**-14.5**	**7.3**	**-11.3**	**6.6**	**4.4**	**36.9**	**7.4**	**-23.7**	**2.0**	**1.3**
	% Incr. / decrease**	**11.0**	**-2.4**	**44.1**	**1.1**	**19.4**	**8.8**	**64.6**	**32.5**	**-21.0**	**20.2**	**14.7**

In spring, 2016, grain yield performance was compared to check hybrid CS5800 (Table 3). QPHM200 produced maximum grain yield of 9704 kg/ha at Arifwala, and 9327 kg/ha at Manga Mandi. Check hybrid CS5800 had lower grain yield, producing 7088 kg/ha and 7687 kg/ha at both locations respectively. At all 10 locations, the proposed hybrid QPHM200 averaged 1.3% higher yields than the check hybrid CS5800. QPHM-300 performed a little better, out yielding the check hybrid in all locations of Punjab except Pir Sabak and Mardan located in Khaiber Pakhtun Khua (KP) Province.

In autumn, 2015, the NUYT results from ten locations showed that the proposed hybrid QPHM-200 yielded more than 10 tons/ ha at four locations (viz; Sahiwal, Islamabad, Yousafwala and Vehari farmer field Vehari). Values of 13921 kg/ha, 11694 kg/ha, 11579 kg/ha and 10967 kg/ha for the hybrid against check values 9298 kg/ha, 9127 kg/ha, 10527 kg/ha and 9976 kg/ha, respectively, were observed. At all 10 locations, the proposed hybrid QPHM200 averaged 18.64 % higher grain yield than check hybrid Y. Wala (Table 4). In autumn, 2016, results from seven locations showed that QPHM-200 produced 0.9% higher grain yield than check Y.H.1898.

Table 4. Means of grain yield (kg/ha) for Maize Hybrid QPHM-200 in NUYT (Yellow) conducted during autumn 2015 and 2016.

Year	Particulars	Locations (Grain Yield kg/ha)										**Ave. Hyb**.
		Islam abad	Yousf wala	Vehari	Sahi wal	Manga Mandi	Lahore	Dadu	Faisal abad	Arif wala	Pir Sabak	
Autumn 2015	**QPHM 200**	11694	11579	10967	13921	7496	7421	4250	8214	8794	8271	**9261**
	Y. WALA (Check)	9127	10527	9976	9298	6002	6211	5127	7699	7806	6288	**7806**
	Ave. Loc	**11421**	**11559**	**11253**	**12015**	**9287**	**7964**	**5012**	**8925**	**7872**	**5925**	**9123**
	LSD (0.05)	1645	1056	1167	1333	932	591	313	493	443	690	-
	C.V %	8.78	5.57	6.32	6.77	6.12	4.53	3.8	3.37	3.43	7.1	**-**
	% Increase / decrease	**28.13**	**9.99**	**9.93**	**49.72**	**24.89**	**19.48**	**-17.11**	**6.69**	**12.66**	**31.54**	**18.64**
Autumn 2016	**QPHM 200**	7636	7595	-	7842	4875	5476	6806	5912	-	-	6680
	Y.H.1898 (Check)	7959	6967	-	8792	4072	5205	6620	5634	-	-	6620
	Ave. hybrid (41)	**9105**	**7501**	-	**4324**	**6234**	**9564**	**9564**	**7827**	-	-	7100
	LSD (0.05)	**611.9**	**1590**	-	**varies**	**varies**	**328.24**	**NS**	**2004**	-	-	-
	C.V %	**4.14**	**13.37**	-	**25.94**	**15.11**	**2.1**	**10.3**	**16.55**	-	-	-
	% Increase / decrease	-4.1	9.0	-	-10.8	19.7	5.2	2.8	4.9	-	-	**0.9**

Quality protein profiles of QPHM200 and QPHM300

Quality Protein Maize (QPM) produces 70-100% more lysine and tryptophan than normal varieties and hybrids of tropical maize. These two amino acids allow the body to manufacture complete proteins, thereby eliminating wet-malnutrition. In addition, tryptophan can be converted to Niacin in the body, which theoretically reduces incidence of Pellagra.

Lab analysis of data received from CIMMYT revealed that parental lines and their crosses (F1) of proposed hybrid QPHM200 contained 11.56% protein, 0.096% tryptophan, 0.436 lysine and quality index of 0.832 (Table 5). On the other hand, QPHM300 showed higher protein levels for hybrid and parental lines, viz: 13.857% to 12.044%, than QPHM200. Lysine and Tryptophan content varied at 0.295% to 0.401%, and 0.068 to 0.102% respectively.

Table 5. Protein profile of proposed hybrid QPHM200 received from CIMMYT AIP Maize, 2014

S. No.	Pedigree	Origin	% Protein	% Trp	% Lys	Quality index
	QPHM200					
1	CML161	PM13A-016-53	11.58	0.095	0.421*	0.816
2	CML165	GW14B-M 2900 - 14	11.55	0.094	0.530*	0.815
3	**CML161/CML165**	**CE14B-14BQ1005-2**	**11.56**	**0.096**	**0.436**	**0.832**
	QPHM300					
1	CML451Q	PM11B-036-51	12.044	0.084	0.401	0.699
2	CL02450Q	PM11A-022-7	13.857	0.068	0.295	0.493
3	**CML451Q/CL02450Q**	**PM10B-022-3/4**	**12.476**	**0.084**	**0.323**	**0.674**
4	CML451Q	PM13A-039-8	13.022	0.102	0.389	0.786
5	CL02450Q	PM13A-039-7	13.393	0.068	0.336	0.509
6	**(CML451Q)/(CL02450Q)**	**PM11A-042-7/042-8**	**12.453**	**0.082**	**0.344**	**0.656**

Conclusion

In Pakistan, maize is planted on an area of 11.3 million hectares, with an annual production of 46.95 million tons. Punjab accounts for 55.5% of total maize acreage and 79.3% of the total maize grain production. The local seed production of maize hybrid and import seed in total only meets 30% of demand (31914 MT).

Demand for maize grain as poultry and livestock feed is increasing because maize plant is considered best for silage. As uses for maize increase, so does seed demand. The QPHM200 for autumn and QPHM300 for spring will be more beneficial as they have enriched quality protein (Lysine and Tryptophan), yellow, dent like, bold and shiny kernel type with higher shelling percentage compared to the existing improved hybrids, without reducing average grain yield. These characteristics make it more acceptable to farmers.

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Present Status and Future Prospects of Maize Crop in Pakistan

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Introduction

Pakistan is blessed with versatile geographic conditions, with altitude ranging from sea level to over 8000 meters above sea level. Pakistan has total geographic area of 79.6 M ha, of which 33.58 M ha area is cultivatable. However, only 23.56 M ha is currently under cultivation with maximum contribution from Punjab province followed by Sindh (Table 1). Agriculture is the lifeline of Pakistan's economy, accounting for 19.5% of the gross domestic product, employing 42.3% of the labor force and providing raw material for several value-added sectors. Maize contributes 2.7% to the value added in agriculture and 0.5% to GDP. Maize area and production are increasing in Pakistan, as is productivity. The area has increased from 1087 thousand hectares in 2011-2012 to 1334 thousand hectares in 2016-2017 (Figure 1). Similarly, yield per hectare has increased from 3991kg/ha in 2011-2012 to 4595 kg/ha in 2016-2017. In 2016-2017, area sown under maize crop increased to 1334 thousand hectares, showing a significant increase of 12.0% over last year's sown area of 1191 thousand hectares. Maize crop production stood at a record high of 6.13 million tons in 2016-2017 showing an increase of 16.3% over last year's production of 5.271 million tons. Pakistan ranks 20th globally with respect to area under maize cultivation, 21st with respect to maize production and 11th regarding average yield of maize. In Asia, Pakistan is at 8th place with respect to area and production of maize and 5th with respect to yield per hectare.

Table 10. Land utilization statistics of Pakistan

	Geographical area	Agriculture land	Arable area	Cultivated area	Cultivable waste	Forest area
Pakistan	79.61	33.58	29.39	21.25	8.14	4.19
Punjab	20.63	14.52	14.04	12.45	1.59	0.48
Sindh	14.09	7.31	6.28	4.9	1.38	1.03
Pakhtunkhwa	10.17	4.4	3.08	1.84	1.24	1.32
Baluchistan	34.72	7.35	5.99	2.06	3.93	1.36

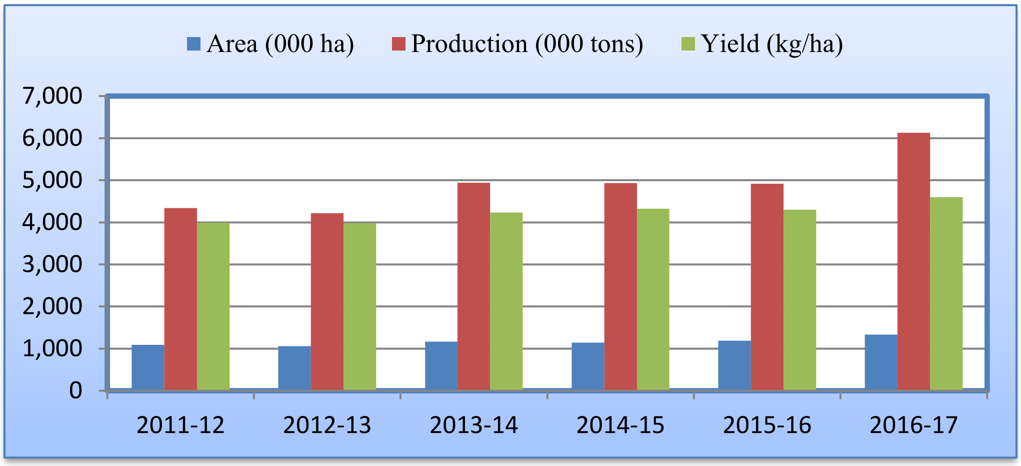

Figure 1. Area, production and yield of maize crop in Pakistan.

Major Maize Growing areas in Pakistan

Punjab is a major contributor of maize area and production followed by KPK. Punjab takes up 62% of total area and 89% of total production which implies that yield per acre of Punjab is higher than all other provinces. Khyber Pakhtunkhwa contributes 37% in area and 10% in production. The last 1% area and production is shared by Baluchistan and Sindh. Basically, maize growing area is centered in Punjab and Khyber Pakhtunkhwa, regions that fall in Agro-ecological zone IV (a&b) known as Northern Irrigated Plains and include Okara, Pakpattan and Sahiwal at the top (Figure 2).

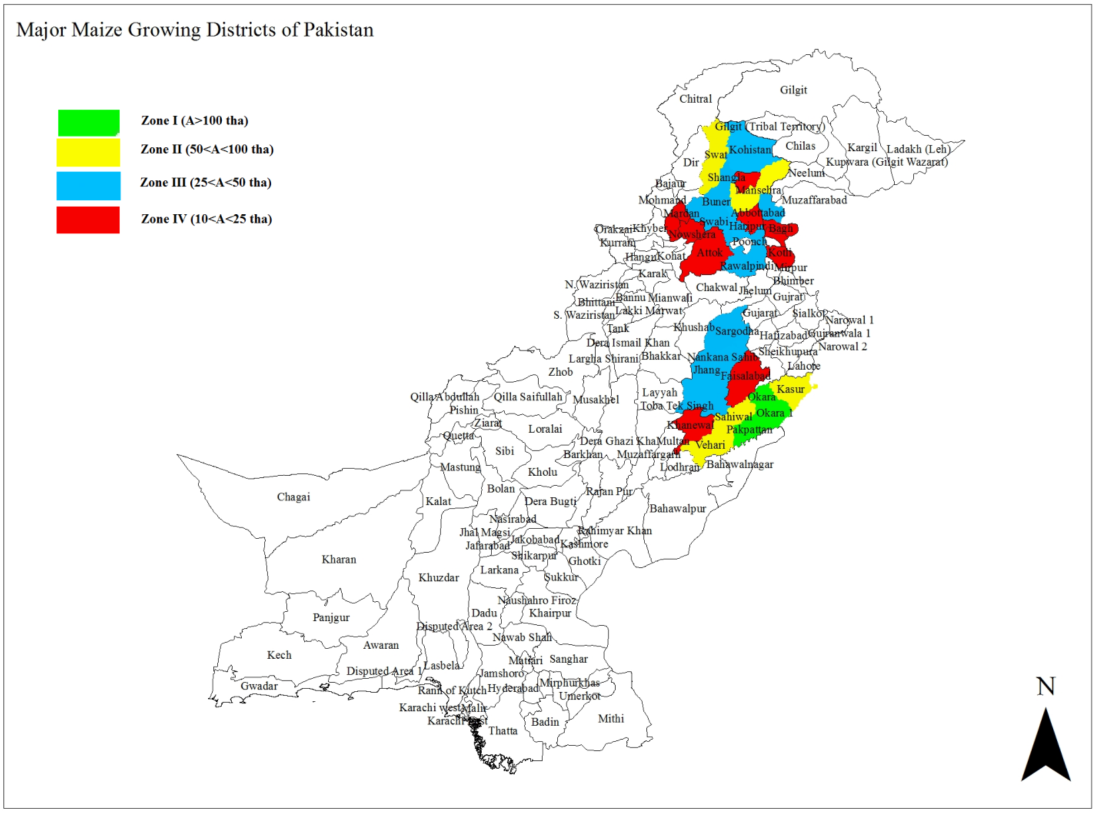

Figure 2. Major maize growing regions in Pakistan.

Research Network on Maize Crop in Pakistan

Pakistan has a vast and well-organized agricultural research system at both federal and provincial levels and crop specific research institutes are established in major zones of the crops. National Agricultural Research Institute (NARI), situated in Islamabad, is a federal institute working on maize crop in Pakistan. NARI is also responsible for coordination of National Uniform Maize Yield Trials (NUMYT) which are distributed twice a year and conducted across the country at national and private institutes. Seed preparation, distribution, data compilation and results distribution are done by NARI. Arid Zone Research Centre, D.I. Khan, KPK, is also working on maize crop under the umbrella of Pakistan Agriculture Research Council. Punjab has a central research institute, Ayub Agricultural Research Institute (AARI) based in Faisalabad with sub-stations throughout Punjab. Maize and Millets Research Institute (MMRI), Yusafwala Sahiwal, is working on maize crop in Punjab under AARI. MMRI is in Punjab with two stations in Faisalabad and Murree. Cereal Crop Research Institute (CCRI), Pirsbak Nowshera, is a provincial research institute working on maize in Khyber Pakhtunkhwa. It is situated on the left bank of Kabul River, near village Pirsabak. Maize and Millet Research Institute (MMRI), Dadu, and Agriculture Research Institute Tandojam, Hyderabad, work on maize and millets in Sindh Province. In addition to public research institutes, national and multi-national private seed companies, are also working on maize crop in Pakistan.

Maize Seed Sector in Pakistan

Seed systems in Pakistan are developing rapidly. In the case of maize crop, availability of certified seed is still not satisfactory, but improving steadily. In 2013-2014, availability of certified seed was at 42%. Improved Government policies resulted in 68% availability of certified seed in 2016-2017. Another important aspect in total availability is the share of local and imported seed. Local seed system has experienced an increase from 19% in 2013-2014 to 65% in 2016-2017 (Figure 3). This change may be attributed to public-private partnerships, Government policies and international collaborations for capacity building. Agriculture Department has been working hard to improve local seed systems through provision of various facilities.

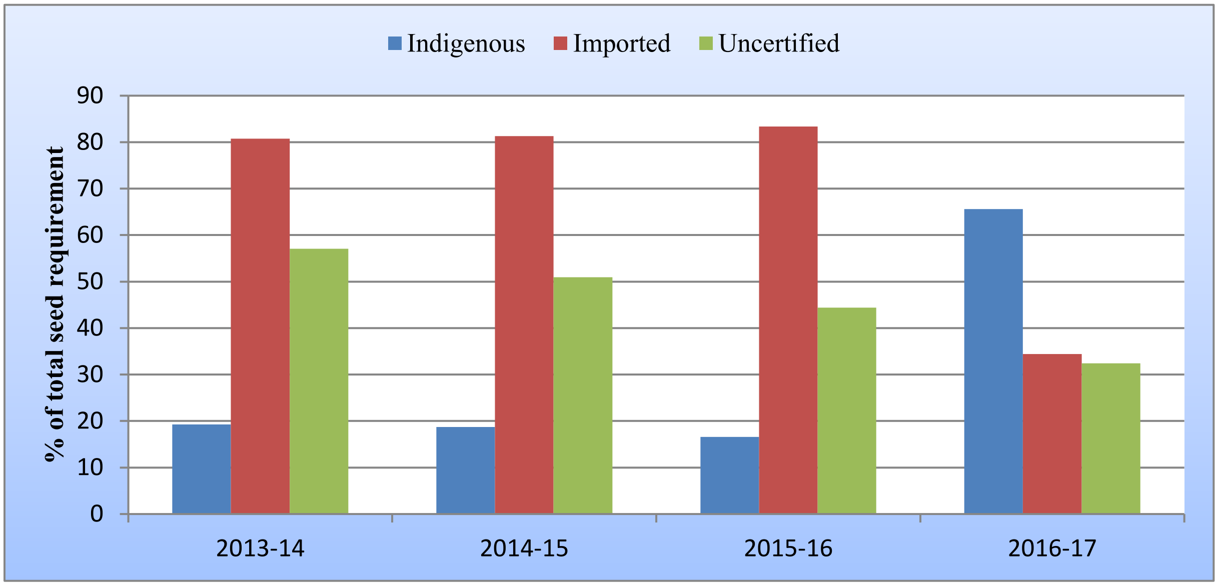

Figure 3. Availability of certified (indigenous & imported) and uncertified seed in Pakistan.

Challenges to Maize Production in Pakistan

Maize production in Pakistan faces challenges such as unavailability of certified seed, high cost of production and non-competitiveness in international markets. Currently, only 68% of certified maize seed is available to farmers while 32% of seed requirement is being fulfilled by the uncertified seed. Cost of maize production is high in Pakistan due to expensive seed, fertilizer and electricity. Estimated cost of production for 100 kg of maize grain is 2012 rupees, which is higher than the price of maize grain in international markets (Figure 4) and explains its non-competitiveness. This has compelled the Government to impose regulatory (30%) and import duty (10%) on maize grain to protect the local maize market. Therefore, it is necessary to formulate effective strategies to reduce the cost of production, increase local market share and ensure 100% availability of certified seed.

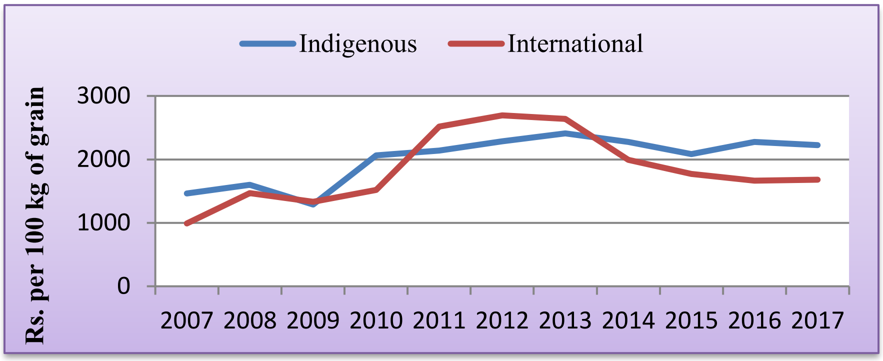

Figure 4. Price of maize grain in indigenous and international market.

Government Policies to Boost Maize Production in Pakistan

Government is working tirelessly to benefit the farmer community, Agricultural Departments and Private industry. Price of maize grain is monitored and regulated by the government throughout the year, which helps to stabilize maize crop production and sale in the country. Government has imposed regulatory (30%) and import duty (10%) on maize grain to protect local maize market. Provision of subsidized fertilizer rates is another admirable project by the Punjab Government to reduce cost of production, as is modernization of markets through pavement of market floors and provision of dryers; this helps to reduce postharvest losses. The Research and Development Board was set up to bridge the gap between farmers and research institutes, while Punjab Maize Advisory Board was constituted to actively solve issues regarding maize crop.

Maize and Millets Research Institute Yusafwala, Sahiwal, Pakistan

MMRI is public research institute working on maize crop in Punjab and has so far contributed 14 maize hybrids and 12 maize OPVs. The institute's top hybrids (YH-1898, FH-1046 and FH-949) and OPVs (Pearl, MMRI yellow and Malka-2016) are popular in Punjab's farming community due to their high yield and heat resistance.

MMRI provides hybrid seeds directly to farmers, whereas Punjab Seed Corporation - a public seed sale agency - is provided with parents of hybrids for seed multiplication and seed sale throughout Punjab. Local seed companies buy pre-basic and basic seed of maize OPVs for seed multiplication. Therefore, MMRI directly and indirectly provides maize seeds to many farmers in Punjab. MMRI is currently working on projects funded by the Provincial Government geared towards:

- Provision of additional research facilities for development of heat resilient maize hybrids.
- Acceleration of maize breeding through inducer line mediated doubled haploid inbred lines for development of climate smart and high yielding maize hybrids.
- Development of maize hybrids/ OPVs with high protein oil and provitamin-A content to overcome malnutrition.

International Collaboration of MMRI

MMRI is working in collaboration with international institutes including CIMMYT and the Bavarian State Research Center for Agriculture, Germany. The institute has collaborated severally with CIMMYT, specifically on the Heat Tolerant Maize in Asia (HTMA) project and the Agriculture Innovation Program (AIP). Sahiwal is a hotspot for screening against heat stress due to the very high temperatures it experiences, especially during reproductive phase of maize crop. Material provided by CIMMYT is evaluated at MMRI and its station, Maize Research Station Faisalabad. Similarly, bio-fortified material under AIP is screened by the Institute. CIMMYT allocated one short duration white maize OPV (CZP-132001) to MMRI, which has been successfully acclimatized and maintained. CZP-132001 has been evaluated at national level and was found superior to the check over two years' National Uniform Maize Yield Trials (NUMYT) across the country. At the same time FSC&RD has recommended the variety as distinct, uniform and stable after two years of DUS studies. The variety has also been evaluated at farm level under 'spot examination' by members of the Expert Sub-Committee of Punjab. It will be approved by the competent authority in near future. Being a short duration variety, it will be highly adopted in arid zones and in maize-potato-maize crop rotation in Sahiwal division.

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