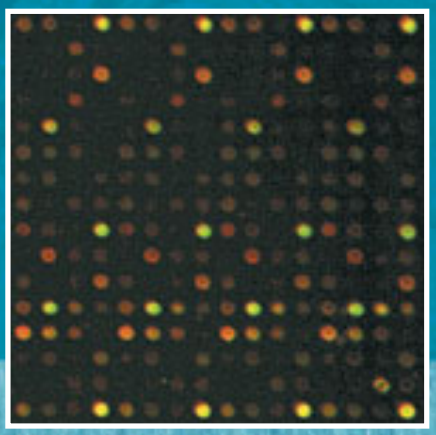
Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments

J–M Ribaut and D. Poland, editors



A Strategic Planning Workshop
held at CIMMYT, El Batan, Mexico,
21–25 June 1999,
with support from the Rockefeller Foundation

CIMMYT®

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Cover photos: (Upper left) Loading a horizontal agarose gel to reveal polymorphism at the DNA level using SSRs as molecular markers. (Photo: CIMMYT Applied Biotechnology Center)

(Lower left) Image of a section from a cDNA microarray that contains maize genes. The DNA on the microarray was hybridized with labeled DNA from stressed and unstressed maize ears. (Photo: Pioneer Hi-bred Int.)

(Right) Bread wheat under different stress regimes. (Photo: CIMMYT Wheat Program)

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Contents

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Before the beginning of the workshop, presentation papers were made available to interested parties on the CIMMYT web page (http://www.cgiar.org/cimmyt). This was possible because of the conscientious efforts of the presenters, and we are particularly grateful to them. Special thanks go to Fernando Garcia, who formatted and posted the papers on our web site, and to Kelly Cassaday, who did the same for the discussion summaries, workshop recommendations, and conclusions, shortly after the workshop concluded.

We would also like to thank all of the CIMMYT support staff for their help in organizing and supporting the workshop activities. Our sincere thanks go to Susana Velazquez for her assistance with the workshop logistics and manuscript submissions; to Linda Ainsworth and the Visitor Services' staff for their efforts that ensured the smooth running of the workshop; and to Eliot Sanchez Pineda for his design and layout work for this proceedings.

Foreword

The Rockefeller Foundation was pleased to cosponsor with CIMMYT the international workshop "Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments." Throughout its history, the Foundation's Agricultural Sciences program has emphasized the salient role of crop genetic improvement as an effective means of enhancing crop productivity and food security in the developing world. This workshop, part of a series of meetings and commissioned studies sponsored by the Foundation over a two-year period (1998–99), evaluated the scientific evidence and probabilities of future success related to genetic modification of cereals for enhanced "drought tolerance." Coincidentally, the Foundation recently completed a process of internal reorganization and programmatic renewal. The Food Security Theme (formerly Agricultural Sciences Division) has identified "water-limited environments" (rainfed or poorly irrigated) as a priority constraint to crop yield and, more importantly, to the stability of poor farm-family livelihoods. Hence this timely workshop, with its prioritized recommendations (see this volume) for future research, may be viewed as a major milestone enroute to the final goal of making genetic crop improvement for "drought tolerance" a reality on the plant breeding landscape. The workshop proceedings incorporate scientific research updates in tandem with solid goal-oriented recommendations. This assures the volume will be of direct value to research scientists and administrators as well as the Rockefeller Foundation and other donor organizations.

Water deficits, whether stemming from rainfall deficits or inadequate irrigation, are major constraints to crop production—surpassing even more obvious and well-researched constraints such as plant disease. Conservative estimates of yield losses for two of the principle cereals addressed in this workshop, and the major food sources for sub-Saharan African and Asian populations—maize and rice, respectively—are illustrative. Global annual yield losses from drought in tropical maize are estimated to be 17% of production or 20 million metric tons per year, representing US$ 2,200 million. Annual losses for the rice crop are estimated to be 4% of global production or approximately 18 million metric tons, valued conservatively at $ 3,600 million.

Regardless of the monetary value placed on these losses, the ramifications for poor farm families are often incalculable. Recent studies of "coping mechanisms" or risk management practices forced on rice farm-families in eastern India illustrate the insidious nature of drought as a destabilizing factor. Varying with intensity of drought-induced crop yield losses, coping mechanisms may range from changing household consumption patterns for food and clothing to postponing educational and medical expenditures, all to often, indefinitely. This in turn can lead to loans and borrowing that may prove ill-advised in the long term, and to more catastrophic ramifications such as selling assets (tools, animals, family heirlooms), and migration in search of employment. The costs and insecurities represented by these "coping mechanisms" for poor farm households in drought-prone regions have not been adequately studied, but intuitively they represent significant justification for renewed efforts to decrease the sensitivity of major crop species to this ubiquitous abiotic stress.

The Rockefeller Foundation joins CIMMYT in the fervent hope that through the determined application of quality science—especially the use of new and powerful tools of plant molecular biology—agricultural research may contribute to more stable livelihoods for farm communities dependent on drought-prone environments for their food and income. CIMMYT is to be especially congratulated for assembling a world-renowned group of experts. Over the past 25–35 years, few scientists have applied themselves over an entire career to this most difficult area of crop genetic improvement. The workshop organizers, however, managed to bring together just such resources, representing the world's foremost scientific experts in this field. Among those who participated are individuals who have dedicated their entire professional careers to solving the mysteries of crop response to water deficit and employing that knowledge for crop breeding to help solve the problem. Coupling this deep understanding of the subject with those scientists conversant with the new tools of DNA molecular marker-assisted breeding and the still untapped power of "functional genomics" has ensured the resounding success of this workshop. The serious and comprehensive dialog across plant science disciplines and across five of the world's most important cereals has led to blueprints for future applied and basic research. These proceedings are especially timely with regard to future global agricultural water resource scenarios. As we look to the pressing realities of the 21st century, we observed that the world's rainfed farms and farmers will benefit from the research advocated by this workshop; further, we are reminded of the fragile nature of agriculture's future water resources—both in terms of quantity and quality. Hence, this workshop's global relevance is compounded as politicians, researchers, and producers are cautioned that *today's irrigated environments may well be tomorrow's water-limited environments.*

John C. O'Toole
The Rockefeller Foundation

Preface

Water is the basis of life. In agriculture, both its overabundance and its scarcity are cause for concern for farmers around the world—rich and poor, subsistence and commercial, and from the North and the South. In tackling water or drought stress, we are taking on one of the oldest and most pervasive threats posed to agriculture by the environment. While some plant species possess extremely efficient mechanisms to cope with water stress, most of the important agricultural species—especially the major cereals such as maize, wheat, rice, sorghum, and pearl millet—are, to varying degrees, susceptible to it. Thus, when the rains fail or are inadequate, the productivity of these species is severely limited, a fact that is especially critical in developing countries where food is often already scarce.

Biotechnology has been proposed as an important tool in the development of improved plant varieties that can make our key crop species more drought tolerant. Indeed, the rapidly growing use of molecular markers is clearly demonstrating the possibility of understanding even complex traits, and in fact, such markers are now being used as indirect selection tools for crop breeding. At the same time, we are recognizing the limitations of approaches based solely on structural genomics. The field of functional genomics, meanwhile, provides an opportunity to greatly expand our ability to understand the genetic basis of complex phenotypes. The challenge is how to apply such powerful approaches to difficult problems such as a plant's response to water-limited environments.

The participants in this workshop undertook this challenge and devoted four days to intense discussion and debate in order to identify the most appropriate technologies and research approaches for improving water-stress tolerance in each of the five targeted crops. The deliberations led to the recommendations that are included in these proceedings. A great deal of thanks must be given to the participants for their willingness to attend, to openly discuss, and to work toward consensus. It is because of their participation that the workshop was a success and that these excellent proceedings could be produced.

The original goal of the workshop was to develop a set of recommendations that could be used by scientists, policymakers, and others interested in the topic. I trust that all those who read these proceedings will agree that the goal has been achieved. But more importantly, I hope that readers will use the carefully considered conclusions to develop further research to address this important challenge.

Dave Hoisington
Director, Applied Biotechnology Center, CIMMYT

Executive Summary

Drought is a major problem for the production of the world's five principal cereals: maize, wheat, rice, pearl millet, and sorghum. Past and current breeding efforts have met the challenge posed by drought with some success. With the knowledge generated by those efforts and the emergence of technologies such as structural and functional genomics, the time has come to identify new strategies that combine advanced molecular technologies with conventional breeding and physiological techniques.

From June 21 to 25, 1999, a group of 37 scientists from around the world met at CIMMYT Headquarters in Mexico to discuss the problem of drought and to design a strategic workplan that identified and prioritized research activities. The goal of the plan is to accelerate cultivar development leading to improved yields under drought conditions.

The strategic workplan drafted by the group of experts includes seven integrated, high-priority approaches for producing drought tolerant crops. The priorities include the following:

- the characterization of target environments;
- the establishment and quantification of screening environments and protocols;
- gene discovery;
- improved marker system and marker-assisted program integration;
- bioinformatics leading to improved databases;
- dissection of physiological traits; and
- utilization of new genomics-based technology.

For each of the approaches, the group developed commodity-specific activities, which provide the basis for future research activities and cross-commodity opportunities. Finally, the group drafted the following four recommendations that it felt represented the major conclusions reached after three days of deliberations.

- Drought is a major problem especially in developing countries facing the production of the five major cereal commodities: maize, wheat, rice, pearl millet, and sorghum.
- New opportunities to improve drought tolerance in cereals have emerged with recent developments in molecular technology and genetics. These should be utilized in combination with conventional breeding and physiological techniques. As reflected in the seven priorities identified during the workshop, ongoing activities should be improved concomitantly with the allocation of resources for new technologies and research.
- Integrated approaches across commodities and disciplines have been clearly identified. The time frame for research and product development in different crops, however, may be quite different. In this regard, the following report, which summarizes the objectives, design, and process used for the workshop, should serve as a reference for the development of further initiatives related to drought tolerance improvement in the five target cereals.
- The anticipated new cultivars and approaches can be used in both developing and developed countries, by resource-poor farmers and researchers.

Workshop Objectives and Outcomes

Objectives

- Provide updates on the ongoing science related to drought tolerance through two days of presentations.
- Discuss ways to apply molecular technology and genetics to plant breeding in order to further the development of drought tolerant crops (wheat, maize, rice, pearl millet, and sorghum).
- Identify commodity-specific activities and techniques that will support the development of drought tolerant crops and that can be shared across commodities.
- Develop a strategic workplan, including priorities, to implement these ideas for improving drought tolerance in farmer's fields, based on current information, approaches, germplasm, and emerging technologies, especially biotechnology.

Outcome

The outcome: A publicly available strategic workplan that identifies priority actions needed to develop drought tolerant crops. The strategic workplan is made available to any institution or organization to set priorities, develop research proposals, and/or seek financial support to implement activities related to the development of drought tolerant commodities.

Participants

Plant breeders, physiologists, and biotechnology experts spent five days at CIMMYT working together, across disciplines, to design the strategic workplan. The group included renowned experts from CGIAR centers, research institutions, universities, and representatives from the private sector. Three Rockefeller Foundation representatives also participated in the discussions. (See Attachment 1 for a complete list of participants.)

Sponsor

The workshop was designed and implemented by CIMMYT, with funding from the Rockefeller Foundation.

Workshop Design

Twenty-nine research papers were presented during the first two days of the workshop (Attachment 2 lists the presentations). The papers may be found in the second section of this document. A strategic workplan that identifies priorities and commodity-specific research activities to accelerate cultivar development for improved crop production under drought conditions was developed during the following three days.

The three-day session was professionally facilitated to allow as much interaction and discussion as possible, while keeping the group focused on the goal of creating a realistic, viable strategic workplan that included specific priorities.

The workshop included both plenary and break-out sessions to allow for adequate exchange of ideas (see Attachment 3 for the agenda of the three-day session). The group was divided by discipline and then by commodity to provide an opportunity to identify multidisciplinary, integrated approaches to solving the problem.

During the first of the discussion sessions, participants worked in three groups, according to discipline (breeders, physiologists, and molecular technology experts) to identify specific priority activities they thought were needed in their field to accelerate production of drought tolerant crops (see Attachment 4).

During the remainder of the workshop, participants worked in four commodity-based teams; wheat, maize, and rice were discussed in separate groups, while the sorghum and pearl millet experts worked together. The commodity groups discussed and reached some consensus on both short- and long-term actions that will be necessary to move toward the development of drought-tolerant crops (see Attachments 5 and 6). The group defined "short term" to mean that results would be available in 3–5 years, while long-term results may take 10–15 years or longer. The commodity teams met three times during the week to identify major activities and their respective components and to discuss how to create productive multidisciplinary approaches.

The objectives of the discussions were as follows:

- Discussion per activity (Wednesday, July 23, morning session, Attachment 4)
  Objective: To identify the different tools available, or to be developed, per activity.
- Discussion per commodities (Wednesday, July 23, afternoon session, Attachment 5)
  Objective: To define and prioritize activities by commodity, considering applied versus basic science.
- Discussion per commodities (Thursday, July 23, morning session, Attachment 6)
  Objective: To give a time frame to the different activities identified on the previous day.

Outcome/Strategic Workplan

In the different sections of the strategic workplan, suggested action steps are grouped into activities. The order of those activities does not necessarily reflect an order of priority, and in most cases, several activities should be developed concomitantly to have an impact on crop improvement. Of course, priorities and time frame defined by breeding programs should be compatible with local resources.

A. Maize

Activity 1: Within ongoing maize breeding programs that target drought environments, deploy proven conventional and molecular techniques. At the same time, screening and selection methods will be optimized and standardized.

- Characterize the target environments (GxE evaluation).
- Continue the development and characterization of germplasm.
- Pyramid elite alleles involved in the expression of yield components and/or a secondary trait of interest (such as ASI), either at identified QTL or through conventional techniques.
- Further improve stress management techniques and develop drought screening protocols.
- Explore genetic variability (phenotypically and through molecular markers) in improved and exotic germplasm.

Activity 2: As more breeders/scientists apply these techniques in a range of environments, more diverse drought-tolerant germplasm will be developed. As a consequence, screening and selection methods will be, and need to be, further developed and optimized. This activity will require strong interactive participation of the different disciplines.

- Provide access to, and development of, marker technology.
- Identify new secondary traits of interest. This might be based on genetic or physiological studies, proteomics, or on the discovery of novel pathways using data produced at the cell-molecular level.
- Develop and test new marker-assisted strategies.
- Explore comparative mapping to take advantage of the genes identified in the related species.

Activity 3: Identify the genes involved in the drought-tolerance response/pathways through genomics approaches.

- Screen microarrays with cDNA from mRNA expressed from different tissues and under different experimental conditions. The screening of cDNA clones fixed on microarrays enables the assessment of gene expression for tens of thousands of genes. In the short term, such DNA clones could be used as DNA markers in the selection and characterization of germplasm. In the long term, such clones identify components of the plant's response to water stress and provide a basis for devising novel strategies for screening and perhaps directly modifying the crop plant.
- Establish the physiological pathways. The DNA sequence of the clones establishes their identity and provides the first clues regarding the biological function of the gene. Further clues are provided by large-scale analyses of the proteome of specific tissues and cells under stress conditions. The combined analyses will reveal the important key genes and pathways comprising and controlling the responses to drought and their interaction with previously unknown pathways.

Activity 4: Considering the existing technology and germplasm, improve the conventional and marker-assisted screening facilities and human resources.

- Create and enhance the infrastructure necessary to screen germplasm for traits known to enhance drought tolerance. Depending on regional circumstances, this would include the addition of field locations that are properly equipped (e.g., irrigation equipment) or modified to facilitate clear discrimination among genotypes.
- Create or enhance the infrastructure necessary to collect and analyze molecular and physiological data for genetic loci, genes, biochemicals, and processes known to enhance tolerance to drought. This would include the establishment and equipping of laboratories needed to service breeding programs of a given region.
- Ensure access to molecular marker technologies by a wide range of researchers.
- Conduct training courses on topics and techniques needed to implement the new and established methods for screening and improving germplasm.

Institutional framework: Maize has been one of the most intensely studied crops at the genomic level.

Several molecular techniques are routine in maize or are on the verge of being available in the public sector. Ongoing research programs are already in place and action should be taken to ensure that new strategies and projects are well integrated in such research. Activities 1-4 assume that existing research activities will result in the following:

- More microsatellite loci, with about 700 already publicly available.
- Public database of ESTs (in progress, for maize and several other grasses).
- Databases of QTLs, genes, sequences, etc. for various grasses, including maize (one option might be to work with the curators of maizeDB to make it more "search friendly" for stress-related information and molecules).
- Maize microarrays are, and will be, available at several places.
- "Gene machines."
- Transformation technology.
- Physical maps for related smaller genomes such as rice and sorghum (useful for gene hunting and comparative mapping).
- A large network of potential cooperators in the private sector.

B. Rice

The order in which the activities are listed approximately reflects the priority assigned by the discussion group. Activities 1 and 2, however, are considered equally important.

Activity 1: Integrated approach of genomics, breeding, and plant physiology for accelerating genetic improvement of rice in water-limited environments. This approach will have the following components:

- Introgress, identify, and characterize drought tolerance gene/QTL as part of breeding efforts.
- Characterize different water-limited environments (upland, rainfed lowland, and irrigated ecosystems).
- Develop and standardize corresponding phenotyping protocols and testing networks, including protocols for inducing realistic drought stress under small-scale experimental field conditions.
- Apply these protocols at drought-sensitive stages of plant development (tillering, panicle induction) to tolerant and susceptible lines and selected lines from mapping populations.
- Identify and understand the physiological basis of secondary traits and corresponding genes/QTLs associated with drought tolerance at different developmental stages.
- Characterize G x E interactions of identified drought tolerance genes/QTLs.
- Characterize and enhance drought tolerance in rice germplasm.
- Exploit allelic and non-allelic diversity of drought tolerance genes/QTL.
- Develop valuable genetic/breeding materials such as NILs for drought tolerance genes/QTLs and cultivars with significantly improved drought tolerance in different target environments.
- Integrate marker-aided pyramiding and marker-aided recurrent selection as routine practices of breeding for drought tolerance in rice.

Activity 2: Functional genomics for drought tolerance (drought tolerance phenotype, biochemical pathways, genes) with the following components:

- Prepare drought-responsive cDNA libraries, ESTs, and 2D protein analyses in different tissues, developmental stages, and genetic backgrounds under stressed and unstressed conditions, and analyze on microarrays.
- Characterize transcriptional profiles under drought in different tissues, developmental stages, and genetic backgrounds using DNA microarrays.
- Identify candidate genes by integration of EST, QTL, and physical mapping.
- Develop high-throughput gene disruption system to accelerate target gene discovery and deployment into drought tolerance breeding programs.
- Establish capacity for allele mining in rice germplasm (from *Oryza sativa* through wild rices and related grasses to the other cereals).
- Develop efficient transformation system and reverse genetic tools to determine gene functions.

Activity 3: High-throughput DNA marker system(s).

- Access to, and development of, high-throughput marker systems such as microsatellites and single-nucleotide polymorphism (SNP) to accelerate genetic mapping and deployment into drought tolerance breeding programs.
- Develop and test new marker-assisted selection strategies.

Activity 4: Comparative genetics.

- Develop integrated genetic and physical maps to facilitate gene hunting and comparative genetics in rice and other cereals.
- Identify and map drought-responsive orthologous loci in cereals.
- Use rice as anchor genome to accelerate gene discovery in all cereals.
- Perform comparative genetic/genomic analysis of drought tolerance genes/QTLs.

Activity 5: Bioinformatics.

- Develop user-friendly database to accelerate the processes of gene discovery and genetic improvement of drought-tolerant rice and other cereals.
- Access to rice genome sequencing data.
- Inventory of germplasm, pedigree, phenotypic data, mapping populations, DNA markers, genetic and physical maps, genes, QTLs, and ESTs.

C. Wheat

Activity 1: Bioinformatics.

- This is an overarching activity for all research areas. Comprehensive databases are crucial to organizing data to permit ready access to new information and to tie the different research areas together. For example, GIS data collected to characterize target environments may be used to develop simulation models of different screening environments. The same information can be used to help establish treatments for functional genomics studies, the results of which may be cross-referenced with data coming from QTL analysis, etc.

Activity 2: Characterize target environments.

- Drought-prone environments are highly variable with respect to rainfall distribution as well as a number of other biotic and abiotic stress factors. Therefore, it is important to characterize these environments in order to target genetic improvement towards the prevailing stress factors. For example, a genotype adapted to growing exclusively on stored soil moisture in South Asia, would not necessarily be well adapted to the Mediterranean environment where rainfall occurs prior to heading, or to South America where moisture stress is relieved after flowering. In addition to differences in rainfall patterns, regions may show local variation in soil chemistry or disease pressures. There is good evidence of interactions between genotypes and factors such as zinc deficiency and nematode infestation under moisture stress. As well as these biological considerations, there are social and economic factors that need to be assessed when prioritizing regions being targeted for crop improvement research.

Activity 3: Establish screening environments/protocols.

- Once target regions are defined, germplasm screening environments need to be developed that resemble the most important target locations in terms of their principal yield-limiting characteristics. This may involve refinement of technologies such as line-source sprinkler irrigation to match water distribution profiles, as well as careful characterization and control of other biotic and abiotic stress factors in breeding nurseries. In addition, the potential interaction of genotypes with commonly used agronomic practices in target environments needs to be established. Selection environments need to be developed that reflect appropriate crop management, or, if possible, that permit selection of superior genotypes under all representative agronomic practices

Activity 4: Functional genomics.

- With the advent of DNA chip technology or microarrays, the relative importance of different genes involved in drought tolerance can be determined. (The technique involves extracting RNA from plant tissue and generating labeled cDNA or cRNA probes that are hybridized with the microarrays. The microarrays are scanned to determine which genes were turned on in the tissue sample.) Due to the large number of genes involved in stress response, a huge amount of information is generated for each sample. To discover candidate genes for crop improvement, it will be necessary to choose only the most appropriate plant organs, stages of phenology, and stress conditions on which to focus the research. Once genes associated with performance under drought are identified, the information can be used to complement empirical plant breeding. After the information has been interpreted in terms of its biochemical and physiological significance, it should permit the identification of traits that can be enhanced through the introgression of new sources of genetic diversity.

Activity 5: Validate secondary traits.

- Many anatomical, physiological, and biochemical traits are reported in the literature as being drought adaptive (e.g., osmotic adjustment, stem reserve mobilization, early vigor, canopy temperature depression, spike photosynthesis, leaf anatomical traits, etc.). Relatively few of these have been introgressed into suitable genetic backgrounds (i.e., recombinant inbred lines, doubled haploid populations, near isogenic lines) to assess potential genetic gains associated with their selection. This work should be conducted for the most promising traits. This activity is highly complementary to several other areas of research that have already been mentioned, including QTL mapping, marker-assisted selection, and assessment of genetic diversity. Information on

secondary traits can also be used in interpreting data generated by research in functional genomics.

Activity 6: QTL mapping (structural genomics).

- When parents that contrast in yield and associated traits under drought have been identified, they can be crossed to develop populations for QTL mapping. Mapping should probably be conducted in several genetic backgrounds in which there is genetic variability for the important traits in order to identify reliable candidate QTLs for marker-assisted selection.

Activity 7: Marker-assisted selection.

- Once reliable secondary traits and QTL molecular markers have been confirmed, it should be possible to speed up the process of selection among breeders' materials using marker-assisted selection (MAS). MAS can be useful in selecting good and potentially complementary parental lines and for screening progeny for the presence of drought-adaptive genes and traits. Use of MAS will reduce the need to rely exclusively on field-based evaluation of germplasm, which is complicated by interactions with the weather, soil heterogeneity, and other factors.

Activity 8: Assess genetic diversity.

- Functional genomics, evaluation of secondary traits, and QTL mapping can help identify the traits associated with drought tolerance and their genetic basis. This information can be used to screen germplasm collections for phenotypes with extreme expression of those traits, as well as for new sources of allelic diversity in the relevant genomic regions. New sources of genetic diversity, once introgressed into the current germplasm base, should permit steady progress in yield under moisture-stressed conditions.

D. Sorghum and Pearl Millet

Activity 1: Establish target environments, testing environments, and screening protocols through a collaborative drought research network.

- Quantify the severity, timing, and frequency of drought stress in testing and target environments via simulation modeling.
- Establish a drought screening network, with strong linkages to national programs, for sorghum and pearl millet, based on a set of global, coordinated, well-quantified stress environments.
- Utilize both natural stress environments—to assess trait value in target environments and managed (specific and repeatable) stress environments—to refine trait assessment protocols, assess productivity costs associated with traits, and improve secondary trait phenotyping.
- Establish and deploy standardized screening methodologies and general and targeted measurement protocols for assessment of both crop performance and the expression and effects of specific resistance/tolerance traits.
- Exploit drought screening network(s) as a mechanism for technology improvement, technology transfer, and human resources development.

Activity 2: Develop better marker technology for QTL detection and trait evaluation.

- Develop improved and integrated molecular marker-based genetic linkage maps for both crops, which are appropriately anchored and have markers (SSRs and ESTs).
- Further evaluate existing mapping populations for the expression and importance of target traits and the identification of additional drought-tolerant traits and QTLs.
- Construct new mapping populations to identify and evaluate additional genes/alleles for the initial complex of tolerance traits and to identify and map new complex(es) of traits.
- Initiate fine mapping of major QTLs (via NILs development) to identify more specific markers and to initiate map-based cloning of effective drought tolerance alleles.
- Develop and test new and more effective methodologies for using marker-assisted selection in improving drought tolerance.

Activity 3: Deploy new molecular technology to accelerate the use of existing genetic diversity in the improvement of performance under drought stress.

- Use fingerprinting to increase the effectiveness of the choice of parents for conventional and marker-assisted drought tolerance breeding programs.
- Use marker-assisted and recurrent selection to introgress drought-tolerance QTLs into adapted breeding materials and cultivars.
- Use marker-assisted selection to develop sets of NILs for
  - Quantification/confirmation of trait benefits;
  - Dissemination of traits in adapted backgrounds;
  - Dissection of physiological traits; and
  - Fine mapping and gene identification.
- Pyramid drought-tolerance QTLs and traits in adapted backgrounds.

Activity 4: Evaluate and dissect complex physiological tolerance traits.

- Exploit available NILs as tools to confirm/validate trait utility, to determine the physiological and biochemical mechanisms underlying trait function, and to identify candidate drought tolerance genes for cloning.
- Develop additional mapping populations to assess the physiological effects and genetic variability of (new) secondary traits and their potential applicability in improving drought tolerance.
- Quantify trait value for a wide range of target environments, using simulation modeling of the physiological effects of the traits.

Activity 5: Use functional genomics tools to enhance the ability to improve drought tolerance.

- Identify genes whose expression is altered in response to drought, primarily through analysis of ESTs from different tissues and various drought regimes.
- Use microarrays to evaluate gene expression under drought conditions, across environments and in different genetic backgrounds, including NILs for drought-tolerance QTLs.
- Identify candidate genes, gene sets, and biochemical pathways that may be involved in drought tolerance.
- Integrate QTL and EST mapping with structural genomics to accelerate gene identification.
- Compare gene expression profiles under drought stress in sorghum, pearl millet, maize, and related grasses.
- Develop acceptable transformation systems to facilitate biotechnology approaches involving reverse genetics.

Activity 6: Link all the research activities described above (across species and target traits) through bioinformatics.

- Strengthen and exploit the International Crop Information System (ICIS) and linkages to other curated databases to convert data generated in Activities 1-5 into information, by facilitating the mining of a broad range of datasets:
  - Test and target environment characterization data (ICIS).
  - Pedigree information (ICIS).
  - Marker genotype datasets (crop-specific genome databases).
  - Phenotypic (physiological, morphological, and agronomic) datasets (ICIS).
  - Putative QTL inventories.
  - Candidate gene datasets.

The sorghum and pearl millet research team wishes to stress that a fully integrated approach, bringing to bear the tools of biotechnology (molecular marker-assisted breeding, gene discovery, and bioinformatics), conventional plant breeding, and crop physiology and modeling, will be far more effective in addressing the improvement of drought tolerance in these cereals for the people that have been left out of the Green Revolution, than would isolated support of any subset of these activities. Thus the activities described above were designed to be implemented as an integrated program, in which progress in any one area directly contributes to improving the rate of progress in the others.

E. Priorities across Commodities

Not surprisingly, the approaches presented for the various cereals have a lot in common. Depending on the crop considered, however, differences in time frame and priorities have been identified, linked principally to differences in germplasm or available technology. To emphasize those common activities and underline some characteristics for each crop, a table summarizing the activities by commodity was developed during the workshop (Attachment 7).

The resulting strategic workplan includes seven priorities that are based on interdependent and complementary approaches for producing drought-tolerant crops.

- Characterize target environment.
- Establish, quantify, and standardize screening environments and protocols.
- Gene discovery.
- Improved marker system and marker-assisted program integration.
- Bioinformatics—leading to an improved database.
- Dissection of physiological traits.
- Utilization of new technology.

Attachments

Attachment 1: List of participants
Attachment 2: Program of the presentations
Attachment 3: Agenda of the three last days
Attachment 4: Roundtable report by activity and plenary session (Wednesday, July 23, morning)
Attachment 5: Roundtable report by commodity and plenary session (Wednesday, July 23, afternoon)
Attachment 6: Plenary session report (Thursday, July 24, morning)
Attachment 7: Activities across commodities (Table)
Attachment 8: Glossary of acronyms and terms

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Attachment 2: Program for the Drought Workshop Presentations

Monday 21 / Morning session (Auditorium) / Chairman: Shivaji Pandey

8h00-8h40: **Opening remarks**
Claudio Cafati (CIMMYT)
David Hoisington (Applied Biotechnology Center)
Shivaji Pandey (Maize Program)
Gary Toenniessen (Rockefeller Foundation)
Linda Ainsworth (Visiting Services)

8h40-9h10: **Abraham Blum**
Towards standard assays of drought resistance in crop plants

9h10-9h40: **Henry Nguyen**
Molecular dissection of drought resistance in crop plants: from traits to genes

9h40-10h10: **Jeff Bennetzen**
Comparative genomics approaches to the study of drought tolerance

10h10-10h40: Discussion

10h40-11h00: Coffee break

11h-11h20: **Richard Trethowan / Wolfgang Pfeiffer**
Challenges and future strategies in breeding wheat for adaptation to drought stressed environments: a CIMMYT Wheat Program perspective

11h20-11h40: **Matthew Reynolds**
Evaluating a conceptual model for drought tolerance

11h40-12h00: **Richard Richards**
Physiological traits to improve the yield of rainfed wheat: can molecular genetics help?

12h00-12h20: Discussion

12h20-12h30: Workshop photo in front of the main building

12h30-14h00: Lunch at "el Rincon Mexicano"

Monday 21 / Afternoon session (Auditorium) / Chairman: Richard Richards

14h00-14h20: **Fran Bidinger**
Genetic improvement of tolerance to terminal drought stress in pearl millet (*Pennisetum glaucum* (L.) R. Br.)

14h20-14h40: **Barry McCarter**
Application of biotechnology in breeding maize for Africa: the perspective of a private company

14h40-15h00: **Marianne Bänziger**
Breeding for drought tolerance in tropical maize: conventional approaches and challenges to molecular approaches

15h00-15h20: **Stephen Mugo**
Prospects of using ABA in selection for drought tolerance in cereal crops

15h20-15h50: Discussion

15h50-16h10: Coffee break

16h10-16h30: **Robert Schaffert**
Genetic control of phosphorus uptake and utilization efficiency in maize and sorghum under marginal soil conditions

16h30-16h50: **Peter Goldsbrough**
Assessing the contribution of glycinebetaine to environmental stress tolerance in sorghum

16h50-17h10 **Arjula Reddy**
Probing the vitality of plants by the JIP-test, a novel non-invasive phenotypic screening technique for performance under water-limited conditions

17h10-17h30: **Dominique This**
Towards a comparative genomics of drought tolerance in cereals: lessons from a QTL analysis in barley

17h30-18h15: Discussion

Tuesday 22 / Morning session (Auditorium) /

Chairman: Mike Lee

8h-8h20: **Renee Lafitte**
Genetic variation in performance under reproductive-stage water deficit in a doubled haploid rice population in upland fields

8h20-8h40: **Zhikang Li**
Development of near isogenic introgression line (NIIL) sets for QTLs associated with drought tolerance in rice

8h40-9h00 **Rattan Yadav**
Identification and utilisation of quantitative trait loci to improve terminal drought tolerance in pearl millet (*Pennisetum glaucum* (L.) R. Br.)

9h00-9h20: **Tom Hash**
Marker-assisted backcrossing to improve terminal drought tolerance in pearl millet

9h20-9h40: **Mark Cooper**
QTL mapping activities and marker assisted selection for yield in the Germplasm Enhancement Program of the Australian Northern Wheat Improvement Program

9h40-10h10: Discussion

10h10-10h30 Coffee break

10h30-10h50: **Michel Ragot**
Efficient selection for adaptation to the environment through QTL mapping and manipulation in maize

10h50-11h20: **Jean-Marcel Ribaut**
QTL analyses, MAS results and perspectives for drought tolerance improvement in tropical maize

11h20-11h40: **Gebisa Ejeta**
Genetic analysis of pre-flowering and post-flowering drought tolerance in sorghum

11h40-12h00: **Andrew Borrell**
Physiological basis, QTL, and MAS of the stay-green drought resistance trait in grain sorghum

12h00-12h30: Discussion

12h30-14h00: Lunch at "el Rincon Mexicano"

Tuesday 22 / Afternoon session (Auditorium) /
Chairman: David Hoisington

14h00-14h30: **Mike Lee**
Understanding and enhancing stress tolerance in maize: what can genetic maps contribute?

14h30-15h00: **Dominique De Vienne**
Proteomic and genetical approach of physiological and molecular responses to drought in maize

15h00-15h30: Discussion

15h30-15h50: Coffee break

15h50-16h20: **Jeff Habben / Chris Zinselmeier**
Utilizing new technologies to investigate drought tolerance in maize: a perspective from industry

16h20-16h50: **Hans Bohnert**
Cataloging stress-inducible genes and pathways leading to stress tolerance

16h50-17h10: **Mark Cooper**
Computer simulation linked to gene information databases as a strategic research tool to evaluate molecular approaches for genetic improvement of crops

17h10-17h40: Discussion

Note: Unfortunately, two presenters who had planned to participate in the workshop were unable to attend. However, their papers are included along with those of the other presenters.

Timothy Close
The dehydrin multigene family in the triticeae and maize

Qifa Zhang
Improving the tolerance of irrigated rice to water-stressed conditions

Attachment 3: Agenda of the Strategic Workplan Sessions (June 23-25)

OBJECTIVES:

- Discuss ways to apply molecular technology and genetics to plant breeding and improvement that will result in the development of drought tolerant crops (wheat, maize, rice, pearl millet, and sorghum).
- Identify commodity-specific activities and techniques that will support the development of drought tolerant crops and that can be shared with those working with other commodities.
- Develop a strategic workplan, including priorities, to implement these ideas.
- Identify next steps.

OUTCOME:

A strategic workplan, available to the public, that identifies the priority actions for developing drought tolerant crops. The workplan can be used by any institution or organization to develop proposals for financial support to implement activities related to development of drought tolerant crops/ commodities.

AGENDA:

Wednesday, June 23, 1999

8h00 Overview of objectives / agenda / guidelines for working together
Kathy Alison

8h15 The Strategic Workplan: Why we are developing it and how we will use it
Jean-Marcel Ribaut, CIMMYT
John O'Toole, Rockefeller Foundation

8h30 Expectations and comments from group on development of the strategic workplan
Kathy Alison

9h00 Introduction of a matrix: activities and commodities
Jean-Marcel and Kathy Alison

9h15 Roundtable discussion on activities
Group A: Breeding
Group B: Physiology
Group C: Molecular tools and strategies

10h45 Coffee

11h00 Plenary Session (15 minute report from each group + discussion)

12h30 Lunch

14h00 Afternoon discussion task: Commodities

14h15 Roundtables (Commodities)

Group A: Maize
Group B: Rice
Group C: Wheat
Group D: Sorghum and Pearl Millet

15h45 Coffee

16h00 Plenary Session (15-minute report from each group + discussion)

17h45 Overview of Thursday sessions

18h00 Adjourn

19h15 Transport available from housing area to restaurant

19h30 Official dinner of the workshop at "La Casona" restaurant, Texcoco.

Thursday, June 24, 1999

9h00 Compilation of matrix for all crops

10h30 Coffee

11h00 Overview of Strategic Workplan process and assignments

12h15 Overview of Friday sessions

12h30 Lunch at "el Rincon Mexicano"

14h30 Visit to the pyramids (optional)

Friday, June 25, 1999

9h00 Refining Strategic Workplan

10h30 Coffee

11h00 Identification of priorities

12h00 Summary and next steps, final comments

12h15 Adjourn

12h30 Lunch at "el Rincon Mexicano"

Attachment 4: Drought Improvement in Crops: Discussion by Activity

(Wednesday, July 23, morning session)

Objective: To identify the different tools available, or to be developed, by activity

I. Roundtable Discussion on Breeding

A. Traits (alternatives)
Yield under drought
Stay-green
Nutrient acquisition / uptake efficiency / acid soil tolerance
Osmotic adjustment / relative water content
Transpiration efficiency
Canopy temperature
Deep root development
Harvest index drought
Short ASI under drought
ABA
Gibberelic acid
Grain number maintenance
Emergence characteristics/vigor
Grain fill (duration and rate)

The group agreed that, concerning whether this work is "basic" or "applied," it depends on the crop and trait. Essentially, all putative traits will be applied in breeding, but breeding work itself is experimental (and therefore, basic) until you have a product that farmers actually adopt.
Criteria: heritability; relation to grain yield; genetic variability; costs; G x E (characterize trait and target environment).

B. Methods / Tools / Implementation
Molecular Biology
Criteria to consider this approach: Cost and solid foundation

- QTL mapping (increases heritability; understanding genetics)
- Marker assisted selection
- Fingerprinting (affects choice of parents, gives idea of genetic variation)
- Bulk segregant analysis (allelic identification in populations)
- Gene cloning
- Transformation
- Reverse genetics

Integration of molecular biology and breeding
Issues: Competition for resources, team formation, choice of germplasm/genetic background on which work will be performed. The relevance of molecular biology research to breeding is not ensured; molecular biologists need to be identified who are interested in crop breeding (students of cutting-edge researchers?). Funding agencies need to help ensure integration, relevance to developing country NARSs, delivery to farmers.

Integration with physiology
Once limited by available breeding materials; now better materials are available.

C. Pathways
Development vs. dissemination
Early, intermediate, final products
Impact: effective delivery

II. Roundtable Discussion on Physiology

Identify activities

Categorize as
- Applied research
- Basic science
- Other

List status
- Beginning
- In progress
- Completed

Begin to identify criteria for selection of priorities.

A. Brainstorming Session
1. Define co-limiting and confounding factors in drought experiments.
   - Develop agreed upon protocols on how to measure drought tolerance.
   - Define testing ground, testing facilities.
   - Develop testing networks.
   - Apply predictive technologies (climate).
   - Apply simulation modeling to predict value of traits.
   - Exploit opportunities for interaction with other disciplines.
2. Support step-by-step improvement.
3. ID critical physiological ideotypes limiting factors in target environments.
   - Establish value of traits in conventional breeding programs.
   - Establish value of genes coming from genomics (screening large numbers of genes).
   - Apply molecular technologies (QTLs, genes) to test value of traits, concepts.
   - Receive materials to test genes/traits.
   - Generate information for breeding programs.

Essential to cooperate across disciplines and not work in isolation.

B. Develop Research Tools for Phenotyping
Develop drought screening protocols to measure drought tolerance (phenotyping: the link between physiology with molecular biologists and breeders); in progress, but slow.

- Need for rigor, support, coordination.
- Need for testing facilities.
- Need for testing networks.
- Quality control (confounding factors).
- Catalogue protocols.

Did not differentiate between "What genes do" (basic) and "How to use them" (applied).

Define and prioritize target environments by crop, who should do it?
(Quantification, magnitude, frequency of stress)

Develop research tools.

- Develop drought tolerant ideotypes using molecular technologies.
- Deal with information on stress-responsive genes.
- Place value on gene WRT (water related trait) target.
- Sieving genes WRT function.
- Sieving genes WRT value.
- Test physiological hypotheses on value of specific traits.

III. Roundtable Discussion on Molecular Tools and Strategies

A. Important Ideas / Technologies: Brainstorming
Bioinformatics
Something to distribute all information to all parties (immediate and application).
Turning data in information.

Marker-assisted selection
How to apply
Candidate genes (QTL)
Genetic dissection of trait
Integration of genomics and molecular breeding
Gene discovery

Miscellaneous
High throughput mapping
Physical mapping
Comparative genetics
Microarrays
ESTs
Proteonics
Transgenics—promoters (stress induced)
Comparative physiology
Homologous recombination
Germplasm—geneflow
SSRs
SNPs
Mutagenesis—reverse genetics

Group says need broader categories to integrate these areas of research.

What components do we need to make use of the above items?
Genetic material, characterization, components from other groups (parents, lines, populations). Need databases for this.

B. Discussion of Items (current status, applied or basic research, time frame for development)
Bioinformatics. Status: Beginning (not integrated). Databases are in progress, but integration is just now being discussed. High priority. Applied and basic research. Needed immediately. Always getting better. Who and how is the curation going to happen? Curation is vital. Often bypassed because it's a nasty job to create a public good. CG centers need ammunition to show why this is a priority to World Bank. Need integration of databases. CG system is natural choice for integrating these various databases. Tough to layer on curation later. Area in which linkages to the private sector are really difficult.

Targeting data as information. Status: Just beginning. Applied and basic research.

MAS. Status: Depends on crop. Applied research; in progress. MASs will evolve; essential for plant improvement and genetic gain. Currently limited by technology, information, resources.

Candidate genes. QTL into loci. In progress, but for drought just beginning; never finished. Mainly basic research, but application potential high. Link between genetics and physiology.

Trait dissection. Status: In progress; (have trait, see what genetic basis and genes involved are—comes from other direction from candidate gene, though goal is the same). Mainly basic research, but application potential is high.

Application of genomics and breeding. This is overall goal of workshop.

High throughput mapping. Status: Industry, in progress, rest of the world, beginning. Applied according to timeline, otherwise, basic research. Resource limited.

Microarrays/ESTs. Status: Beginning. There's a perception that it will be finished (routine data) in general step within five years. For drought, basic research.

Protoeomics. At beginning; basic research; long term to finish.

Transgenics, homologous recombination, promoters (w/ candidate genes?). Status: Transgenics are in progress; homologous recombination just beginning; promoters in progress. Both applied and basic; limitation in terms of IP, public acceptance, etc. Promoter may be a key for application in drought.

Physiological mapping. Status: In progress; completion envisioned within 3-4 years; basic tool.

Comparative genetics and physiology. Status: Beginning. Basic research. No time frame for completion—continuing effort.

Germplasm in context of geneflow, characterization. Status: In progress—rapid acceleration. Applied research, but also basic in a broader sense. Never finished.

Better marker systems (High throughput marker systems). Applied research.

SSRs and SNPs. Status: In progress. Applied research. Finished in 2-5 years; limited by resources.

Mutagenesis, reverse genetics. Status: Industry in progress; everyone else just beginning Basic research.

Forward genetics. Basic research.

Interactions with other groups. Status: beginning; Can (must) be implemented now. Applied research, ESSENTIAL. Basic, ESSENTIAL.

C. Discussion of Criteria: Time: Short and Long Term, Resources, Impact, Likelihood.

Priorities:

#1. Question on databases is not about databases themselves, but accessible and user-friendly databases—hence integration of databases is a key issue.
#1. Interactions with other groups and shared components.
#2. MAS, SSRs, SNPs , trait dissection, candidate genes-QTLs (short-term) Point made that progress must be made in order to convince breeders and others that these technologies will have payoffs to support further efforts.
#2. Gene discovery: microarrays, ESTs, Proteonics. Long term, but could make great impact on the drought questions. In the past it was argued that public sector can't afford it, so it gets left to the private sector.
#3. Comparative genetics, comparative physiology, geneflow.
#4. Promoters, homologous recombination, reporters, transgenics.
#5. Physical maps.

IV. Plenary Discussion

A. Breeding Group

Physiology, ABC, and breeding. Need to have a "helicopter view" to see how they all fit together. Need to identify traits and genes. All these areas must lead to understanding drought.

Discussion topics:

1. Traits and environments analysis
2. Integration of molecular biology with breeding
3. Delivery systems thru germplasms to clients-pathways from varieties to farmers

Across categories paramount to identify successes and failures and then onto the process of learning and identifying opportunities.

Traits and environments analysis

Criteria for traits:

1. Heritabiltiy (yield and other traits) and existing and potential genetic variation (sources)
2. Costs and economics
3. Which environments to assess traits in
4. Comparative advantage to other screening methods?

List of tools that are important :

Target trait identification:
Stay-green
Tolerance to deficiency
Transpiration efficiency
Deep root development
Harvest index under drought
Traits related to anthesis and flowering
Traits connected to emergence
Grain fill—rate and duration
(list not complete)

Methodology:

Crop dependent and environment dependent:

1. Mapping—QTL, heritability—get improved knowledge of trait
2. MAS
3. Fingerprinting for parental choice and to get better handle on genetic variation.
4. BSA—gene allele identification in a population
5. Genetic transformation
6. Gene cloning
7. Reverse genetics

Integration

- Learning about potential—now isolated knowledge islands—need to fuse these islands though communication to answer key questions.
- Communication is required for guidance of research and involvement.
- Team formation—must be an incentive or some pressure to form such teams.
- Competition for resources has led of lack of integration. Different donors involved with different units/programs works against team approach. Must be addressed through joint projects.

Delivery system

- Top—lab work—upstream
- Middle applied
- Bottom
- Farmers and NARS
- Links need to be strengthen to deliver products needed at the bottom. Impact assessment is key. Intermediate bodies could strengthen these links(farmers and researchers for bottom links and roundtables to connect top links).

Comment: Need to involve plant physiologists to establish measurements for crop expression. Reinforce need to link those three areas together (helicopter view).

Comment: Thought it might be useful to break down deep rooting problems into biotic and abiotic stresses. Broke categories down even further.

B. Physiology Group
Did not follow guidelines directly.

Need to define who will define our activities.

Trying to establish value of traits in conventional breeding programs. Is it better to go from yield to traits or from traits to yields. As a group of physiologists, most said must go from yield to traits but that it must be defined in the target environments. Simulations could be used for some drought scenarios (ongoing).

Another activity—when genes or QTLs come out—physiologists will be asked to establish value in the target environments. With genes the tasks could be overwhelming. It will be very important to have large-scale screening techniques. Primarily applied activity.

Want to be applying molecular techniques to test value of traits.

**None of this can be done in isolation. To do work, we need to procure materials to test these hypotheses. So need to work with breeders and geneticists to get these materials.

Need to generate information for breeding programs.

Development of drought screening protocols

- Need to continue developing screening protocols for phenotypes for drought tolerance. Resources however are decreasing for field tests. There is a need for testing facilities and testing networks.
- Activity is to have some sense of quality control that is often confounded by soil acidity, etc.
- For phenotyping there is a need for definitions with rigor.
- Need for catalog protocols—need a new vocabulary to talk about drought screening

What do they do?
What do protocols do? Basic.

How to use them –Applied—This was not a useful concept for this group.

Comment: Because drought is so complex, after talking with breeders, we should define a simple target environment and start there. Breaking it down by commodities could be useful.

Comment: We're most likely to make the greatest leaps when we're working together with groups (helicopter reference).

C. Biotechnology Group
We felt the goal was to identify pertinent technologies that would be of value in bringing about drought improved crops and then set priorities for reaching that goal.

Criteria for priority setting were time, resources, impact of change, likelihood of success.

Found it particularly difficult to distinguish basic and applied because of rapid rate of change in the field. There are cases where we will be surprised how quickly technologies in one of these categories will go to another.

Highest priority was bioinformatics. Have information that is underutilized. Components include databases, databases with "Day 1 Curation," and linkages. All part of one suite. They have to be set up in a way that you can use them in terms of user-friendliness and in terms of turning data into information. This priority activity was both basic and applied.
This work is basically just beginning.

"Other highest priority." We saw this as a suite of activities—the synergy here is enormous. Linking people up across disciplines, across institutions, and across commodities.

Second-level priority was MAS. It's more on the applied end, but it will also tell us basic information. Deserves considerable resources. Marker development—currently microsatellites, with SNPs on the horizon. Efforts can be greatly accelerated using these technologies. We could have a full set of microsatellites in the short term. It hasn't been developed because of a lack of resources and "its not sexy."

Industry people have been thinking about this for many more years than we have. We don't do it in the public sector, but it still needs to be done.

Third-level is gene discovery through a suite of technologies—ESTs, high-throughput mapping, microarrays, mutagenesis (forward and reverse), proteomics, and physical maps. ESTs could be done in a few years time as could microarrays.

Another "third-level" priority is comparative genetics and comparative physiology and germplasm.

Fourth level is transgenics. Low priority because other people will be doing a lot regardless of public sector activities.

D. Clarification Comments
The physiology group said we need isogenics for further studies.

Isn't bioinformatics strongly covered by outside groups? What about implications of IPR?
Response 1: IPR was slightly discussed because the whole group needs to discuss it. Avoided it because of time constraints. The question about whether such databases already exist didn't come up. Personal experience is that there is not many linkages out there.
Response 2. Apparently there are a lot of difficulties in making data usable as information.
Response 3. Money allocated for databases, but I don't know of any truly curated database. People fill them up and that's about it. The ones that exist would be of no value to this group.
Response 4. We should try and learn something from the databases established in the private sector.

Would like to have more conversation about the freedom to operate, especially related to the activities cited by the biotech group and how it relates to what the public sector can achieve in the years to come.

Response 1. Databases in the public sector are underfunded and are used basically for the people who are filling them. Some efforts in USA and UK to link between crops. Industry has gone much further. But question of freedom to operate is key to all this. From experience in genomics on sharing information from companies, there are strings attached—he does not have complete freedom to operate. We should proceed as if the private sector doesn't even exist. We are the scientific community.

Response 2. The CG system has to take essentially the same course just described. Now getting advice on how to achieve freedom to operate, complicated by working in other parts of the world where the IPR questions are still in flux. Think its going to get resolved fairly quickly, but we shouldn't limit what we're going to do on that account. Transgenics have lower priority in his view because of IPR difficulties.

Response 3. The only way my relatively small institution can work because of freedom to operate issues is to be part of a network and partnership arrangements. The private sector serves as benchmark for progress. We could start identifying linkages to strengthen the public sector efforts.

Response 4. I think organizations concerned about international research are waking up to the fact that they will have to deal with IPR issues. Of relevance to this group is that were talking about putting together teams. And there certainly has to be a willingness to share information to be part of such teams. Discouraged by public sector universities that are not encouraging freedom to operate of late. Our own public institutions need to think about how they continue to be productive members of teams.

Attachment 5: Drought Improvement in Crops: Discussion by Commodities

(Wednesday, July 23, afternoon session)

Objective: To define and prioritize activities by commodities, considering applied versus basic science

I. Roundtable Discussion on Maize

A. Breeding
Applied
Priority 1: Deployment of proven techniques in a range of environments (e.g., ASI).
MAS—deploy where cost-effective.
Build up screening facilities and human resources.
Priority 2: Assess native genetic diversity (molecular, phenotypic, heterotic groups).
Better define target environments (biophysical).
Define features/patterns of G x E (germplasm performance data).
Priority 3: Establish testing network specific for drought (screening sites, partners, quality control).

Basic
Priority 1: QTL pyramiding via recurrent selection; explore new, cost-effective MAS schemes.
Priority 2: Assess new secondary traits.
Priority 3: Search for tolerance in wild relatives.
Explore relationships with nutrient deficiency/toxicity and other stresses.

B. Physiology
Applied
Priority 1: Establish and standardize useful protocols.
Quality control.
Priority 2: Training
Develop screening facilities.

Basic
Priority 1: Assess new secondary traits for selection:
-Transpiration efficiency.
-Canopy temperature (aerial photography).
-Flowering process.
-Root development.
-Ear shoot development.
Priority 2: Cell/molecular-level physiology (native and novel pathways—evaluate suggestions from biotech).
Priority 3: Explore linkages of nutrient deficiencies/soil toxicity to drought tolerance.
Comparative physiology.

C. Molecular Markers
Applied
Priority 1: Accessing and developing marker technology (e.g., SSR, SNP, Ab).
Priority 2: QTL detection in new segregating populations.
Capacity building (people and places).

Basic
Priority 1: Gene "hunting":
- Arrays
- Insert mutagens
- EST
- Promoters
- QTL definition
- Proteomics
Priority 2: Comparative genetics.
Cell-molecular level/proteomix physiology (novel pathways).

Explore opportunities for collaboration with private companies.

Breeders and physiologists need to work together to assess new secondary traits.

II. Roundtable Discussion on Rice

A. Breeding
Applied
Priority 1: Materials development and development of new isogenic lines.
Priority 2: Movement toward integrated molecular approaches.
Priority 3: Follow QTLs that already exist (deep roots, osmotic adjustment, etc.). This item also applied to basic research.

Basic
Priority 1: Germplasm enhancement, using molecular techniques to funnel new materials

B. Physiology
Applied
Priority 1: Get good screening techniques and facilities.
Standardize phenotyping and protocols (networking).

Physiology must be synchronous to breeding and molecular-level work.

Basic
Priority 2: Comparative mapping

C. Molecular Techniques

Applied
Priority 1: Develop/use microarrays and ESTs. They are currently available and can be used. But money is a constraint. Database consolidation and efforts to improve access and user-friendliness.
Priority 2: Explore comparative genetics (use rice as model), use high throughput marker generation, and develop more drought specific markers.

Basic
Priority 1. Generate populations of transgenic mutants with appropriate lines that should remain in the public domain; including stress promoters, transposon, etc.
Additional priority: Networking of existing resources by connecting breeding, physiology, and molecular techniques.

III. Roundtable Discussion on Wheat

A. Breeding (applied or basic not noted)
Improve empirical approach
1. Better characterization of environments.
   - Including confounding factors, e.g., Zn, Na+
   - Probe nurseries
   - Simulation modeling
2. Better selection environments, match target and selection environments.
   - Crop management—test materials under appropriate management
   - Irrigation strategies
   - Soil factors
3. Better use of global data.
   - Nurseries
   - Traits
   - Markers
   - ICIS
4. Expand existing genetic base, e.g., screening of:
   - Landraces
   - Cultivars
   - Diploids/tetraploids
   - Synthetics
5. Modeling breeding strategies to optimize breeding systems.
6. Fingerprinting.

B. Physiology
1. Identify yield-limiting traits in elite germplasm.
2. Find new sources of variation for yield limiting traits.
3. Develop appropriate populations to evaluate genetic gains of trait (also useful for markers).
4. Test integrative physiological tools and compare with MAS.

C. Molecular Markers
Genomic characterization.

Generate new markers: Expressed Sequence Tags (ESTs) to find out which genes are turned on under stress.

Gene mapping for known traits.

Transformation.

IV. Roundtable Discussion on Sorghum and Pearl Millet

A. Breeding
Applied
Priority 1: Evaluation of existing RIL populations in selected environment (pre-flowering drought tolerance, lodging resistance, charcoal rot, seed filling, stem reserve QTL). Group thinks there is a lot of material out there that could be useful.
Trait use/MAS:
- NILs (near isogenic lines), stay-green, P uptake, Al tolerance, seed filling)
- Elite breeding materials—particularly tropical materials
- Pyramiding traits—using MAS

Basic
Priority 2. Exploration of "new" traits
- Genetics/physiology
- RIL population improvement when data merits it

B. Physiology
Applied
Priority 1. Research tools for phenotyping.
- Network of evaluation environments
- Characterization work to relate network sites to broader characterization environments
- Evaluation protocols

Basic
Priority 2. Establishing the environmental value of traits and genes.
-RILs/NILs
-New traits/genes

C. Molecular Tools and Strategies
Applied
Priority 1: Need an integrated map.
- SSR maps that are saturated
- Anchor probe

Priority 2/3: (sorghum/millet) Informatics

Basic
Priority 2/3: (millet/sorghum) Gene discovery and fine mapping.
Priority 4: Selected transgenics particularly for sorghum.
- Citrate marker
- Anti-senescence gene

Additional consideration: Integration of NARS scientists.

V. Plenary Discussion

A. Maize

Note: Points are ordered according to priority
(** indicates a significantly higher priority)

Breeding

Applied

**1. Tropical maize: apply established techniques (conventional and molecular) in a range of environments. [links to physiology and biotech]
2. Assess genetic diversity (phenotype and fingerprinting) [links with biotech]
3. Establish lasting network specific for drought. (e.g., contract research)

Basic

**1. QTL pyramiding/new MAS strategies (recurrent selection). [links with biotech]
**2. Assessing new secondary traits. [links with physiology]
3. Search for tolerance traits in teosinte and *Tripsacum* (phenotype, genetic level)

Physiology

Applied

**1. Establish drought screening protocols.
**2. Build up screening facilities and human resources. [links with breeding]

Basic

**1. New secondary traits: Assess with breeders (Most promising for impact in 2-5 years: transpiration efficiency, canopy temp., flowering and ear shoot development, root development). [links with breeding]
2. Cell-molecular level physiology (novel pathways). [links with biotech]
3. Explore relationships with nutrient deficiencies/toxicity.

Molecular techniques

Applied

**1. Access to and development of marker technologies (SSR, SNP, etc.) (links with breeding).
2. QTL detection (links with breeding and physiology).
3. Capacity development (people and places).

Basic

**1. Gene hunting (assays, insertion mutagenics, EST, promoters, proteomics, QTL detection).
2. Comparative genetics.

B. Rice

Breeding

Highest priority was given to materials development and development of new isogenic lines.

Lower priority given to integrated molecular approaches.

Follow QTLs that exist (deep roots or osmotic adjustment).

Long term—Germplasm enhancement, using molecular techniques to "funnel" new materials.

Physiology

Highest priority given to two items:

1. Immediate—physiological pheneotyping (including testing, networking, and protocol establishment) AND physiology must go in synchrony with breeding and molecular approaches.

2. Long term—not clear.

Molecular techniques

Long term

Priority 1. Generate population of transgenic mutants with appropriate line that should remain in the public domain, including stress promoter, transposon.

Priority 2. Rice could serve as model for other cereals.

Short term

Priority 1. Use of microarrays and ESTs. They are currently available and can be used. But money is a constraint.

Priority 1. Database consolidation and merging of database.

Priority 2. Comparative genetics, and hi-thruput marker generation, and development of more drought specific markers.

Final conclusion and major priority: Networking of existing resources by connecting breeding, physiology, and molecular techniques.

Discussion

Comment: Doesn't seem to be much uniformity across the commodity groups.

Comment: For sorghum, millet: Fine mapping is only a part of gene discovery. Should be moved to the plant breeding portion. Also microarrays and ESTs.

Question: Maize group did not mention greater database and bioinformatics. Why?

Response1 : It was listed under molecular technologies as database mining, but did not receive a number of votes in the group.

Response 2: Data mining is only a part of bioinformatics, but it also comes under comparative physiology.

Thinks there is a lot of convergence among the groups.

Question about molecular characterization with wheat. What does it mean?.

Response. Genome characterization. Fingerprinting. Valuable with wheat because it's a pedigreed crop back to landraces so will help with phenotypic analysis with diversity investigations and gene characterization.

Comment: Useful to have two or three common themes to focus on. ESTs or possibly bioinformatics. Might work better than working on a commodity by commodity basis.

C. Wheat

Breeding

Immediate

1. Improve empirical approach.
   - Characterize drought-stressed environments.
   - As a result, develop better selection environments (will not cover all stress situations). Resources/specialists are needed.
   - Consider crop management strategies in those environments, test germplasm there.
   - Irrigation strategies need further development.

Environmental factors tend to confound results.

2. Better use of available information.
   - International nursery data
   - Markers
   - Traits literature
   - International Crop Information System (ICIS)

3. Expanding existing genetic base (genetic diversity spectrum).
   - Landraces
   - Cultivars
   - Aliens
   - Synthetics

Fingerprinting (getting a handle of degree of diversity to use material to greatest effect).

Physiology

Modeling breeding strategies (recurrent selection, Q-gene software, others)

1. Identify yield-limiting traits in elite germplasm (information used to produce new germplasm or eliminate yield-limiting traits)
2. New sources of yield enhancing traits under drought (develop RILs, etc, and characterize). The fact that this might be done in one or two populations could limit the usefulness.
3. Develop appropriate populations to evaluate genetic gains of traits.
4. Evaluate/integrate physiological selection tools and MAS.

Molecular Markers

1. Molecular characterization of elite material.
2. Generate new markers to improve ability to characterize
   - ESTs
   - What genes are turned on under stress?
3. Mapping for known traits.
4. Transformation.

D. Sorghum and Pearl Millet (level of priority indicated by number)

Breeding

#1. Evaluation of existing RIL populations in selected environments (pre-flowering drought tolerance, lodging resistance, charcoal rot, seed filling, stem reserve QTL). Think there is a lot of material out there that could be useful.

#1. Trait use/MAS
   - NILs (near isogenic lines) (stay-green, P uptake, Al tolerance, seed filling).
   - Elite breeding materials—particularly tropical materials.
   - Pyramiding traits—using MAS.

2. Exploration of "new" traits
   - Genetics/physiology
   - RIL population improvement when data merits it.

Physiology

#1. Research tools for phenotyping.
   - Network of evaluation environments
   - Characterization work to relate network sites to broader characterization environments.
   - Evaluation protocols

#2. Establishing the environmental value of traits and genes.
   - RILs/NILs
   - New traits/genes

Molecular Tools and Strategies

#1. Need an integrated map
   - SSR maps that are saturated
   - Anchor probe

#2/3. Informatics

#2/3. Gene /discovery/ Fine mapping

#4. Selected transgenics particularly for sorghum
   - Citrate marker
   - Anti-senescence gene

Additional consideration: integration of NARS scientists.

Comment: Important to evaluate sorghum hybrids under stress. Consensus is that it is not an issue.

Attachment 6: Drought Improvement in Crops: Discussion by Commodities

(Thursday, July 24, morning session)

Objective: To give a time frame to the different activities identified at the Wednesday session.

The group defined "short term" to mean that results would be available in 3–5 years, while "long term" results may take 10–15 years or longer

I. Plenary Discussion

A. Maize

Short Term

1. Deploy QTL/MAS in elite tropical maize germplasm in connection with conventional techniques (breeding).
2. Environment characterization.
3. ASI seems to be working and important in molecular germplasm—find out what are its mechanisms and explore it more physiologically.
4. High throughput marker systems.
5. Continue to create appropriate segregating populations to allow many QTLs to be characterized at physical and molecular level for priority traits.
6. Assess genetic diversity (conventional and molecular approach).

Long Term

1. Comparative genetics and physiology.
   - What does maize have and what does it lack in terms of the genome?
   - New and better genes and pathways
2. Assess new secondary traits.
3. Run correlations with other stresses.

ST and LT impact on release of new cultivars of ongoing breeding programs—faster gains, larger gains.

B. Rice

Short Term

1. QTL for drought tolerance and connecting the identified/mapped QTLs to known secondary traits.
2. Defining and setting-up drought environments to conduct field trials in different target environments and develop appropriate breeding strategies based on what is known about the genetics of drought tolerance in rice.
3. Simultaneous enhancement/development of molecular technologies and materials. These include ESTs/arrays for drought tolerance and also defined mutants.
4. Simultaneously develop additional phenotypes related to drought tolerance. Example—sterility, epidermal structure/composition, roots.
5. Identify QTLs, add new markers, and develop NILs.
6. The use of methods and protocols for drought tolerance are dependent on the development methods that approach the problem as a cross-commodity endeavor.
7. High throughput marker systems can be immediately taken up to accelerate the mapping program.
8. Data accessing/contribution/sharing activity—the bioinformatics platform requires improved methods, formats, assured curation, weighted information to make it easier for the breeder to get the data/information in a discernible or useful manner.

Long Term

1. Cloning (drought tolerant QTLs), candidate genes for major QTLs.
2. Develop mutant lines in the background of drought tolerant rice lines.
3. Comparative mapping.
4. Enhancement of germplasm for QTLs, molecular information QTL pyramiding.
5. Development of gene expression system using genetic maps.

C. Wheat

Short Term / Long Term

Five groups of activities that need to run in parallel

1. Information use.
   - ***Get ICIS running
   - Link Qu-gene to this also.

2. Environmental characterization (define selection environments).
   - International nursery analysis
   - Define selection environments
   - Target population environments (GRDC project)
   - Characterization of Mexican and international test sites

3. Expanding genetic base (must have appropriate genetic material to make gains in dry environments).
   - Ongoing experiment
   - Screening of sources of variation particularly within landraces
   - Genetic characterization of gene pool

4. Trait identification, identication and validation of the yield-limiting traits, mapping and MAS.
   - Screening of parental material
   - Development of appropriate populations
   - Conduct genetic analysis of each of these traits
   - Develop most appropriate MAS strategies

   Traits assigned highest priority
   - Root system
   - Osmotic adjustment and water relations traits
   - Establishment and early vigor
   - Transpiration efficiency
   - Cabodydrate storage and remobilization
   - Spike photosynthesis

   (investigate recurrent selection)

5. Gene identification and gene discovery.
   - Larger library of ESTs
   - Gene expression assays
   - Transformation

D. Sorghum and Pearl Millet

Short Term

1. Produce an integrated map with anchor probes.
2. More extensive evaluation of existing mapping populations.
3. Establish information network and testing network at global scale (primary and secondary sites). Good way to link to NARS scientists.
4. Use of traits that we have identified—stay-green—several. Developing backcross populations to make it more useful in tropical backgrounds for sorghum breeders.

Long Term

1. Gene discovery activity. Look at existing QTLs and identify genes associated with them and dissect those traits.
2. Look at new traits and genes in terms of their field value and their molecular characterization. A lot of untapped germplasm.
3. Informatics, transgenics, (citrate marker).

Attachment 7

This table was developed by the organizers of the workshop as a rough summary of what was discussed during the breakout sessions by commodity and activities. This document is not to be considered to be an **exhaustive document**. However, the table does provide a simple method to identify the common approaches identified across the different commodities, with some activities characteristic to a given crop. When reported by a group, the number after an activity indicates the level of priority (1 being highest priority), and the letter refers to short (S) or long (L) term.

Maize	Rice	Wheat	Sorghum/Pearl Millet
Bioinformatics Link and fill existing databases Maize DB, private sector	**Bioinformatics** Data access/sharing (1/S)	**Bioinformatics** Data access/sharing (1/S) ICIS/Qu-Gene	**Bioinformatics** Data access/sharing (2/S)
Environment characterization GxE analysis, GIS (2/S)	**Environment characterization**	**Environment characterization** Establish screening protocol based on the environment	**Environment characterization**
Deployment of proven techniques in a range of environments (e.g., screening for ASI) (1/S)	**Deployment of proven techniques in a range of environments** (1/S)	**Deployment of proven techniques in a range of environments** (1/S)	**Deployment of proven techniques in a range of environments** (2/S)
Establish/standardize drought screening protocols (1/S)	**Establish/standardize drought screening protocols** (1/S)	**Establish/standardize drought screening protocols** (1/S)	**Establish/standardize drought screening protocols** (1/S)
Assess new second traits (1/L) Grain yield, stay-green root structure, transpiration efficiency CTD, flowering grain abortion	**Assess new second traits** S Sterility, root structure Epidermal structures	**Assess new second traits** 2/S Roots, OA, early vigor, CTD Transpiration efficiency Carbohydrate storage/remob.	**Assess new second traits** 2/L RIL/NIL population
Establish lasting network specific for drought (3/S) Screening testing sites Germplasm exchange Private sector collaboration	**Establish lasting network specific for drought** (1/S)	**Establish lasting network specific for drought** (1/S) Inter-nursery (S) Characterization of environment.	**Establish lasting network specific for drought** (1/S) Selected environment
Access to and development of DNA markers (1/S) SSR/STS/SNP	**Access to and development of DNA markers** (1/S) SSR/STS/SNP	**Access to and development of DNA markers** (1/S) SSR/STS/SNP	**Access to and development of DNA markers** (1/S) SSR/STS/SNP
QTL detection (2/S) New secondary traits Segregating population (F2:3/RILs/NILs)	**QTL detection** S New secondary traits Segregating populations (RILs/NILs)	**QTL detection** (3/S) New secondary traits Segregating population (RILs/NILs)	**QTL detection** (1/S) New secondary traits Segregating population (RILs/NILs)
Gene discovery (1/L) High throughput marker sys (S) Microarray/gene expression (L) (gene machine) ESTs (1/S) Transgenics Physiol pathways (L)	**Gene discovery** (1/L) High throughput marker sys S Microarray/gene expression L ESTs (S) Transgenics Candidate gene Mutagenesis pop. (e.g., NILs)	**Gene discovery** (1/L) High throughput marker sys (2) Microarray/gene expression (2) ESTs (2/S) Transgenics (4)	**Gene discovery** (2/L) Fine mapping Microarray/gene expression ESTs Transgenics (3/L) (Citrate marker, anti-senescence gene)
MAS strategies (1/S) Ongoing experiment New strategies	**MAS strategies** (1/L) Germplasm enhancement Integration with breed/physiol.	**MAS strategies**	
QTL pyramiding (1/S) (e.g., ASI)	**QTL pyramiding** (3/L) Deep roots, OA		**QTL pyramiding** (1/S) Stay green, P uptake Al tolerance, seed filling
Assess genetic diversity (2/S) Explore exotic germplasm and species (genebank) Fingerprinting Survey for gene expression		**Assess genetic diversity** (2/S) Synthetics/cultivars Landraces/gene-pool Fingerprinting (1/S)	**Assess genetic diversity** Fingerprinting
Comparative genetics (2/L) Cell-molecular level/proteomics physiology, novel pathways (2/L)	**Comparative genetics** (2/L)		
Build-up screening facilities and human resources (1/S) Linkage with Conventional breeding and MAS)		**Build-up screening facilities and human resources** (S)	
Other Explore relationships with nutrient def./toxicity and other stresses (3/L)		**Other** Modeling breeding strategies	**Other** Select for secondary traits (1/S) Lodging, charcoal rot, seed filling, stay-green, stem reserve

Attachment 8:

Glossary of Acronyms and Terms

ABA: Abscisic Acid; hormone.

ABC: Applied Biotechnology Center, at CIMMYT.

ASI: Anthesis Silking Interval; the asynchrony between silk emergency and pollen shading at flowering stage in maize.

BSA: Bulk Segregant Analysis; molecular marker technique that identifies polymorphisms between the bulked DNAs of two segregating progeny groups, where each group contains individuals that share a particular characteristic. This strategy is useful in identifying and mapping genes that control simple inherited traits.

cDNA: A single stranded DNA molecule produced from (and complementary to) an RNA template.

BACs: Bacterial Artificial Chromosomes; used for cloning large DNA sequences (hundreds of kb).

Candidate gene: Gene that, on the basis of prior physiological, genetic or biochemical characterization, we suspect may be contributing to a quantitative trait.

Candidate gene approach: Search for candidate genes and implementation of any genetic, molecular, or physiological techniques to validate the candidates.

CGIAR: The Consultative Group on International Agricultural Research.

CTD: Canopy Temperature Depression; physiological parameter.

DNA chip: DNA chip technology provides efficient access to genetic information using miniaturized, high-density arrays of oligonucleotide probes. A set of oligonucleotides is defined, synthesized, immobilized on silica wafers or chips to construct a high-density array; each probe having a predefined position in the array. Labeled (fluorescence) nucleic acids from the analyzed sample are hybridized on the array, and hybridization intensities are detected by a scanner that reports quantitative assessment of RNA level in the sample for each gene represented in the array.

EST: Expressed Sequence Tag; a sequenced cDNA that can be used as a marker for genetic mapping.

Gene machine: Slang term for a reverse genetic technology that allows the investigator to identify an insertional mutation (due to a transposable element or Agrobacterium T-DNA) in any gene of interest. Hence, the investigator can determine the phenotype, if any, of an inactivational mutation in any candidate gene in the organism.

Genomics: In a narrow sense, genomics refers to the study of genome composition, structure and function, which can be classified into classical genomics (crossover-based), physical genomics (DNA sequence-based) and genome informatics. However, most researchers using genomics do not investigate the question of genome structure/function/evolution, but rather use this technology to efficiently pursue questions in development, pathology, cell biology, physiology, etc. The terms genomics, structural genomics, and functional genomics are widely used, but have many different interpretations (for review see Hieter and Boguski 1997, Science 278, 601-02).

GxE: Genotype by Environment interaction.

GIS: Geographic Information System.

ICIS: International Crop Information System.

IP: Intellectual Propriety.

IPR: Intellectual Propriety Right.

MaizeDB: Maize Data Base (University of Missouri, Columbia, Missouri).

Map-based cloning: Isolation of a gene based on knowledge of its location on a genetic map. The first step of this approach is to identify DNA markers tightly linked to a gene of interest, and then to "walk" to the gene via overlapping clones (e.g., cosmids, BACs or YACs). Also called positional cloning.

MAS: Marker-Assisted Selection; genetic selection through DNA markers in segregating population to trace, and/or pyramid, favorable allele at target loci in a given genome.

Microarray: Similar approach to the DNA chip, except that microarrays use cDNAs (EST clone inserts, for instance), and not oligonucleotides, and are immobilized on glass. Quicker and cheaper when compared to DNA chips, but less precise.

NARS: National Agricultural Research System.

NIILs: Near Isogenic Introgression Lines. See NILs.

NILs: Near Isogenic Lines; they are generated by a process of repeated backcrossing into a recurrent parent, with selection for the desired character at each round of crossing.

OA: Osmotic Adjustment; physiological process of accumulation of solute molecules inside the cells in response to a decline in external water potential. This adjustment may postpone and contribute to lessen tissue death after desiccation by maintaining cell turgor pressure.

Proteome: Protein complement expressed by a genome.

Proteomics: Study of the proteome; technically and conceptually similar to functional genomics, but with the aim of studying biological aspects of all proteins at once in a systematic manner.

QTL: Quantitative Trait Loci; gene(s) controlling quantitative traits.

RILs: Recombinant Inbred Lines; population of lines brought toward homozygosity through several cycles of self-pollination and single seed descent from an original F2 population.

SNP: Single Nucleotide Polymorphism. Most precise DNA marker technology, with excellent automation potential, but requires a great deal of additional genome characterization and technology optimization before it can be routinely applied to any plant species.

SSR: Simple Sequence Repeat; repetitive DNA with repeats ranging in size from 1 to 6 bp; it is also referred to as a microsatellite.

YACs: Yeast Artificial Chromosomes; like BACs but for much larger DNA fragment.

Contributed Research Papers

Towards Standard Assays of Drought Resistance in Crop Plants

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Summary

Recent interest in the genetic improvement of crop drought resistance by conventional breeding and molecular techniques underscores the urgent need for standard assays of drought resistance. The lack of such standards is becoming a major obstruction to the proper assessment of genetic modifications towards drought resistance. This presentation offers a conceptual framework for defining and developing a standard testing system for drought resistance. It is an invitation to pursue a pragmatic discussion towards the creation of standards. The main postulate put forward is that the test of drought resistance must be performed with whole plants and/or plant communities, even if compelling evidence for the prevalence of high resistance can be derived or implied from data at lower levels of plant organization.

Drought resistance is attained within three major physiological domains: (a) the maintenance of a high (favorable) plant water status during stress; (b) the maintenance of plant function at low (unfavorable) plant water status, and (c) the recovery of plant water status and plant function after stress. Possible tests are discussed for each domain, in terms of principles, problems and possible solutions, but not in terms of the final protocols. The integrated response to drought stress in terms of plant production must be tested in the field. An outline of the available field-tests for assessing plant production under drought stress is summarized.

It is concluded that any claim for a genetic modification of stress resistance that is presumed to impact crop performance in agriculture will remain on paper unless proven with whole-plant testing systems and under field conditions. It is our responsibility to agree upon a standard testing system to serve this purpose

The Problem

Crop drought resistance is a major factor in the stabilization of crop performance in drought prone environments. Drought resistance is now considered by both breeders and molecular biologists to be a valid breeding target. Consequently, the proper evaluation of genetic modifications towards improved drought resistance is becoming an acute issue. There is a serious lack of concept, direction, and protocol for measuring drought resistance. The methodological issue becomes an obstacle in applying molecular work to the genetic improvement of drought resistance, whereas the measurement of drought resistance in plant molecular work is often unclear or insufficient with respect to the practical significance of the results. The seriousness of the problem is reflected in the publication of letters in scientific journals calling for proper measurement and interpretation of drought resistance in molecular work (e.g., Blum et al. 1996; Gaff 1966).

There is therefore a need for a standard system of testing stress tolerance in general, and drought resistance in particular, to the same extent that we have standard protocols for assaying plant disease resistance or any other selected trait in plant breeding. The designation of standard tests should provide the necessary yardstick by which molecular geneticists, plant physiologists, and plant breeders can scale their work.

However, while we have suitable methods for measuring plant water relations on one-hand and plant physiological functions on the other, we do not have a comprehensive standard system for measuring drought resistance. This is mainly because drought resistance and its

impact on plant production under stress involve interactions between plant water relations and plant physiological functions. These interactions and their impact on crop yield are open to debate and misinterpretation. This is exemplified in the debate over water-use efficiency and carbon isotope discrimination as indices of plant production under drought stress and as selection criteria for drought resistance (e.g., Hall et al., 1994 and numerous recent publications).

The intricate interactions of plant function with its internal water status are at the root of the complex relationship between the plant and its environment. Partly because of this interaction plant response to drought stress varies with the rate of stress development, the duration of stress, and plant age when stress develops.

Henceforth, methods for measurement of drought resistance must account for such interactions and resolve the order of events, by which drought resistance can be defined and measured. A reasonable order can be achieved in this domain if one considers all the important literature on the subject, beginning even with the early treaties of Ashton (1948) and Levitt (1972). The discussion presented here is therefore an attempt to establish a stepping stone towards the definition and the formulation of standard assays of drought resistance for use in practical applications in agriculture. This is not a final thesis but an invitation to pursue a pragmatic discussion towards the construction of a standard testing system.

Drought Resistance

Drought resistance is addressed here in terms of plant water relations and plant function as the plant desiccates. It does not address issues related to plant developmental plasticity, plant phenology or various constitutive traits which affect plant performance under stress (e.g., Blum 1996). These are relatively simpler traits and methods for their measurements are fairly known and acceptable.

The main postulate I presently put forward is that the test of drought resistance must be performed with whole plants and/or plant communities, even if compelling evidence for the prevalence of high resistance can be derived or implied from data at tissue or cellular levels. This is a necessary link at least between science and application in agriculture.

A proposal of detailed test protocols is avoided here in preference for outlining some of the major principles involved in designating such tests. When the principles are acceptable, then the design of the specific test protocols should become simplified. At that stage, further considerations should be given to the technical, economical, and logistical aspects of the test protocols.

A review of the large volume of published information on genetic variations in drought resistance within crop species shows that genetic variations can exit within three major domains:

1. Maintenance of high plant water status and the delay of the typical symptoms of water deficit such as wilting. Maintenance of plant water status is taken here in its wide context where cells and plant tissues retain hydration by various mechanisms.
2. Maintenance of plant function at low plant water status (*syn.* 'drought tolerance'; Levitt 1972).
3. Recovery of hydration and function from very low plant-water status (near-lethal desiccation), a capacity that is often associated with or referred to as 'survival.'

There are several general considerations common to all possible assays of drought resistance for all three domains.

Plant adaptation to drought stress requires time. This has been amply demonstrated for both drought (Jones and Rawson 1979; Babu et al. 1999) and salinity (Munns et al. 1995) stress. It is well established that a very short (and fast) drying cycle, in the range of few days does not allow the full adaptive potential of the plant to be expressed. Protocols for assaying maintenance of plant water status must be defined in terms of the minimum time duration under given drought stress conditions. Plant dehydration rate and the response to dehydration vary with plant developmental stage, as a function of tissue age and plant size. Some of these aspects are further touched upon below.

Many of the reports attempting to characterize drought resistance very often do not discern among the three domains stated above. For example, photosynthesis (P) is often justly advocated as a measure of plant response to drought stress and drought resistance. P changes with leaf water status. When P is measured across genotypes on a

given day during a drying cycle, it may simply represent plant water status in each genotype on that day so that genotypic differences in P may simply reflect genotypic differences in plant water status. In such a case, a difference in P among genotypes does not represent any information on P and its tolerance to leaf desiccation but rather it represents the genotypic variations in the capacity to sustain leaf water status under drought stress. In such a case plant water status is the proper (and sometimes a simpler) assay of drought resistance. This distinction is not only a question of formality. It bears upon any further interpretation and research into the findings represented by the test. An assumption that a given genotype is resistant in terms of P, for example, may lead to a decision to investigate P tolerance to desiccation in this genotype. However, this genotype may express superior P function under drought stress because of a capacity to maintain leaf water status — which has nothing to do with P function at low leaf water status.

The conclusion underlined here is that the measurement of plant function should be clearly distinguished from the measurement of plant water status in assessing drought resistance. If plant function is evaluated as a possible explanation of drought resistance, it must be measured in all genotypes at a given plant water status.

Plant Water Status

Plant water status is estimated by several major variables such as water potential (WP), turgor potential (TP), and relative water content (RWC). Each variable carries its own merit in terms of analyzing plant water relations. Following previous arguments (Sinclair and Ludlow 1985) which are augmented by subsequent studies and personal experiences, RWC is taken here as the best integrated measure of plant water status, which represents also variations in WP, TP, and osmotic adjustment (OA). A simple case for the advantage in RWC over WP for assessing genetic differences in drought resistance is, of course, the common observation that while having the same WP, genotypes may vary in their RWC due to a respective difference in OA. The choice of RWC as the best representation of plant water status in terms of genetic variation is also supported by founded genetic association between RWC and plant production under drought stress (e.g., Tehara et al. 1990; Rodriguezmaribona et al. 1992; Blum et al. 1998).

In the field a favorable plant water status, as expressed in high RWC can be maintained via three mechanisms:

1. The capability to sustain high WP by deep soil moisture extraction.
2. The capacity for osmotic adjustment (OA), which allows maintaining RWC and TP to lower WP.
3. Stomata closure in response to leaf desiccation and/or a transported hormonal signal produced in the root in response to root desiccation (e.g., Davies et al. 1994).

An additional factor affecting tissue water status is the change in tissue extension capacity as a result of hardening of the expanding cell walls when cells dehydrate (e.g., Neumann 1995). Cell wall hardening increases the capacity of expanding cells to maintain TP at given RWC, at the cost of limiting cell expansion. Cell wall hardening is estimated by the tissue bulk modulus of elasticity, which can be derived from the rate of change in TP relative to the rate of change in RWC. The role of cell wall hardening in sustaining TP has been well demonstrated for seedling axial extension in an artificial test environment (e.g., Chazen and Neumann 1994). Indirect evidence for the possible importance of cell wall mechanics on crop drought resistance has been presented (Sanchez et al. 1998). However, its importance in crop drought resistance relative to other components of the plant water status in the field remains to be quantified (e.g., White et al. 1992).

Variations among genetic materials in observed RWC could also be derived from variations in plant size. The rate of leaf canopy development and LAI in the field can affect plant water status when soil moisture is limited. Larger plants use more water than smaller plants and after a given time under stress the former is likely to express relatively lower RWC. The effect of plant size on phenotypic variations in RWC is amplified and accentuated when the different genetic materials are grown in pots with restricted root volume. A recent example concerning the evaluation of certain genetic transformations on tobacco drought resistance can be referenced in Serrano et al. (1999).

A suitable assay of plant water status as an explanation of drought resistance must account for the source of variation in RWC among genotypes, being it root function, OA, stomata closure, or simply plant size.

Possible tests for the maintenance of plant water status in terms of RWC

- Test for assessing root capacity for deep soil moisture extraction.
- Test for estimating OA capacity.
- Test for assessing stomatal closure as a water-saving mechanism.
- An integrated test for plant maintenance of RWC at given root medium water status.

Plant Function

Plant tolerance in terms of physiological function at low RWC is a rare occurrence. This conclusion stands especially if one discounts all the reports on genetic differences in physiological function in which genotypes were not shown to be at the same plant water status when measured for function under stress.

Following the analysis of Turner (1986), OA is sometimes being regarded as a component of drought (dehydration) tolerance, However, OA is a dehydration-responsive plant function which ascribes dehydration avoidance as it helps to sustain higher RWC (relative *water content*) at a given LWP.

Plant processes depend on plant water status and there is a general relationship between the rate of the process and tissue RWC, TP, or WP. Only if this relationship is unique to the tested genotype can we conclude that plant function is a genetic component of drought resistance. An ominous example for an oversight of this fact can be seen in the attempt to outline molecular markers for OA in barley (Teulat et al. 1998). In that study an effort was made to standardize all measurements of OA by holding pot soil moisture content constant (at 14% of 'field capacity') for all genotypes. Whether soil moisture was indeed constant or not is irrelevant because RWC at the time when OA was measured varied greatly among genotypes, from about 70% to 90%. Since OA changes plant water status, OA data collected in that study did not represent the genetic variation in OA capacity, but rather the differences in plant water status (RWC) among genotypes at the time of measurement.

Plant function at a given plant water status may be affected by the history of stress, most prominently by the time taken to reach the water status at which measurements are performed. Time is needed for what is defined as 'hardening,' which involves osmotic adjustment, various biochemical modifications, and cellular membrane and cell wall transitions. Undoubtedly, photosynthesis at a RWC of 70%, for example, is expected to differ if a plant reaches this point in 3 days or in 3 weeks. A test protocol must take these effects into consideration and the simplest way to achieve this is by "saturating" the requirement for hardening. Hardening is a finite process and after sufficient time under stress, it may be assumed to have been maximized, at least for the purpose of resolving large genetic differences. Hence, for OA in rice or wheat, 3 to 4 weeks of drought stress to reach a leaf RWC of 65-70% was found to be sufficient for expressing the full adaptive response of plants. Munns et al. (1995) demonstrated a generally similar period of time for the proper expression of genotypic variation for salinity tolerance in wheat.

Possible tests for measuring plant function at given RWC:

- Plant or organ growth (by weight or extension) at given span of RWC should represent an integration of many functions under stress.
- Chlorophyll fluorescence and/or chlorophyll loss at given RWC should represent a function central to mass accumulation capacity under stress.
- Since cellular membrane stability under heat shock (CMS) has been shown to have a remarkable association with yield under heat stress (Saadalla et al. 1990; Reynolds et al. 1998; Fokar et al. 1998), an assay of CMS at given level of leaf desiccation may constitute a potential assay of function at the cellular level.

Because plant functions depend on various environmental conditions aside from water supply, such tests should be performed mainly in controlled environments.

Plant Recovery

The capacity for plant recovery, which is often referred to as 'survival,' is a very common phenomenon in the plant kingdom, especially in native vegetation. It culminates in "resurrection plants," which present the highest capacity in this respect. It is often heard that survival is not an important trait in crop production. This may be generally true in developed agriculture. However, in subsistence farming in developing countries the capacity for plant survival may at times translate into human survival.

Plant recovery from desiccation in agricultural crops is primarily a function of the capacity for

maintaining RWC during desiccation. This is exemplified in tall fescue (Huang et al. 1998) in which both plant function during stress and its recovery after stress were related to the difference in RWC between the resistant and susceptible genotypes. Another example for tef (*Eragrostis tef*) cultivars is presented in Fig.1. Therefore, survival will be promoted by any factor supporting the maintenance of RWC during stress, most notably OA (e.g., Volaire and Thomas 1995). The results of assays for the maintenance of RWC during stress (above) also predict the potential capacity of the tested genotypes for survival and recovery.

Abscisic acid (ABA) may have a role in affecting plant recovery from drought stress and that role can be mediated by its effect on the maintenance of RWC, as can be seen in Table 1.

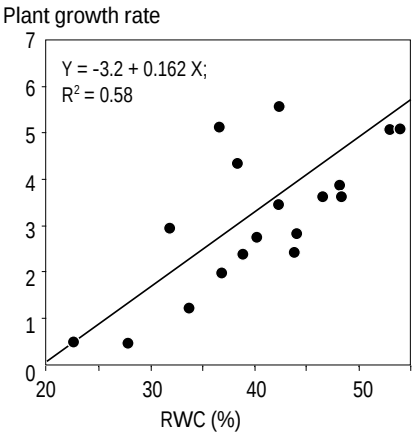

Figure 1. The regression of plant growth rate upon recovery from drought stress on plant relative water content (RWC) at peak stress across 20 cultivars of tef (*Eragrostis tef*). (25-day old plants were stressed until all leaf laminae were killed, when the remaining plant tissues RWC were measured. Irrigation was then applied. Growth was estimated by the increase in total shoot dry weight 10 days after irrigation).

Recovery capacity, which is independent of the maintenance of plant water status, has rarely been explored in a critical fashion. A test of proof in this respect must evaluate the recovery of different genotypes when all are desiccated to the same RWC.

Recovery after rehydration can be assessed by visual scores of recovered plant number or recovered leaf area (e.g., Babu et al. 1999). In the extreme case, plants of the susceptible genotype die while those of the resistant one recover (e.g., Table 1).

Field Tests

The ultimate test of 'value' of a genetic modification toward crop drought resistance is a field trial performed in the relevant agroecosystem. For most cases, this is an intricate requirement. The problem lies in achieving proper control over the field stress environment in order to assure the relevant test drought profile. Installations such as rain-exclusion shelters were developed in order to overcome the problem and they may serve well for small area tests. For larger area tests, a reasonable solution is to develop a test site in an arid environment where any water regime can be simulated by applied irrigation in the absence or near-absence of effective rainfall.

The common test criterion is yield when yield under stress is the target of the breeding program. Yield under stress may be affected by the genetic makeup of yield potential and by specific genes affecting drought resistance. In order to elucidate the phenotypic effect of a specific genetic modification towards stress resistance, the field test must separate between the effect of this modification from the impact of the yield potential of the given genotype on yield under stress. Estimating drought resistance in terms of the yield difference between potential and stress growing conditions can isolate the two effects. Ideally this may be expressed by the crossover interaction between the tested genotypes and the environment (nonstress and stress) (Blum 1993). The crossover interaction can be recognized if the compared genotypes are tested over a range of stress levels or at least under nonstress and sufficiently severe stress conditions. For example, in

Table 1. The effect of exogenous abscisic acid (ABA) on the relative water content (RWC) of wheat seedlings and their recovery from severe drought stress. Seedlings were grown in vermiculite. Daily irrigation was terminated at 5 days after emergence (DAE), when the last irrigation water contained 1 μmol ABA, as compared with plain water in the controls. Irrigation with plain water of all plants was resumed at 19 DAE.

		RWC (%)		Recovery assessment at 7 days after re-watering (26 DAE)
Treatment	Cultivar	At the onset of stress (6 DAE)	At peak stress (19 DAE)	
ABA	K1056	94.1	91.5	Plants alive
ABA	Sunstar	97.0	91.6	Plants alive
ABA	Barkaee	92.6	93.4	Plants alive
ABA	Sundor	94.0	92.6	Plants alive
Control	K1056	94.3	61.7	Plants dead
Control	Sunstar	96.6	47.3	Plants dead
Control	Barkaee	92.9	61.5	Plants dead
Control	Sundor	95.7	35.3	Plants dead

Mediterranean wheat and barley, a sufficient stress level for this purpose is expressed by a yield level that must be reduced to about a third of that in the nonstress controls (Blum and Pnuel 1990; Ceccarelli and Grando 1991).

Two major test systems are warranted and used for this purpose.

1. The line source irrigation system, which allows us to test materials over a gradient of drought (e.g., Mahalakshmi et al. 1990) or salinity (Isla et al., 1997) conditions. Here the analysis of the data allows comparing the response curves of the tested genotypes to a full range of stress conditions.
2. An orthogonal comparison of the tested genotypes between nonstress and appropriate stress conditions or over a range of stress conditions. Such a test can be developed at one site by using irrigation to control stress (e.g., Mahalakshmi et al., 1990), or it can be performed by testing materials over different locations that differ mainly in their water regime. Here, data can be analyzed by the appropriate statistical procedure (ANOVA or linear regression), provided that a sufficient level of stress (see above) has been achieved in at least one test. This analysis can be supplemented by deriving a 'stress susceptibility index' (Fischer and Maurer, 1978) or by normalizing cultivar performance under drought stress for genetic variation in yield potential and phenology (Bidinger et al., 1982). The value of these yield tests for deriving an estimate of drought resistance in terms of yield depends on the extent to which the main variable causing yield variations among tests is indeed the water regime.

Final Paragraph

After all that has been said and written it must be clear that any claim for a genetic modification of stress resistance that is to impact crop performance in agriculture or forestry will remain on paper unless proven with whole-plant testing systems and under field conditions. It is our responsibility to agree upon a standard testing system to serve this purpose. This presentation serves only to promote further discussion and work towards this goal.

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Molecular Dissection of Drought Resistance in Crop Plants: from Traits to Genes

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Summary

Breeding for drought tolerance is a challenging task because of the complexity of drought responses, environmental factors, and their interactions. Conventional breeding approaches have been successful, but progress has been slow. Recent advances in genome mapping and functional genomic technologies provide new powerful tools for the genetic dissection of drought tolerance components. It is anticipated that molecular genetic research will provide high-throughput DNA marker systems for marker-assisted selection that will be more efficient and effective in combing out favorable drought tolerance traits in breeding programs. It will also lead to a better understanding of the molecular basis of the genes underlying drought tolerance, which can be used in a genetic engineering program for drought tolerance improvement.

Introduction

Drought is a major production constraint, reducing crop yields in water-limited areas where many of the world's poorest farmers live. Development of drought tolerant crops will enhance food production and the livelihoods of farmers in these areas. Moreover, as the world population continues to grow and water resources for crop production decline, development of drought tolerant cultivars and water-use efficient crops is a global concern.

Breeding for drought tolerance has produced improved cultivars for drought-prone environments, but progress has been slow due to the complex physiological responses to drought, various environmental factors, and their interactions. The objective of this paper is to provide an overview of molecular approaches that could be used to tag and dissect the genetic basis of drought tolerance traits in plants.

Conventional and Molecular Breeding Approaches

Drought resistance is a complex phenomenon involving drought escape, drought (dehydration) avoidance and drought (dehydration) tolerance, and desiccation tolerance mechanisms (Blum 1988; Zhang et al. 1999; and Blum in this volume). Drought resistance can be defined based on the relative yield or survival of a genotype, compared with other genotypes subjected to the same drought, and where drought escape is not a major factor (Hall 1993). This definition of drought resistance involves genotypic comparisons and is, therefore, useful in the context of plant breeding in which plant productivity is a primary aim. Conventional breeding methods have depended mainly on plant performance such as yield or secondary traits highly associated with yield (e.g., anthesis-silking interval in maize or stay green in sorghum) under stress environments as a selection criterion. This approach has produced crop cultivars with improved adaptation and performance under stress, but progress has been slow on genotype x environmental interactions because of year-to-year variations in the timing and intensity of drought stress in field breeding nurseries. Molecular mapping and genomics approaches offer new opportunities and strategies to dissect major genes and quantitative trait loci (QTL) underlying drought tolerance. New molecular tools are available that can be integrated with conventional breeding and physiology to accelerate a basic understanding of drought tolerance in plants and the development of drought tolerant crops, as illustrated in Figure 1.

Molecular biology of drought stress responses

Plants respond to drought stress at the molecular and cellular levels as well as at the physiological level. Exposure of almost any plant to dehydration stress causes increased expression of a variety of functional and regulatory proteins (Klueva et al. 1998; Yamaguchi-Shinozaki and Shinozaki 1999). These highly conserved inducible responses among plants represent a fundamental response to the disruption of cellular homeostasis caused by water potential extremes. Molecular analysis of the signal transduction pathways reveals a connection between changes in turgor pressure, synthesis of hormones such as ABA, and the induction of one set of genes involved in dehydration tolerance, while other genes are activated through a cellular dehydration signal transduction pathway that does not involve ABA. Genetic engineering of a single structural protein (such as HVA1) or a single transcription factor (DREB1A) has resulted in striking improvements in plant tolerance to drought (see recent review by Baja et al. 1999 and Zhang et al. 2000). These results indicate that there is considerable potential for the transgenic approach to enhance drought tolerance in plants.

Analysis of quantitative variation in drought tolerance in plants

Drought tolerance is associated with many different morphological and physiological traits or responses including stomatal regulation, variation in leaf cuticle thickness, root morphology and depth, osmotic adjustment, antioxidant capacity, desiccation tolerance (membrane and protein stability), maintenance of photosynthesis and the timing of events during reproduction (Bohnert et al. 1995; Bray 1997; Nguyen et al. 1997; Klueva et al. 1998). The complexity of these responses is not surprising because well-adapted plants must be able to tolerate significant variation in water status during all phases of development.

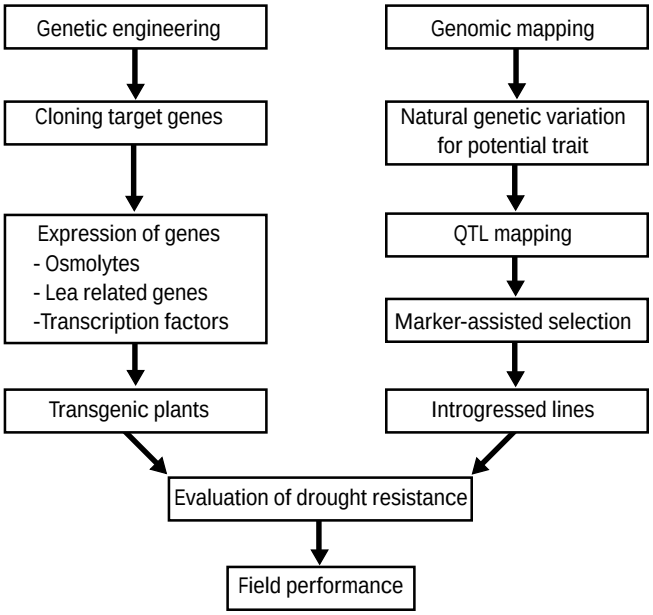

Figure 1. Strategies for improving drought resistance.

Moreover, the quantitative nature of drought tolerance is easy to understand in this context because numerous genes (Champoux et al. 1995; Lilley et al. 1996; Ray et al. 1995) control traits/responses like osmotic adjustment and root morphology. With the development of advanced molecular marker technology, it is now possible to understand the complexity of quantitative trait inheritance through its dissection into underlying Mendelian units. This advanced technology has had a significant impact upon applied breeding programs. Genetic studies of drought tolerance in sorghum and maize show that multiple genes control tolerance associated with the stay-green trait in sorghum and regulation of the anthesis-silking interval in maize (Ribaut et al. 1996; Crasta et al. 1999). More information about drought resistance QTLs in plants can be found in a recent proceeding edited by Ito et al. (1999). Based on the initial QTL mapping results, research efforts are underway aimed at marker-assisted selection to validate the usefulness of this molecular breeding approach for drought tolerance improvement. These efforts will also lead to the development of near-isogenic lines that will be extremely useful in the investigation of physiological functions and gene discovery.

As indicated previously, plant response to drought stress is quite complex, and is associated with a large number of physiological and biochemical changes. Some of those changes, such as ABA accumulation, osmotic stress adjustment, and root morphology, are known to be controlled by multiple genes

(Tuberosa et al. 1998; Champoux et al. 1995; Lilley et al.1996; Lebreton et al. 1995). At present, we have very limited understanding of the nature of QTLs. Theoretically, they could be structural or regulatory genes. The recent work of Tuberosa et al. (1998) in maize did not support the structural genic nature of the identified QTLs for ABA concentration (Schwartz et al. 1997). Similarly, the role of regulatory loci in cold and drought tolerance has been demonstrated in *Arabidopsis* by Thomashow and colleagues (Jaglo-Ottosen et al. 1998) and Shinozaki and colleagues (Liu et al. 1998). Although QTL mapping studies on several traits in different crops generated a wealth of provocative information, definitive demonstration of genes or mechanisms that provide naturally significant drought resistance remains elusive. Recent progress toward the positional cloning of QTLs for heading date in rice (Katayose et al. 1999) demonstrates the feasibility of isolating those determinants involved in drought tolerance with the help of modern genomic tools. Knowledge of the genes underlying these drought tolerance QTLs would be extremely useful both for the understanding of the biological basis of tolerance, and for utilization. Advances in molecular marker technology and the development of integrated genetic and physical maps make gene discovery in QTLs possible (Figure 2).

Functional Genomic and Candidate Gene Approaches

Expressed sequence tags and microarray technology

Microarray technology allows one to monitor the expression of thousands of genes systematically in a single hybridization using small quantities of the RNA sample, thereby requiring relatively small amounts of source tissue (Schena et al. 1995; Schena et al. 1996). Compared to oligo-based DNA chips, microarrays are quicker and less expensive to make, and more adaptable regarding the nucleic acid source that can be arrayed. DeRisi et al. (1997) pointed out that "perhaps the greatest challenge now is to develop efficient methods for organizing, distributing, interpreting, and extracting insights from the large volumes of data these experiments will provide." Software tools are commercially available to facilitate this analysis. The utility of this genome-based approach for studying a set of complex biological processes in a multicellular organism was recently demonstrated in *Drosophila* (White et al. 1999). This technology should be extremely useful in determining the genetic foundations of complex trait like drought tolerance when coupled with drought-regulated expressed sequenced tags (ESTs) and unique genetic materials (mutants or near-isogenic lines). Dedicated research efforts on the isolation and development of unique drought-related ESTs are needed in most crops to allow the utilization of microarray technology in drought tolerance gene discovery.

Candidate gene analysis

Candidate gene analysis starts with selection of some target genes based on biological pathway or genome location relative to a known QTL identified for the target trait (Byrne and McMullen 1996; Rothschild and Soller 1997). A drought-related EST database, microarray analysis, and the mutagenesis approach will yield a large number of valuable candidates for verification of their association with the drought tolerance traits. Alternatively, searches can be conducted for orthologs in existing literature and databases for information on drought and related abiotic stress genes. After genetic mapping is accomplished, ESTs that map very near the trait QTLs can be targeted for further candidate gene analysis. This fine mapping of the genes that generate a QTL will only be possible in NIL populations, in which a single QTL provides all of the population variation for drought tolerance. For

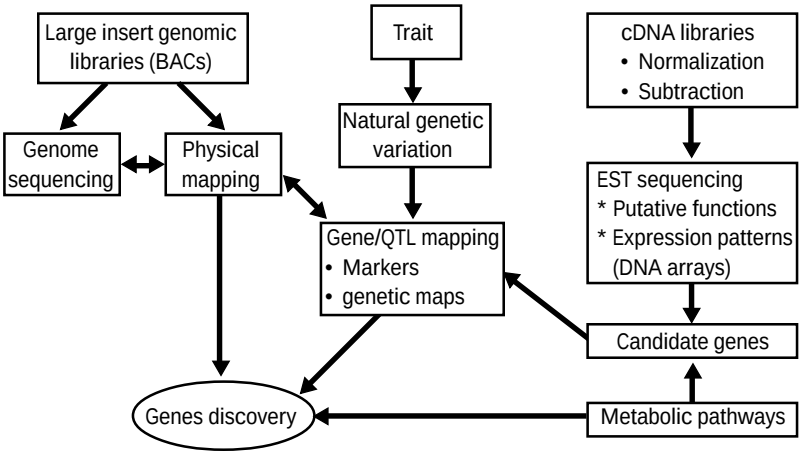

Figure 2. From traits to genes.

those ESTs that could not be mapped because of a lack of polymorphism, the physical map will help to confirm their location. Since most of the ESTs can be located on the physical map, one will be able to target a subset of candidate genes for co-segregation analysis in different populations. If several candidate genes with different functions in different QTL locations could be verified, a model can be hypothesized regarding how drought tolerance is manifested. With the integration of QTL mapping, comparative mapping information, growing EST databases, expression (including microarray) results, and the identification of more and more genes in the future, the candidate gene approach will become an important and powerful tool to uncover the mystery behind the expression of quantitative traits. Finally, with the development of high quality physical map and high-throughput genomic sequencing technology, a combination of cDNA capture and one-pass sequencing of several BAC contigs that comprise QTLs will be a powerful strategy in search for candidate genes.

Conclusions and Perspectives

Bohnert et al. (1995) rightly pointed out that "one promising genetic avenue is the mapping of quantitative trait loci that relate performance and yield to drought, low-temperature, or salinity tolerance. Thus, regions of chromosomes can be identified that carry genes that improve stress tolerance." Therefore, the genomic approach provides an unprecedented opportunity to isolate and understand the QTL that condition stress adaptation. Giving the complexity of drought tolerance in most crops and production systems, selection for yield QTLs per se is unlikely to be fruitful. The most promising approach would be targeting specific adaptive traits, complemented by marker-assisted selection. This will require QTL mapping for the target traits with relevant phenotyping. Tightly linked DNA markers then will provide a powerful tool for marker-assisted selection. This approach will enhance selection efficiency and pyramiding of favorable QTLs in a trait-based drought tolerance improvement program. The QTL mapping followed up by gene discovery in crop genomes is becoming a reality with the help of high-throughput microarray and genomic sequencing technologies. The insertional mutagenesis or "gene machine" approach, as presented by Lee and Bennetzen in this volume, will greatly complement the candidate gene analysis in the discovery of genes for transgenics. Finally, the comparative genetic analysis will be extremely useful for the molecular dissection of drought tolerance QTLs in large, complex genome species (such as wheat and maize) by using co-linear regions from a smaller genome species (such as rice and sorghum).

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Comparative Genomics Approaches to the Study of Drought Tolerance

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Summary

Genomics represents an entirely new conceptual approach to the study and application of biology. Structural genomics uses the rapid generation of huge quantities of precise DNA sequence data to identify genes, and the structures of genes and other elements in a genome. The functions of these genes can be assessed by a number of high-throughput approaches, so-called "functional genomics."

These techniques include the mapping of complex traits in very large populations, the characterization of correlated expression patterns of every gene within a species under all possible circumstances, and the use of reverse genetics and high throughput mutant screening to identify the phenotypes of mutations in all of the genes within a species. The ultimate goal of genomics is to find every gene and to determine the roles of each of these genes. Comparative genomics takes this goal several steps further: to identify and find the role of every gene in every species, to see what changes are significant in making one species different (in phenotype, growth habit, adapted environment) from another, and to determine how these changes came about.

Drought tolerance is a highly appropriate target for comparative plant genomics because only such an information-rich approach is likely to unveil the key genetic contributors to the complex physiological processes involved. The applied goal of comparative plant genomics might be described as identifying all of the genetic variation in the biosphere, whether in crops or in wild species, that can be used to design the most productive, benign and sustainable agricultural systems. One use of this technology could be to find and appropriately utilize the best drought tolerance alleles in nature, regardless of source, for crop improvement. The technology and biological materials needed to accomplish this ambitious goal now exist. All that is lacking are the appropriate team and resources to undertake this important task.

Introduction

Drought is one of the major limitations to food production worldwide. In some parts of the world, particularly the semi-arid tropics and other locations where most of the world's poor people reside, drought is endemic. But even the most productive agricultural regions experience short periods of drought within almost any year and occasional years with severe droughts. Moreover, many parts of the Earth's surface are not arable, primarily because of severe water limitations, and the amount of land with these problems grows ever year.

Hence, improved tolerance to drought has been a goal of crop improvement programs since the dawn of agriculture. With some crops under some conditions, significant improvements in genetic tolerance to drought have been achieved. However, drought tolerance appears to be a complex problem, with many contributing loci that show efficacy only in a subset of circumstances (Lebreton et al. 1995; Ribaut et al. 1996, 1997; Tuinstra et al. 1996, 1997; Nguyen et al. 1997). Thus, progress in understanding the basic molecular and physiological nature of drought tolerance has been slow (Shinozaki and Yamaguchi-Shinozaki 1996; Bray 1998). Genomics offers a new approach that may allow relatively rapid progress in producing crops with improved drought tolerance.

Genomics might simply be viewed as a collection of technologies that permit high throughput genetics. However, like any major new

technical advance, genomics provides the opportunity for a new conception of what is feasible in both applied and basic research arenas. Plant genomics allows the mapping of complex traits in very large populations and under many different environmental circumstances. Hence, even minor contributing loci can be identified and mapped (for reviews, see Dudley, 1993; Kearsey and Farquhar 1998). Fast DNA sequencing technologies allow the eventual identification of all of the genes that might be involved in drought tolerance. Rapid technologies for the study of gene expression allow the investigator to determine the level and timing of activation of these genes during development and in response to various environmental perturbations (Schena et al. 1996; Ruan et al. 1998). Efficient technologies for isolating mutations in any targeted gene (Das and Martienssen 1995; Krysan et al. 1996; Hirochika 1997) will allow a relatively rapid determination of whether that candidate gene (Byrne and McMullen 1996) actually contributes to drought tolerance. The major current limitation to genomics is the need to assemble the tools and, most importantly, talented scientific team that can carry out the complex multidimensional tasks required to understand and engineer improved drought tolerance in crop plants.

Components of a Plant Genomics Project Targeted on Drought Tolerance in Crops

The major tools available to a plant geneticist or genomicist involve the identification of genes involved in a trait, and correlations of alleles and/or expression patterns in those genes with the investigated trait. The particular technologies available for improvement in the drought tolerance of crop plants are no different from those available for any genomic approach to the study or enhancement of crops. These tools are comparative analyses across species and populations, high throughput mapping, identification of drought-associated genes by expression, and characterization of mutations in candidate genes.

Comparative analyses across species and populations

Recent studies have indicated that all grasses share very similar gene contents and stretches of conserved gene order along the chromosomes (reviewed in Bennetzen and Freeling 1997; Gale and Devos 1998). Less numerous studies also suggest significant common gene content and colinear gene order in closely related dicotyledenous plants (Bonierbale et al. 1988; Fatokun et al. 1992; Kowalski et al. 1994). Hence, different plant species appear to differ less in the genes they carry than they do in the alleles of genes that they contain. Moreover, wide cross and comparative mapping studies suggest that the genetic differences responsible for very different morphologies and physiologies might be caused by differences in just a handful of genes, probably regulatory loci (Doebley et al. 1995; Paterson et al. 1995). This suggests that most biochemical pathways will exist in most plant species, and that the differences observed are due to possibly subtle changes in the timing, tissue, or magnitude of expression of such pathways. This conclusion makes it both possible and imperative to use the information from one species to enhance our understanding of every other species.

In drought tolerance, for instance, it is likely that maize and sorghum share the same basic tolerance pathways, but that sorghum has acquired superior allelic versions of the genes in that pathway because it evolved in drought-prone environments. Comparative genomics of these and other species will allow the identification of traits that are shared by all plants (and, hence, are required for general survival) and those that are only shared by some or all drought-tolerant individuals (and, hence, presumably are required only to survive in drought-prone regions). If one can identify the sorghum genes that are responsible for superior drought tolerance, then it is likely that these genes could function in maize to provide superior drought tolerance as well. This approach may be viewed as merely expanding the allelic variation available to maize, in a transgenic sense. Of course, the number of improved genes that would need to be added to have a significant effect remains an open question. Still, it is clear that using information (and eventually genes) from all pertinent species will provide a synergistic route for the improvement of any and all individual crops (Bennetzen and Freeling 1997).

High throughput mapping

Automated technologies for DNA preparation and the mapping of DNA markers by polymerase chain reaction (PCR) assays can provide the route for mapping hundreds of markers in many thousands of progeny in just a few months' time

(Paterson and Wing 1993). The costs of this technology are currently fairly high, but they are not nearly as high as the costs of mapping one trait at a time with traditional morphological scores by the individual scientist. High throughput mapping costs will continue to plummet, allowing the mapping of large populations across many environments for numerous traits, many of them with complex patterns of inheritance. If the populations are large enough, one can identify and crudely map all of the quantitative trait loci (QTL) involved in drought tolerance that are segregating in that population under the environment(s) tested, even those that contribute small amounts to the trait (W. Beavis, pers. comm.).

If QTL are major, if they are found to function across multiple environments, and if they are seen to co-map in several different species and/or populations, they then provide optimal targets for cloning by map-based approaches. Near isolines (NILs) can be generated that differ for a single QTL, thereby making that trait responsible for 100% of the variation in that population. In that NIL population, the contributing genes can be precisely mapped as a qualitative trait in large populations, thereby providing the raw material for map-based cloning (Martin et al. 1993).

Identification of drought-associated genes by expression

By "traditional" molecular approaches (i.e., ones in use since the late 1970s), researchers identified genes that were induced or repressed by a particular environmental cue, such as drought. However, these technologies could never yield more than a subset of the genes involved, and were biased toward recovering genes with very major changes in expression that were observed over (usually) two chosen points in time. The genomic approach to this same question is to test all of the genes in a species for their RNA levels at all times before and after drought stress induction (Schena et al. 1996; Ruan et al. 1998). Because these characterizations are fairly quantitative, quite sensitive, and quick to perform, they allow the identification of genes that are induced or repressed by only a few fold, are expressed at low levels, and are altered in expression only during part of the exposure program. Compared to the traditional approach, one expects to more than triple the number of affected genes that are identified. Once again, a comparative approach would see the significant commonalties and differences in these expression patterns relative to species and populations that differ in drought tolerance. These genes with affected expression can also be located on the genetic map and, when they appear to co-localize with a QTL for drought tolerance, make attractive candidate loci (Byrne and McMullen 1996).

Characterization of mutations and other allelic variations in candidate genes

Once genes that may play an important role in drought tolerance have been identified by mapping and/or expression studies (preferably both), they can serve as candidates for direct genetic characterization. One possibility would be to clone a candidate drought tolerance gene from sorghum, and then transform it into maize. This transgenic maize plant and its progeny could then be tested for drought tolerance. This is an arduous approach, however, and would probably require a major genetic contribution by the candidate in order for the test to be successful. With many candidate genes, this would probably be a poor use of limited resources. A better initial route (quicker and less expensive) to screen candidates for a possible role in drought tolerance would be to analyze knockout mutations. If a candidate allele truly is both dominant in action and involved in drought tolerance, one expects that inactivation of that allele would give rise to increased drought susceptibility. Current PCR technologies allow one to rapidly screen for inactivational insertion mutations in several plant species, including Arabidopsis (Krysan et al. 1996), maize (Das and Martienssen 1995), and rice (Hirochika 1997), while many more species have this technology under development. Of course, this knockout approach is far from foolproof, because as one might imagine, there are many genes not directly involved in drought tolerance whose loss would make a plant sickly and, hence, more sensitive to drought. Moreover, some drought tolerance genes may be essential loci, such that a knockout mutation would yield no homozygous progeny. Still, any candidates that showed no phenotypic alteration in drought tolerance after an insertion mutation in coding regions could be simply removed from the candidate list. Eventually, once the candidate list was pared to a manageable few, the direct assay of superior gene function in transgenics could be performed, thus providing the only direct proof

of a role. If one or more drought tolerance genes were identified, they could then be entered into superior germplasm to see if they provide improved drought tolerance.

Conclusions

Comparative genomic approaches to drought tolerance are now sufficiently competent to identify the genes responsible for drought tolerance in crop plants and their close relatives. These genes can be used to help understand the nature of drought tolerance, because they will indicate the pathways involved and how their actions/expression differs in tolerant and susceptible plants. The same loci can serve as probes for marker-assisted selection of these traits into elite germplasms, with the added knowledge of the set of traits (and their germplasm sources) that can provide an optimal balance of tolerance and yield. Moreover, some of these genes may be used in transgenics to provide drought tolerance superior to that within any of the germplasm traditionally available to a particular crop species. Once the correct resources and research team are assembled, comparative genomics can begin the process of characterizing and improving crop drought tolerance.

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Challenges and Future Strategies in Breeding Wheat for Adaptation to Drought Stressed Environments: A CIMMYT Wheat Program Perspective

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Summary

The genetics of drought tolerance in wheat is poorly understood and the highly variable nature of rainfall in most rainfed environments makes genetic progress extremely difficult. Nevertheless, considerable improvement in the adaptation of wheat to dry areas has been made by plant breeders over the last 50 years. The adoption of modern varieties, however, has lagged behind that of irrigated areas and the percentage yield advance has been considerably lower.

Various strategies can be employed or developed to improve the efficiency of germplasm development targeted to dry environments. These strategies include the improved use of information, better characterization of testing locations, refinement of selection environments, implementation of suitable management practices, expansion of genetic variability, identification of drought adaptive traits and the implementation of molecular strategies, including the identification of QTLs and DNA fingerprinting.

Introduction

Most wheat breeders working in dry environments long ago gave up attempting to screen for drought tolerance per se. The genetics of drought tolerance is poorly understood and the highly variable nature of rainfall in these environments makes genetic progress for drought tolerance extremely difficult, as drought patterns are not consistent among years. In addition, many biotic and abiotic factors are frequently misinterpreted as expressions of drought tolerance. For example, plants tolerant to nematodes or micronutrient imbalances may be selected as drought tolerant by the plant breeder, simply because they have healthier root systems. Breeders have therefore concentrated on improving tolerance to those factors, particularly diseases, for which they have known and repeatable variation. The key variables influencing yield in dry environments are outlined in Table 1.

Table 1. Factors effecting yield in dry environments

Patterns of Moisture Stress	Temperature Extremes	Nutrient Stress & pH Extremes	Biotic stress	Agronomic Practices
Terminal	Heat Stress Humid	P and N Deficiency/ Efficiency	Root rots	Stubble retention
Pre-Anthesis	Heat Stress Dry	Deficiency (e.g., zinc)	Nematodes	Zero tillage
Residual Moisture	Cold Stress	Toxicity (e.g., boron)	Foliar pathogens	Crop rotations
Reduced Irrigation	Cold Stress – Late Frost	Acid Soils Mineral		Shifting cultivation
General Low Rainfall		Acid Soils Volcanic/Organic		Water harvesting
Shallow, Marginal, Infertile, Eroded Lands		Alkaline Soils		

The Nature of the Target Environment Globally

Half the area sown to wheat in developing countries and up to 70% of that grown in developed countries suffers from periodic drought (Rajaram, personal comm.). Drought can occur at any time during the cropping cycle in all rainfed environments. However, when examined over the long term, certain regions are more likely to suffer a particular stress pattern. Terminal or post-flowering stress is typical of many Mediterranean-type environments, including North Africa/West Asia, southern Australia and South Africa. Pre-anthesis stress is frequently encountered in the Southern Cone of Latin America while continuous stress, typically experienced when farmers sow on stored soil moisture, is characteristic of the monsoonal areas of central India.

To examine the challenges facing wheat researchers more closely, Singh and Byerlee (1990) analyzed wheat yield variability in 57 countries over 35 years. Yield variability was measured by calculating coefficients of variation of yields around linear trends. The amount and distribution of rainfall was the predominant factor influencing yield variability: countries in which half the wheat was sown in dryland conditions experienced twice as much variability as countries in which wheat was mostly grown under well-watered conditions. Yield variability also tends to be higher in warmer subtropical countries due to heat stress.

Advances in the Development of Drought Tolerant Wheat

Over the last 50 years, breeders have been successful in increasing the adaptation of wheat to dry environments. In developing countries, farmers have traditionally grown landrace cultivars, which are well adapted to serious moisture stress conditions. However, these traditional cultivars are generally poor yielding in the "good years" when rainfall is more plentiful. Modern cultivars now yield the same as the traditional cultivars in the dry years, yet will respond to more favorable moisture and nutrient conditions (Osmanzai et al. 1987). Additionally, improved disease resistance in the modern cultivars also "protects" the yield during the more humid, high-yielding years.

Current adoption rates indicate that modern varieties are increasingly grown in the dry regions and rates of adoption approach those in irrigated and high rainfall areas (Fig 1). After a time lag, adoption rates in drought-prone rainfed areas in Argentina, Pakistan, and Syria are above 80% (90% in 1998). Data from 1998 exhibit a similar situation—adoption rates of between 80% and 90% for North Africa, Morocco, and Tunisia have been realized. Only Algeria lags behind with adoption rates of modern varieties below 50%. These are striking figures, particularly when compared with other small grains, such as barley. The adoption rate for modern barley in Syria is only 4%.

Strategies for the Continued Improvement of Wheat in Dry Environments

To maintain and improve current rates of advance in dry environments, researchers must address the following six areas:

(A) Improved collection/use of information and characterization of testing environments

The CIMMYT wheat program distributes wheat germplasm around the world each year. Cooperators from many countries return yield and disease information collected on these germplasm sets. The information on the performance of key lines in low-yielding environments is used to drive the crossing program at base in Mexico. CIMMYT's breeders divide the tested germplasm into those that

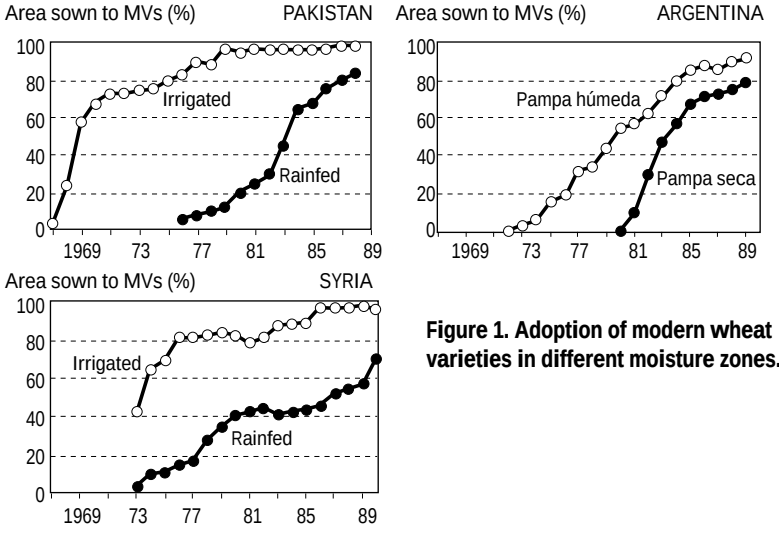

Figure 1. Adoption of modern wheat varieties in different moisture zones.

perform well across environments and those that perform well in specific dry environments. In the absence of information on quantitative trait loci (QTL), environments are characterized on the basis of their stress patterns and crosses are made among the various specific and general performers. The aim of this strategy is to combine those parts of the genome contributing to drought tolerance in different stress environments.

While most cooperators will return yield data, there is scant information returned on environmental parameters or other potentially confounding stresses, such as disease and micronutrient imbalances. The mechanism contributing to the superior performance of a particular genotype in one environment cannot be properly understood. It is therefore critical that the global testing environments are properly characterized on a year to year basis. The same principle applies to multilocation yield evaluation networks within smaller regional wheat improvement programs. One way in which environments can be better understood is to deploy a probe genotype set (Cooper et al. 1994). This is a group of lines that will differentiate a particular environment for a defined set of stresses. Probe lines will likely include lines isogenic for certain key characters.

(B) Refinement of selection environments to better predict drought tolerance per se

The variable nature of rainfall makes selection of segregating generations for drought tolerance in most dry environments extremely difficult. Genoptype x year interactions are large and they obscure genetic effects. The selection environment must, therefore, be repeatable year to year. The CIMMYT wheat program utilizes a site in northwestern Mexico to screen for drought tolerance per se. It is an arid environment and water application through gravity fed irrigation is strictly controlled. Soils have been thoroughly characterized for both abiotic and biotic factors. The heritability of selection in this environment is high. Germplasm is developed by alternating the selection of segregating generations between this dry environment and a high rainfall site in the central Mexican highlands. The strategy was developed to combine drought tolerance with input responsiveness and resistance to the foliar diseases. CIMMYT has also employed Line Source yield testing, in which genotypes are grown perpendicular to a central water line to generate a stress gradient (Pfeiffer et al. 1990). Heritabilites of selection were very high when utilizing these gradients. It is critical that selection environments of this nature be developed if genetic variation for drought tolerance is to be understood and molecular markers ultimately developed.

(C) Development and implementation of suitable crop management practices

To realize the genetic gains in drought tolerance in farmers' fields, suitable agronomic practices must be implemented. Moisture conservation practices such as reduced or zero tillage and stubble retention require a change in infrastructure. Many farmers, particularly those from developing countries, are unable to cope with the associated expense of implementation of these new techniques, however, the interaction of tillage regime x genotype will be very important in realizing significant gains in productivity in dry environments. Other practices, like shifting cultivation or periods of fallow and water harvesting (collecting run-off after rainfall for irrigation use), will also better utilize available moisture.

(D) Expansion of genetic variability

To build upon past successes in the development of drought tolerant wheat, it will be necessary to expand the variability currently available in both the hexaploid and tetraploid gene pools. Among the hexaploid bread wheats, new and useful variation is being exploited through the production of synthetic wheats. These wheats are the result of a cross between two putative progenitors of wheat (*Aegilops tauchii* and *Triticum durum*) with subsequent chromosome doubling. Historically, this cross has probably occurred on few occasions and, consequently, there has been limited sampling of the genetic resources of these two species in the development of bread wheat. The *A. tauchii* accessions currently available have been collected in some of the harshest environments on earth and have evolved over thousands of years in conditions of periodic drought, heat, flooding, and frosting. These genetic stocks must be more fully exploited in the future. This material should also be more amenable to the identification and application of molecular marker technology because the frequency of polymorphisms can be expected to be considerably higher than that found in conventional

wheat. The dicoccum wheats and tetraploid landraces also provide useful potential sources of variation that must be further exploited. The use of genetic transformation, once perfected, may also provide a way of expanding the variability available to the wheat breeder.

(E) Identification and inheritance of drought adaptive traits

To improve genetic progress for drought tolerance, existing variation must be properly characterized and their physiological, morphological, and genetic basis understood. While a number of traits or trait combinations have been proposed for indirect selection (Marshall 1987; Richards and Condon 1994), there has been little progress in the practical application of selection for these traits in wheat breeding programs. Once repeatable genetic variation has been determined (see part B), those traits contributing to improved performance can be identified. Examination of differences among drought tolerant and intolerant populations in repeatable environments will be an important first step in understanding the molecular basis of drought tolerance.

(F) Development and implementation of molecular strategies

Once key areas of the genome are identified that contribute to drought tolerance under a particular set of environmental conditions, the plant breeder can then begin combining adaptation to various types of drought. The development of QTLs may also identify regions of the genome that are constant to all moisture-limiting conditions. To date, this technology is not available to the wheat breeder, however QTLs offer great potential for the enhancement of drought tolerance per se in wheat improvement programs around the world. Among the available technologies, DNA fingerprinting of key germplasm, once characterized for drought tolerance, could be applied immediately. Fingerprinting should provide useful information on those regions of the genome contributing to drought tolerance.

Conclusion

This paper discusses past advances and outlines key research areas for future emphasis. However, just as drought tolerance involves an extremely complex set of interactions among many characters, future improvements in wheat productivity in drought-stressed environments will require the integration of the skills of scientists from many disciplines. At CIMMYT, these efforts have been concentrated under the banner of a global project for abiotic stress tolerance. This project combines the skills of plant breeders, molecular biologists, physiologists, pathologists, agronomists, and economists with the single objective of improving agricultural production from wheat in dry environments. Collaborative approaches like this will be fundamental for the effective initiation, conduct, and implementation of research into the future.

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Evaluating a Conceptual Model for Drought Tolerance

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Summary

Wheat yields are reduced by 50–90% of their irrigated potential by drought on at least 60 million ha in the developing world. CIMMYT's wheat program is attempting to further improve drought tolerance by introgressing stress adaptive traits into empirically selected drought tolerant germplasm. Our current conceptual model for drought encompasses high expression of the following traits: seed size, coleoptile length, early ground cover, pre-anthesis biomass, stem reserves/remobilization, spike photosynthesis, stomatal conductance, osmotic adjustment, accumulation of abscisic acid (ABA), heat tolerance, leaf anatomical traits (waxiness, pubescence, rolling, thickness), high tiller survival, and stay-green. CIMMYT's germplasm collection is being screened for high expression of these traits. The traits will be tested systematically either in recombinant inbred lines, near isogenics, or synthetic hexaploids. Molecular markers will be developed for those traits showing genetic gains to selection.

Introduction

At least 60 million ha of wheat is grown in marginal rainfed environments in developing countries. National average yields range from 0.8 to 1.5 t/ha, approximately 10 to 50% of their theoretical irrigated potential (Morris et al. 1991). Rainfall distribution patterns vary considerably among locations and years, and additional stresses may include heat and cold stress, soil micro-element deficiency or toxicity, and a range of biotic stresses. Physiological assessment of drought tolerance characteristics in the field is therefore a complex task. Research at CIMMYT using a line-source gradient to create different intensities of drought stress demonstrated a linear relationship between grain yield and water application (Sayre et al. 1995). This suggests that wheat is relatively drought hardy, unlike maize for example, which may fail completely if the anthesis-silking interval is delayed beyond a critical threshold due to drought (Bolaños and Edmeades 1993). Breeding for drought tolerance in wheat, therefore, should focus more on improving overall radiation use efficiency under stress rather than reproductive stages of growth and partitioning. This conclusion is backed by recent studies with *Rht* isolines in which the shorter growth habit normally associated with better partitioning to yield was of no benefit under drought (Singh, personal communication).

CIMMYT's breeding work for moisture-stressed environments has been largely empirical to date (Pfeiffer and Trethowan 1999), but recent emphasis on breeding for marginal environments has increased the focus on dry environments, and a multidisciplinary effort has been initiated to improve drought tolerance. The main inputs from a physiological point of view will be (i) to develop conceptual models of trait combinations which may enhance drought tolerance; (ii) identify sources of those traits among current breeders materials and germplasm bank accessions including landraces; (iii) evaluate genetic gains associated with specific traits or trait combinations when introgressed into different adapted backgrounds; (iv) pre-screen diploid and tetraploid genotypes for use in development of synthetic wheat lines so as to increase the probability of favorable traits being expressed in hexaploid and tetraploid combinations; (v) evaluate traits in genetically mapped populations to identify molecular markers for drought tolerance genes; and (vi) establish stress treatments for functional genomics studies and identify traits for crop improvement based on genetic dissection.

For the purposes of this workshop, the focus will be on a drought environment broadly characterized as follows: average yield 1.0–2.0 t/ha (approximately 25% of irrigated yield potential), moisture deficit starting after approximately 30 days (jointing) and gradually intensifying until maturity, possible heat stress (air temperature > 30°C) during grain filling. This environment is reasonably representative of most rainfed wheat growing regions in Asia and North Africa. Nonetheless, within this broad region, significant differences occur between sites for factors such as rainfall distribution pattern, soil water holding capacity, agronomic practices etc., and breeding objectives need to take account of this variability.

A Conceptual Model for Drought Tolerance

Many anatomical, physiological and biochemical traits are mentioned in the literature as being drought adaptive (Blum 1988; Loss and Siddique 1994; Richards 1996). This model will include those which are currently considered of most potential value to the environment described (Figure 1), bearing in mind that not all traits are appropriate for all drought environments. The development of molecular probes for marker-assisted screening of these traits would be an important objective, assuming their use is more efficient at identifying superior genotypes than conventional screening approaches.

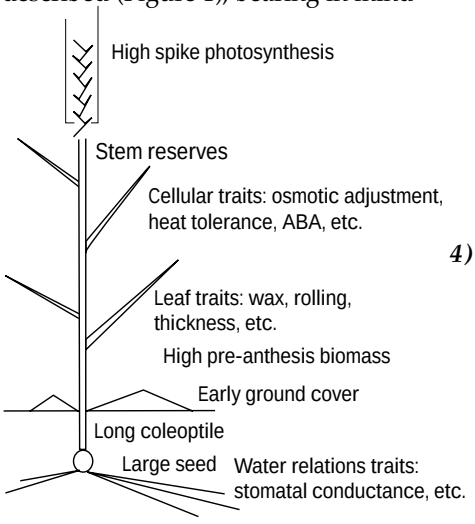

Figure 1. Conceptual model of drought tolerant wheat plant.

1) ***Large seed size.*** Helps emergence, early ground cover, and initial biomass.
2) ***Long coleoptiles.*** For emergence from deep sowing (Radford 1987). This is practiced to help seedlings reach the receding moisture profile, and to avoid high soil surface temperatures which inhibit germination.
3) ***Early ground cover.*** Thinner, wider leaves (i.e., with a relatively low specific leaf weight) and a more prostrate growth habit help to increase ground cover, thus conserving soil moisture and potentially increasing radiation use efficiency. (Richards 1996). This trait would be more important in the Mediterranean type of drought environment where rain may occur during the early part of the cycle. It would be less useful in regions where the crop grows exclusively on stored soil moisture where dust mulching is practiced, or where residue retention is practiced to avoid evaporation from the soil surface.
4) ***High pre-anthesis biomass.*** Potential for vigorous growth prior to heading provides the opportunity to take advantage of relatively good growing temperatures and moisture availability earlier in the cycle. Up to 40% of available water may be lost by evaporation directly from the soil in Mediterranean types of environments (Loss and Siddique 1994), so high early ground cover and biomass production may permit a more efficient use of soil water. Although most drought studies show that high water use efficiency (WUE) is not associated with better performance (e.g., Sayre et al. 1995), ideally early biomass should be achieved with maximal water use efficiency to improve water availability during grain filling. Recent work in Australia (Richards, personal communication) indicate an advantage of high WUE genotypes under severe drought conditions.
5) ***Good capacity for stem reserves and remobilization.*** Stored fructans can contribute substantially to grain filling, especially when canopy photosynthesis is inhibited by drought (Rawson and Evans 1971). Traits that may contribute include long and thick stem internodes, with extra storage tissue perhaps in the form of solid stems. In studies where crosses where made between lines contrasting in the solid stem trait, the solid-stem progeny contained more soluble carbohydrate per unit of stem length, though total stem carbohydrate was unaffected due to narrower and shorter stems (Ford et al. 1979).
6) ***High spike photosynthetic capacity***. Spikes have higher WUE than leaves and have been shown to contribute up to 40% of total carbon fixation under moisture stress (Evans et al. 1972). Awns contribute substantially to spike photosynthesis and longer awns are a possible selection criterion.

While gas exchange measurement of spikes is time consuming and difficult to standardize, chlorophyll fluorescence should be considered as a more rapid means of screening for spike photosynthetic capacity under stress (P. Horton, personal communication). The trait could be measured at any time after heading.

7) ***High RLWC/Gs/CTD during grain filling to indicate ability to extract water.*** A root system that can extract whatever water is available in the soil profile is clearly drought adaptive (Hurd 1968), but difficult to measure. Traits affected by the water relations of the plant, such as relative leaf water content (RLWC) measured pre-dawn, stomatal conductance (Gs), or canopy temperature depression (CTD), during the day, and C_{13} discrimination or ash content of grain or other tissues, can give indications of water extraction patterns.
8) ***Osmotic adjustment.*** (Morgan and Condon 1986). Adjustment will help maintain leaf metabolism and root growth at relatively low leaf water potentials by maintaining turgor pressure in the cells. Some research suggests that the trait can be assayed relatively easily by measuring coleoptile growth rate of seedlings in polyethylene glycol (PEG) solution.
9) ***Accumulation of ABA***. The benefit of ABA accumulation under drought has been demonstrated (Innes et al. 1984). It appears to pre-adapt plants to stress by reducing stomatal conductance, rates of cell division, organ size, and increasing development rate. However, high ABA can also result in sterility problems since high ABA levels may abort developing florets.
10) ***Heat Tolerance.*** The contribution of heat tolerance to performance under moisture stress needs to be quantified, but it is relatively easy to screen for (Reynolds et al. 1998).
11) ***Leaf anatomy: waxiness, pubescence, rolling, thickness, posture*** (Richards 1996). These traits decrease radiation load to the leaf surface. Benefits include a lower evapotranspiration rate and reduced risk of irreversible photo-inhibition. However, they may also be associated with reduce radiation use efficiency, which would reduce yield under more favorable conditions.
12) ***High tiller survival.*** Comparison of old and new varieties have shown that under drought older varieties over-produce tillers many of which fail to set grain while modern drought tolerant lines produce fewer tillers most of which survive (Siddique and Loss 1994).
13) ***Stay-green.*** The trait may indicate the presence of drought avoidance mechanisms, but probably does not contribute to yield per se if there is no water left in the soil profile by the end of the cycle to support leaf gas exchange. It may be detrimental if it indicates lack of ability to remobilize stem reserves (Blum 1998). However, research in sorghum has indicated that stay-green is associated with higher leaf chlorophyll content at all stages of development and both were associated with improved yield and transpiration efficiency under drought (Borrel et al. 2000)

Identification of Sources with High Expression of Drought-adaptive Traits

Germplasm bank accessions, for example land race collections from heat or drought stressed regions such as Iran and Mexico, are being systematically screened for potentially valuable traits. Sources have been identified with high chlorophyll at heading (Hede et al. 1999), high leaf conductance (Villhelmsen et al. 1999), high pubescence (Trethowan et al. 1998), peduncle volume, stay-green, and heat tolerance. Searches are currently under way for long awns, high osmotic adjustment, and biomass under drought and high temperature stress.

Pre-screening Diploid and Tetraploid Genotypes for Making Synthetic Wheat

Dicoccums, durum land races and diploid genome donors are being systematically screened for a number of the traits described above. Lines showing drought adaptive traits can be used to generate synthetic wheat.

Evaluation of Genetic Gains for Traits Introgressed into Drought-tolerant Backgrounds

So far studies have only been accomplished in recombinant inbred lines (RILs). The main focus has been measurement of canopy temperature depression (CTD) to indicate differences in stomatal conductance of water. CTD showed a highly significant association with yield under drought when measured pre-

anthesis (Figure 2), suggesting an advantage from higher growth rates pre-anthesis. When measured during grain filling, CTD also showed a good association with final yield (Figure 3). Genetic variation for awn length, stem thickness and solid stem were estimated in two populations of RILs. Average awn length ranged from 5.5 to 9 mm, average stem thickness of the main tiller (3 cm below the spike) ranged from 1.6 mm to 2.6 mm, but no relationship between yield and these traits was revealed. The solid stem trait was estimated on a visual basis and rated from 1 to 5 where 5 was completely solid. The trait appeared to be facultative in as much as under irrigation all lines scored 1, while under stress there was a full range of expression. However, the trait was negatively associated with yield in two populations of RILs.

Evaluate Traits in Genetically Mapped Populations to Identify Molecular Markers

To date, the only mapped material available at CIMMYT has been the ITMI population (International Triticale Mapping Initiative). The lines are the progeny of a wide cross between a synthetic line (having good drought tolerance) and Opata-M85 (a relatively drought susceptible semi-dwarf). This material has been evaluated for yield, yield loss (relative to lines without stress), and CTD under both drought (Figure 3) and heat. Yield loss under drought ranged from 50% to 95% of well-watered controls, and there was a significant association between yield under drought and yield under heat stress (Figure 4), indicating the value of heat tolerance as a drought adaptive trait. When QTL analysis has been realized, it should be possible to detect QTL markers associated with yield stability under drought. In addition, QTLs for CTD can be compared under drought, heat, and well watered conditions to determine genomic regions which are associated with higher CTD specifically under stressed conditions. Since CTD is determined by stomatal conductance, if there is such a unique linkage of QTLs associated with high CTD under drought, they would be expected to be associated with traits permitting better water relations in these conditions. A new population has also been developed for stress work between two semidwarf lines (Seri-M82 and Babax) which contrast in performance under drought, but whose progeny are quite similar in height and phenology and thus very amenable to physiological studies.

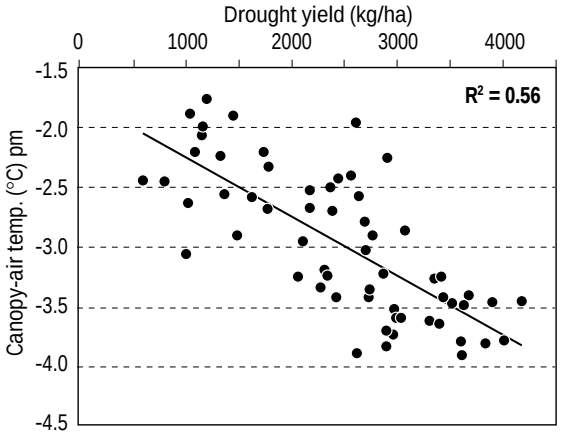

Figure 2. Association of canopy temperature depression measured pre-heading with yield under drought of recombinant inbred lines from the cross Babax/Lucero-Mexicano, Obregon,1998-99.

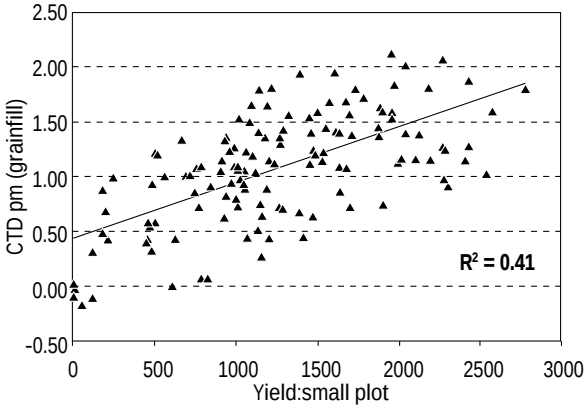

Figure 3. Association of canopy temperature depression measured during grain filling with yield under drought of recombinant inbred lines of the ITMI population (Synthetic/Opata-M85) Obregon 1997-98.

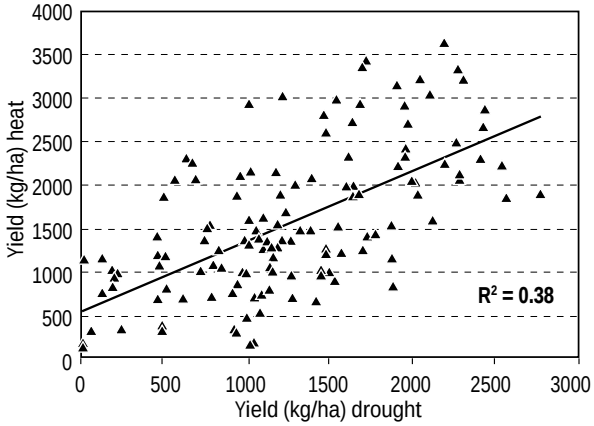

Figure 4. Association of yield under drought with yield under heat stress for recombinant inbred lines of the ITMI population (Synthetic/Opata-M85) Obregon 1997-98.

This will be mapped and evaluated for a number of drought adaptive traits in subsequent cycles.

Establish Stress Treatments for Functional Genomics Studies and Identify Traits for Genetic Improvement Based on Genetic Dissection

With the advent of DNA chip technology or micro-arrays (Brownstein et al. 1998), the relative importance of different genes involved in drought tolerance could be determined. The technique involves extracting RNA from plant tissue and generating labelled cDNA or cRNA probes that are hybridized with the microarrays. The microarrays are scanned to determine which genes were turned on in the tissue sample. Since so many genes are involved, a very large amount of information is generated for each sample. Therefore, it will be important to use physiological understanding to chose the most appropriate plant organs, stages of phenology and stress conditions to focus the research, in order to discover candidate genes for crop improvement. Once the microarray data has been interpreted, it should indicate the types of physiological traits that need to be exploited in terms of introgression of new sources of genetic diversity to improve drought tolerance.

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Physiological Traits to Improve the Yield of Rain-Fed Wheat: Can Molecular Genetics Help?

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Summary

An understanding of the physiological limitations to yield in dry environments, together with molecular genetics, offers important opportunities to hasten yield progress. This approach is particularly important to more accurately target factors limiting yield, to speed up selection, and to broaden the genetic base. In this paper we list morphological and physiological traits we consider important to increasing the yield of temperate cereals grown in dry environments. We also provide an assessment of the heritability of the traits, the ease of selection, whether g x e is important, and whether molecular markers have been identified in wheat. We then provide a rating to indicate the importance of molecular markers to select for each trait. A brief description is also given of our research and our breeding efforts to improve the performance of wheat under drought by increasing transpiration efficiency, crop establishment using alternative dwarfing genes, increased early vigor, and a higher harvest index. Molecular opportunities are emphasized in this description.

Introduction

Any genetic advance in yield in a dry environment must have a physiological basis. If our physiological understanding of yield is adequate, then the most likely traits to improve yields in a given environment can be identified. The use of physiological traits in a breeding program, either by direct selection or through a surrogate such as molecular markers, can then result in more accurately targeting factors limiting yield and may result in faster rates of yield improvement. It can also result in a broadening of the genetic base. The use of these traits as indirect selection criteria for yield in a breeding program will then depend on their relative importance (genetic correlation with yield), ease and cost of measurement, extent of genetic variation, heritability, genotype X environment interactions, and whether they are associated with adverse pleiotropic effects or genetic linkage.

Molecular Enhancement of a Physiological Approach

Molecular techniques can enhance a physiological approach in a variety of ways. Markers can provide more efficient procedures for selection of some physiological traits, particularly if they are used early in a breeding program, so as to allow culling and plant selection before hybridisation. Molecular markers will also provide a better understanding of the genetic basis of the trait; they may also present an opportunity to dissect and understand the key processes involved in plant growth and development. Genomics and gene discovery is a natural follow on which can then lead to further genetic understanding and manipulation of the key traits using conventional and transformation methods.

Backcrossing is often the chosen method in wheat to introduce a new trait into a breeding program. Molecular methods can very effectively increase the efficiency of backcrossing by selection for the genotype of the recurrent parent. This will ensure rapid progress in breeding and should result in a high frequency of progeny with the trait in question and the desirable features of the recurrent parent.

Trait Identification and a Useful Framework to Improve Yield

The success of a physiological approach to improve yield under drought will depend on the effective identification of the limiting trait and then on its genetic complexity. The identity proposed by Passioura (1977) provides the most valuable framework in which to identify critical physiological traits limiting yield under drought. Passioura proposed that grain yield in water-limited environments is the product of three factors, *viz*:

Grain yield = crop water use x water-use efficiency x harvest index.

Improving any one of these should improve grain yield providing the components are largely independent of each other. This identity should be considered in relation to the target environment since the relative importance of traits will vary with the amount and distribution of rainfall in relation to crop growth and development.

In devising ways to improve yield under drought, both biotic and abiotic factors other than drought must first be ruled out as being major determinants of yield. For example, root diseases often induce drought-like symptoms in plants as can soil nutrient deficiencies and toxicities. Molecular methods to screen for resistance are available for some of these problems. Furthermore, molecular methods to detect soil-borne pathogens are now available.

In this paper we list morphological and physiological traits considered important to increase the yield of wheat under drought in different environments. The list of traits is derived from (Richards et al. 1999). A connection between the listed trait and factor in the above identity may not be immediately apparent. Further information on the connection is given in Richards et al. (1999). Also shown is an assessment of the heritability, ease of selection and whether G x E for the trait is expected to be important. We indicate whether known molecular markers have been identified for the traits and also develop an index to indicate the importance of molecular markers to select for the trait. This index is developed from the heritability, ease of phenotypic selection, and expected G x E. Extra weighting is given to the ease of selection as this determines the number of plants that can be screened. The index should be used only as a guide, because factors like ease of selection, heritability, and expected G x E are based on the experience of the authors and these may not always translate to other environments or testing methods. Also, no consideration is given to cost.

Selection for Increased Crop Water Use

Table 1 lists characteristics that may increase water use by the crop or deeper root growth. Selection for deeper roots (provided water is available at depth) is very difficult, and the most effective methods of selection may be to use indicators that are more rapidly and easily measured. Examples of these indicators are canopy temperature, stomatal conductance, stay-green, and leaf rolling. It is unlikely that molecular markers for deep roots will be accurately identified until important variation in rooting depth of wheat has been found and environments where the deep root trait is expressed are characterised. Such variation still seems to be elusive.

Selection for Increased Water-use Efficiency

Water-use efficiency is usually defined as the ratio of above-ground biomass to evapotranspiration. It can be increased by (i) increasing transpiration efficiency (TE = the ratio of biomass to transpiration) or (ii) increasing the transpiration

Table 1. Plant characteristics that may increase soil water use or root growth

For each trait an assessment is given for the broad sense heritability whether it is easy to select, the expected importance of genotype x environment interactions, whether molecular markers have been identified and a rating for the importance of marker-assisted selection (MAS). Also, whether the trait is universally important in all rainfed environments or is associated with a particular form of drought (E specific).

	Heritability	Ease of selection	Expected GxE	Molecular marker	Importance of MAS	Universal trait or Environment specific
deeper roots	Low	Difficult	High	No	****	E specific
late flowering	High	Easy	Low	Yes	*	E specific
seedling vigour	Intermediate	Easy	Low	No	*	E specific
tiller inhibition	High	Easy	Intermediate	Yes	**	E specific
osmotic adjustment	Low	Difficult	High	Yes (?)	****	E specific

Scale of importance from low (*) to high (****).

component of evapotranspiration (i.e., reducing soil evaporation). The latter can be very low, sometimes less than 50%, where crops are reliant on current rainfall. Opportunities to improve both factors and thereby yield by breeding are large.

Reducing soil evaporation

Traits to reduce soil evaporation are given in Table 2. All traits are associated with increasing early leaf area development (increasing vigour) so that a crop canopy forms as quickly as possible and the soil surface is shaded, enabling more soil water to be used for transpiration. This is particularly important when the crop is reliant on current rainfall.

A focus of our research is to use alternative dwarfing genes to the commonly used GA-insensitive dwarfing genes *Rht-Blb* (*Rht1*) and *Rht-Dlb* (*Rht2*). These GA-insensitive dwarfing genes reduce cell length, thereby reducing coleoptile length and slowing early leaf growth to reduce crop establishment and vigour (Richards 1992; Rebetzke et al. 1999). A number of GA-sensitive dwarfing genes, both major and minor, are available to overcome the problems of poor establishment and low vigour. Molecular markers are available for some of these genes. Minor genes for coleoptile length, independent of plant height, are also important for which we are identifying molecular markers.

The traits influencing early vigour such as a large embryo size, a high specific leaf area (Lòpez-Castañeda et al. 1996) and the appearance of a coleoptile tiller (Liang and Richards 1994), which are often absent in most of our spring wheats, are being incorporated into current wheats. As yet we do not have markers for these traits.

Greater transpiration efficiency

There are numerous ways to increase TE of wheat and these are shown in Table 3. We have shown that the carbon isotope discrimination (D) of plant material is closely related to TE integrated over the life of the plant material sampled (Farquhar and Richards 1984; Condon et al. 1990). We are now selecting for D to breed wheats with a high TE and results show it to be associated with higher yields in eastern Australia.

A number of surrogates for D have also been suggested to help cull populations and these are shown in Table 3 under carbon isotope discrimination.

Harvest Index

Morphological and physiological traits discussed so far all contribute to greater yields through increases in total biomass. This biomass must then be converted to yield via a high harvest index (HI). Breeding has been effective in improving HI, and therefore yield, by reducing plant height and by reducing the duration of vegetative growth. It is likely that we are approaching the limit to gains possible by these means. However, opportunities to improve HI still remain. A list of traits to improve HI are given in Table 4.

Traits we are focusing on to increase HI are tiller inhibition and carbohydrate storage and remobilisation. Dryland cereal crops continue to produce an excessive number of tillers, about half of which die around the beginning of stem elongation. We have identified a gene on chromosome 1AS for tiller inhibition (Richards 1988) and have

Table 2. Traits to improve establishment and early canopy development of wheat. Refer to legend in Table 1 for description of headings.

	Heritability	Ease of selection	Expected GxE	Molecular marker	Importance of MAS	Universal trait or Environment specific
High priority						
long coleoptiles	High	Easy	Low	Yes	*	Universal
broad seedling leaves	High	Easy	Low	No	*	E specific
embryo size	Intermediate	Intermediate	Low	No	**	E specific
specific leaf area	Intermediate	Intermediate	High	No	****	E specific
large coleoptile tiller	Low	Easy	High	No	***	E specific
Lower priority						
large grains	High	Easy	Low	Yes (?)	*	Universal
fast emergence	Low	Easy	Low	No	**	E specific
fast leaf expansion rate	Intermediate	Easy	Low	No	*	E specific
low temperature tolerance	Intermediate	Intermediate	Low	No	**	E specific
crown depth	Intermediate	Intermediate	Intermediate	No	***	E specific
crown to shoot partitioning	Intermediate	Difficult	Low	No	***	Universal
leaf area ratio	Intermediate	Difficult	Low	No	***	E specific

identified an AFLP marker for it. This gene may also be important to maximise the storage of carbohydrate reserves in stems that are then remobilised to the grain.

Transgenic Opportunities to Manipulate Traits in Wheat for Dry Environments

Although there is considerable risk in these approaches, the technology is rapidly developing and it is appropriate to identify important processes to target. There are several physiological processes that seem worthy of pursuing for dryland environments. Firstly, the manipulation of sugar metabolism. There is evidence that pollen sterility, poor seed set, and seed abortion under drought may be due to the supply of sucrose during reproductive growth. Also, sucrose movement away from the source leaves to the most actively growing organs may limit any feedback inhibition of photosynthesis and enhance the growth of the growing organs. Manipulation of sucrose phosphate synthase, sucrose transporter, and invertase genes may be important in these processes. Secondly, the manipulation of gibberellic acid (GA) sensitivity in different organs offers potential to influence growth. GA expression may be important early in a crop's life for fast emergence and early growth but less important during stem elongation. Differential expression of genes regulating GA metabolism and reception in different organs may be important to regulate growth. Thirdly, growth and stomatal conductance may be altered by manipulating organ sensitivity to ABA. Fourthly, manipulation or synthesis of metabolites influencing plant water relations. Genomics will be important to enable mapping of these traits as well as the identification and characterisation of critical genes.

Table 3. Traits to improve the transpiration efficiency of wheat. Refer to legend in Table 1 for description of headings.

	Heritability	Ease of selection	Expected GxE	Molecular marker	Importance of MAS	Universal trait or Environment specific
Flowering time	High	Easy	Low	Yes	*	E specific
Growth at low temperature	Intermediate	Intermediate	Low	No	**	E specific
Carbon isotope discrimination	High	Easy	Low	No	*	Universal
Ash content	Intermediate	Intermediate	High	No	***	
NIR*	Intermediate	Easy	High	No	**	
SPAD*	Intermediate	Easy	Intermediate	No	**	
SLA*	Intermediate	Easy	Intermediate	No	**	
Stomatal	conductance	Intermediate	Easy	High	No	**
Canopy	temperature	Intermediate	Easy	Intermediate	No	**
Glaucousness	High	Easy	Low	Yes	*	Universal
Pubescence	High	Easy	Low	Yes	*	Universal
Residual transpiration	Intermediate	Easy	High	No	**	Universal
Leaf size and habit	Intermediate	Easy	High	No	**	Universal

*NIR: near infrared reflectance; SLA: specific leaf area, SPAD chlorophyll meter

Table 4. Traits to improve the harvest index of wheat. Refer to legend in Table 1 for description of headings.

	Heritability	Ease of selection	Expected GxE	Molecular marker	Importance of MAS	Universal trait or Environment specific
Drought Independent						
Flowering time	High	Easy	Low	Yes	*	E specific
Height and peduncle length	High	Easy	Low	Yes (?)	*	Universal
Tiller inhibition	High	Easy	Intermediate	Yes	**	E specific
Assimilate retranslocation	Intermediate	Difficult	High	No	****	Universal
Drought Dependent						
Flowering time	High	Easy	Low	Yes	*	E specific
Tiller inhibition	High	Easy	Intermediate	Yes	**	E specific
Xylem vessel diameter	Intermediate	Easy	Low	No	**	E specific
Leaf conductance	Intermediate	Intermediate	Intermediate	No	**	E specific
Stay green	Intermediate	Easy	Intermediate	No	****	E specific
Leaf rolling	Intermediate	Intermediate	High		****	
Assimilate retranslocation	Intermediate	Difficult	High	No	***	Universal

Concluding Remarks

There is no easy route to achieve genetic improvements in yield in dryland environments. Even the most assured methods, i.e., empirical breeding where plot yield is the unit of selection, is difficult and slow because of the unpredictable variation in temperature and rainfall. A physiological approach, where the underlying physiological limitations to yield can be identified, so as to more accurately target the limiting factors, also carries risks. However, if successful, the benefits are likely to be substantial. The importance of molecular methods to aid in the selection of desirable physiological types will increase. The use of these methods will depend on cost, degree of association with the trait, and the relative ease of measurement of the trait. Better definition of the limiting traits in target environments will also enable the pyramiding of traits. This will be aided by appropriate molecular markers.

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Genetic Improvement of Tolerance to Terminal Drought Stress in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Summary

Panicle harvest index (PNHI) is a useful measure of genotype response to terminal drought stress in pearl millet and therefore a potential selection criterion for terminal stress tolerance. Selection for combining ability for PNHI in potential hybrid parent test cross nurseries improved combining ability for PNHI itself and for grain yield by 5-10% in terminal stress environments (compared to mean of high and low PNHI selections), in materials otherwise unselected for stress tolerance. Selection of experimental varieties on the basis of composite progeny PNHI in terminal stress environments improved PNHI by 1–3% and grain yield by 2–8% under terminal stress (in comparison to control experimental varieties, based on randomly selected progenies). Selection of experimental varieties on the basis of grain yield in nonstressed environments resulted in decreases in PNHI of 1–7% and a decrease in grain yield of 5–19% in terminal stress environments, compared to equivalent random checks. Estimation of the components' correlated response in grain yield to selection for PNHI in mapping population progeny test crosses, indicated that the (genetic) conditions for PNHI to be an effective indirect selection criterion for grain yield under stress needs to be clearly defined.

Introduction

Genetic improvement of drought tolerance has traditionally been a problematic topic in plant breeding for a variety of reasons, among which is the lack of clearly defined selection criteria for tolerance. The effectiveness of direct selection for yield itself in stress environments is limited by the (usually substantial) magnitude of environmental and genotype x environmental variances common in drought environments. In addition, yield under stress is often not a criterion useful of tolerance per se, because yield differences under stress are strongly and variably influenced by differences in drought escape and inherent yield potential. Finally, putative physiological or biochemical resistance mechanisms are commonly too expensive to measure and are often too poorly correlated to final yield differences to use as indirect selection criteria.

The objective of the research reported in this paper has been the identification and evaluation of an effective selection criterion for improving tolerance to terminal (unrelieved post-flowering) drought stress in pearl millet (*Pennisetum glaucum* (L.) R. Br.). Our approach has been to work backwards from measured differences in grain yield in managed drought environments, to readily measurable aspects of field performance which explain those differences (Fussell et al. 1991). This led us to a model of grain yield in terminal stress environments as a function of the following (Bidinger et al. 1987a):

- yield potential (yield in the test environment, but in absence of stress)
- drought escape (estimated in terminal stress environments by time to flowering)
- drought tolerance/susceptibility (estimated from measured yield potential, drought escape and stress yield (Bidinger et al. 1987b).

Correlation analyses associated estimated drought tolerance of individual test entries with a higher percentage grain set (especially when stress began during flowering) and

with better grain filling. Both of these parameters are too expensive to measure to be used as selection criteria in routine breeding programs. However, stress-induced reduction in either or both grain number per panicle or individual grain mass directly affect panicle harvest index (PNHI = the ratio of grain mass to total panicle mass), which is readily and inexpensively measured. PNHI is a particularly effective variable for post-flowering stress, because the mass of the structural parts of the panicle (which complete their growth prior to flowering) is largely unaffected by stress, whereas the grain mass is significantly affected by both floret abortion and reduced grain filling (Bidinger and Mukuru 1995). Analyses of a number of data sets indicated positive and significant correlation between PNHI and both estimated drought tolerance/susceptibility and grain yield in terminal stress environments (F. R. Bidinger, unpublished).

Evaluation of PNHI in Hybrid Parent Breeding

We evaluated PNHI as a selection criterion in hybrid parent breeding by using the following procedure: (1) conducting divergent selection for combining ability for high and low PNHI in replicated potential B and R line test cross nurseries, grown in managed terminal drought stress environments; (2) crossing selected parents on three different A or R line testers from those used in the original test cross nurseries in which we practiced selection; and (3) evaluating these test crosses for combining ability for PNHI, grain yield and yield components, in both fully irrigated control environments and in managed stress environments. The original potential B and R line test cross nurseries were a part of the regular ICRISAT hybrid parent breeding program and had not been selected previously for terminal drought tolerance.

Table 1 presents the results of two such experiments, which compare the mean combining ability of nine high and nine low PNHI B and R line selections, in both irrigated control and managed terminal stress nurseries. In both experiments, the differences between the high and low PNHI selections in the irrigated control environments were small and generally not statistically significant (1% for PNHI itself, 2% for grain yield and 3% for seed mass, Table 1). Differences in the terminal stress environment between the high and low selections were generally statistically significant and of a greater magnitude. For example, the combining ability of high PNHI selections exceeded that of the low PNHI selections by approximately 5–8% for PNHI itself, by 9–13 % for grain yield and by 6–7% for seed mass (Table 1).

Thus selection for or against combining ability for PNHI under terminal stress had little effect on the combining ability of elite parental lines in nonstress conditions, but resulted in a significant difference in their combining ability for both PNHI itself and for grain yield under terminal stress. The actual gain in combining ability for grain yield—assuming that one half of the difference between the means of the divergent selections represent the difference between the high PNHI selections and the mean of the original set of parental lines—was about 6% (Table 1). This gain was not large in absolute terms, but was

Table 1. Combining ability for panicle harvest index (PNHI), yield and yield components of restorer and maintainer lines selected for high (9 lines) and low (9 lines) combining ability for PNHI, in test cross nurseries grown under terminal drought stress at ICRISAT Patancheru. Data are means of three test crosses per line and 3 years replicated evaluations in fully irrigated control and managed terminal stress environments in the dry season at ICRISAT, Patancheru.

	PNHI (%)	Grain yield (g m^{-2})	Grain no. (10^3 m^{-2})	Seed mass (mg $seed^{-1}$)
Restorer lines				
Irrigated environments				
high PNHI selections	76.8	355	38.2	9.45
low PNHI selections	75.3	347	38.6	9.22
SED	0.22	2.9	3.7	0.552
Terminal stress environments				
high PNHI selections	64.8	218	31.1	6.86
low PNHI selections	59.8	192	29.5	6.38
SED	0.44	2.7	3.74	0.691
Maintainer lines				
Irrigated environments				
high PNHI selections	75.5	327	38.3	8.62
low PNHI selections	74.7	321	39.2	8.35
SED	0.21	3.1	4.22	0.468
Terminal stress environments				
high PNHI selections	63.6	189	29.7	6.31
low PNHI selections	60.4	173	28.9	5.93
SED	0.40	2.8	3.87	0.564

achieved at a very reasonable cost—an additional evaluation environment for the potential parental line test cross trial—and without any evident penalty in non-stress environments.

The combining ability of best individual parental lines in the high PNHI selection group exceeded the overall mean of all lines in the trial by a greater margin in the terminal stress environment (data not presented). The mean of the best two R lines was 67.2% for PNHI, compared to a trial mean of 62.3% (13% gain). This was accompanied by a grain yield of 224 g m^{-2}, compared to a trial mean 205 g m^{-2} (9% gain). Similarly the combining ability of the two best B lines for PNHI was 67.5%, compared to a trial mean of 62.0% (9% gain) which was accompanied by a yield of 199 g m^{-2} compared to a trial mean of 181 g m^{-2} (10% gain).

Evaluation of PNHI in Open-pollinated Variety Breeding

We also evaluated PNHI as a selection criterion for experimental varieties with improved tolerance to terminal stress using S1 progeny selection in a random mating population. We compared selection on the basis of PNHI under terminal stress (PNHI/stress) to two controls: selection on the basis of grain yield in a paired irrigated control environment (yield/control) and selection of random S1 progenies (random check). We conducted two cycles of selection, using S1's from the parent population in the first cycle, and S1's from two subpopulations (formed from 50 progenies from the first cycle) representing the PNHI/stress and yield/control selection alternatives, in the second cycle.

The first cycle PNHI/stress experimental variety significantly outperformed the yield/control variety in both the irrigated control and terminal stress evaluation environments. The PNHI/stress variety had a 3% greater PNHI and an 8% greater yield in the control environment and a 9% greater PNHI and 27% greater yield in the terminal stress environment (Table 2). The difference however, seemed to be due to a poor performance of the yield/control variety, rather than an exceptional performance of the PNHI/stress variety. The PNHI/stress variety had a 1% greater PNHI than the random check in both evaluation environments and 7% and 2% greater yields in the control and stress environments, respectively, indicating a positive, but modest, response to selection.

In the second cycle of selection the PNHI/stress variety had a 1% greater PNHI and a 3% greater yield than the yield/control variety in the control environment and a 2% greater PNHI and a 5% greater yield in the stress environments (Table 2).

Table 2. Panicle harvest index (PNHI), yield and yield components of experimental varieties made by random-mating 25 S1 progenies selected from the pearl millet Early Composite 1987 on the basis of either PNHI in terminal stress environments or grain yield in irrigated control environments at ICRISAT Patancheru. The random check is the mean of two experimental varieties made by random mating 25 different randomly selected S1 progenies, from the base population in cycle 1 and from subpopulations derived from selection for PNHI in stress or yield in control in cycle 2. Data are means of 4 years replicated evaluations for cycle 1 and 3 years for cycle 2, in fully irrigated control and managed terminal stress environments in the dry season at ICRISAT, Patancheru.

	PNHI (%)	Grain yield (g m^{-2})	Grain no. (10^3 m^{-2})	Seed mass (mg $seed^{-1}$)
First cycle of selection				
Irrigated environments				
PNHI/stress sel.	80.0	384	38.7	9.98
yield control sel.	77.9	354	38.8	9.10
random check	79.3	360	37.1	9.69
SED	0.58	11.3	1.48	0.222
Terminal stress environments				
PNHI/stress sel.	67.7	171	25.9	6.59
yield control sel.	62.3	135	23.0	5.83
random check	67.1	167	25.4	6.57
SED	1.43	9.7	1.44	0.255
Second cycle of selection				
Irrigated environments				
PNHI/stress sel.	79.2	355	36.1	9.84
yield control sel.	78.5	344	37.5	9.22
random check (PNHI)	77.9	321	33.6	9.57
random check (yield)	77.1	313	32.8	9.62
SED	0.98	13.0	1.68	0.256
Terminal stress environments				
PNHI/stress sel.	70.6	203	25.2	7.90
yield control sel.	69.1	193	25.8	7.46
random check (PNHI)	68.4	188	24.4	7.61
random check (yield)	70.0	203	25.9	7.77
SED	1.26	13.0	1.68	0.303

None of these differences were statistically significant. Since the cycle 2 varieties were selected from different subpopulations, a better evaluation of the effectiveness of the selection criteria is the relative gain over the appropriate check variety. The PNHI/stress variety had 2% greater PNHI and 11% greater yield in the control environment and a 3% greater PNHI and an 8% greater yield in the stress environment, in comparison to its check variety. The yield/control variety had a similar gain over its check in the control (2 and 10% for PNHI and yield, respectively), but had a 1% lower PNHI and a 5% lower yield than its check variety in the stress (Table 2).

Thus in both cycles of selection, the performance of the PNHI/stress variety was at least equal to that of the yield control/variety in the irrigated control evaluation environments, indicating again that selection on the basis of PNHI in a terminal stress environment does not carry a yield penalty in nonstress environments. Selecting on the basis of PNHI in a terminal stress environment resulted in modest gains in performance in this environment in comparison to the randomly selected check variety (1–3% in PNHI and 2–8% in yield). In contrast, selection for yield in irrigated control environments resulted in negative progress in the terminal stress environment, in comparison to the check variety (-1 to -7% in PNHI and -5 to -19% in yield). Therefore, where terminal stress in a major feature of the target environment, it would appear to be very effective to integrate selection for PNHI under stress into a variety breeding program .

Genetics of PNHI as an Indirect Selection Criterion

An analysis of the components of the correlated response to selection provides a means of evaluating the likely effectiveness of PNHI as an indirect selection criterion for grain yield. For PNHI to be an effective indirect selection criterion, the correlated response of grain yield to selection for PNHI should exceed the response to direct selection for yield itself, at the same selection intensity. This will occur when the product of the genetic correlation of PNHI with grain yield by the heritability of PNHI exceeds the heritability of grain yield ($r_g h_{PNHI} > h_{YLD}$, Falconer 1981). Situations in which indirect selection is likely to be superior to direct selection are mainly those in which the application of direct selection is technically difficult, for example, the target trait may be difficult to measure with precision resulting in low heritability (Falconer 1981). This is frequently the case with grain yield in stress environments. Indirect selection may also be of interest when the target trait is costlier to measure than the indirect selection criterion.

We used data from test crosses of 92 randomly selected F4 lines, derived from a cross of two inbred pollinators (Yadav et al. 1999), to estimate the necessary genetic parameters to compare direct selection for grain yield with indirect selection for PNHI. The estimates are based on a random linear model and a 5% selection intensity. The 92 lines, along with their parents, were tested in a randomized complete block design (trials DN 96, DN 97, ROS 97) or an alpha design (trial ROS 98) with three replications both under stress and irrigated control conditions. We felt that this data set better met the requirements for estimation of selection response than the data from our individual selection experiments.

In the four individual trials, PNHI generally had a greater genetic variance under stress than in irrigated conditions. Grain yield, in contrast, exhibited consistently lower genetic variance under stress than in irrigated control conditions (Table 3). This is reflected in PNHI generally having an equal or higher heritability than grain yield under stress. Phenotypic correlations between PNHI and grain yield under stress were generally higher than or equal to those in irrigated conditions. Genetic correlations , however, were generally higher under irrigated than stress conditions, except in trial ROS 98 (Table 3). In all four trials, under stress as well as under irrigation, the predicted correlated response to selection for PNHI was much lower than the response to direct selection for grain yield.

Pooled analysis of the four trials indicated no genotype x year (G x E) interactions for grain yield in either stress or irrigated environments, nor for PNHI under irrigation. Under stress, however, PNHI was subject to a considerable genotype-environment interaction (P=0.009). This, coupled with a lower genetic variance under stress, resulted in a heritability for PNHI of 23% under stress. This, in contrast to the trend in individual trials, was lower in stress than under irrigation, and was effectively the same as that for yield

under stress. This, plus a low genetic correlation of PNHI and yield, resulted in a lower predicted response to indirect than to direct selection, under stress as well as under irrigation. The analysis of predicted response to selection for PNHI thus did not indicate that PNHI would be an effective indirect selection criterion for improved yield under stress in the test crossed mapping population lines. This is in contrast to the results achieved in the actual selection experiments, and suggests that the requisite (genetic) pre-conditions for PNHI to be an effective indirect selection criterion for improved terminal stress tolerance need to be carefully defined.

Table 3. Estimates of genetic parameters for panicle harvest index (PNHI, %) and grain yield (YLD, gm^{-2}) measured under terminal stress and irrigated control environments in four replicated trials conducted during 1996-98. Data are from 92 random F4 lines derived from the cross between inbred pollinators H77/833-2 and PRLT 2/89-33. σ^2_g = genetic variance, h^2 = line-mean heritability, r_g and r_p = genetic and phenotypic correlation between PNHI and grain yield, and DR and CR = direct and correlated response to selection at a 5% selection intensity.

Trial Parameter	Environment	DN 96	DN 97	ROS 97	ROS 98	Pooled
$\sigma^2_{g\ (PNHI)}$	Control	7.28	1.52	0.61	0.97	1.02
	Stress	0.00	6.65	1.25	5.36	0.57
$\sigma^2_{g\ (YLD)}$	Control	267.8	466.1	503.9	1110.7	311.0
	Stress	68.5	13.5	0.00	288.2	43.0
$h^2_{(PNHI)}$	Control	29.18	27.42	6.28	22.12	27.72
	Stress	0.00	63.70	10.89	31.60	23.16
$h^2_{(YLD)}$	Control	22.12	53.37	29.23	58.02	55.31
	Stress	7.15	38.75	0.00	34.26	23.51
r_g	Control	0.65	1.00	-0.12	0.38	0.78
	Stress	0.00	0.55	0.00	0.61	0.37
r_p	Control	0.34	0.47	0.38	0.42	0.39
	Stress	0.48	0.47	0.56	0.76	0.50
$DR_{(YLD)}$	Control	15.88	32.54	25.04	52.37	27.06
	Stress	4.57	18.76	0.00	20.50	6.56
$CR_{(YLD)}$	Control	11.85	23.32	-1.39	12.29	14.94
	Stress	0.00	13.23	0.00	12.01	2.41
$CR_{(YLD)}/DR_{(YLD)}$	Control	0.75	0.72	-0.06	0.23	0.55
	Stress	0.00	0.71	0.00	0.59	0.37

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Can Biotechnology Bridge the Gap for Resource Poor Farmers?

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Introduction

Effective maize breeding in Zimbabwe created the need to create business structures to multiply and market superior hybrids. The seed company created has been successful to the point that large volumes of adapted, high-quality seed have been made available and are purchased annually, even by smallholder farmers. The almost total adoption of hybrid seed would tend to indicate that these farmers are ready to apply reasonably priced improved technology.

History of Research and its Adoption in Zimbabwe

Research focused on breeding hybrid maize began in 1932 at the Harare Research Station, which is still today part of the Ministry of Agriculture. The first commercial maize hybrids were released by this station and introduced for sale by the Seed Maize Association in 1947. SR52, the world's first commercial single cross hybrid, was officially released in 1960. In the 1970s several earlier maturing hybrids better adapted to smallholder production were released. The availability of these hybrids together with improved seed quality stimulated the adoption of hybrid seed by Zimbabwean smallholder farmers.

An important key in the development of the Zimbabwean seed industry was the signing of legal agreements between the Ministry of Agriculture and Seed Co that gave the company the exclusive right to multiply and market a range of government-bred products. In exchange, the company had to undertake to produce agreed upon volumes of seed, including a 20–30% carryover, and to sell seed at agreed prices. These agreements have resulted in large volumes of quality seed being made available to Zimbabwean farmers at prices three times lower than those in South Africa and approximately one-ninth the price in the United States. These agreements have frequently been criticised, but they have been the cornerstone of a public/private partnership that has facilitated the development of the seed industry. In other developing countries, where the seed industry has yet to emerge, this model may be worth emulating.

Ongoing Commitment to Research

- Seed Co now employs nine breeders and owns two research stations;
- Rattray Arnold, purchased in 1973, is in a high potential area and best serves the needs of commercial farmers;
- Kadoma, purchased in 1996, is in an environment that is more typical of the hot dry conditions encountered by the majority of Zimbabwe's smallholder farmers.

The biotechnology revolution is expected to produce technology that will have significant benefits for resource poor farmers. However, since seed will be the vehicle for deploying these technologies, an analysis of maize yields, possible biotechnology costs, and returns are being evaluated.

Extensive trials are also carried out country-wide, in both large- and small-scale farming environments. Collectively, in 1998/99, more than 700 replicated trials on over 100,000 field plots were planted and will be analysed before next summer. In addition, demonstration blocks at approximately 200 sites featuring newly released materials are being grown and used effectively for field day events. By far the majority of these efforts are aimed at smallholder farmers.

These research programmes now operate competitively with those from Cargill, Pannar, and Pioneer, in parallel with the national programmes and increasingly in partnership with those from CIMMYT and ICRISAT. The clear message is that with appropriate marketing strategies, it is economically attractive for the private sector to invest in crop breeding focused on resource poor smallholder farmers.

The Development of the Zimbabwe Maize Seed Market

After the first double hybrids were released in 1949, adoption by commercial farmers was so rapid that within two years more than half the commercial crop was planted to hybrids. The release of SR52 in 1960 further stimulated the use of hybrid seed, and by 1970, 98% of the commercial area was planted to this hybrid. This coincided with the development of the fertiliser industry and the widespread application of fertilisers on maize. Consequently, between 1949 and 1970, commercial maize yields increased from approximately 1 t/ha to 5 t/ha.

With the release of short season maize hybrids in the early 1970s, adoption of hybrid seed by smallholders gained momentum. The area planted to smallholder maize increased from 600,000 ha in 1979 to 1,074,000 ha in 1986, a 79% increase. Over the same period, seed sales trebled. Yields over this period increased from approximately 0.7 to 1.2 t/ha.

Today, almost 100% of smallholder maize is grown to hybrid seed and is sold in package sizes between 0.5 and 50 kg. Everybody, irrespective of plot size, purchases hybrid seed! There were a number of factors that have contributed to this remarkable story:

- Return to peace and political stability at Independence in 1980;
- Attractive commodity prices;
- Effective commodity purchasing by the Grain Marketing Board;
- Commitment and field demonstrations by the government extension agency;
- Cost benefits are clearly evident even to smallholder farmers; and;
- Production and wide distribution of small packs of quality seed at relatively low prices (US$500 - 600/t)

The success of this model both can and needs to be replicated in other developing countries. This model can become a powerful tool in the deployment of conventional technologies and biotechnologies to smallholder farmers.

Seed Co Limited—The Result of Evolution

The origins of Seed Co date back to 1940, with the formation of the Seed Maize Association, which was requested by the Government to multiply and market popular open pollinated maize varieties.

The Crop Seeds Association was formed in 1957, initially to concentrate on improved wheat, soybean, and groundnut seed production. Later developments embraced sunflower, sorghum, millets, barley, and bean seed crops.

In 1983 the Zimbabwe Seed Maize and Crop Seeds Associations merged to form the Seed Co-operative Company of Zimbabwe Limited. Given their similar objectives, this merger promoted more efficient resource use.

In mid-1996, the company was renamed Seed Co Limited and was listed on the Zimbabwe Stock Exchange. This significant event would made shares available to all interested investors; provided the means to raise additional capital; and made management more accountable for the company's performance.

This evolution of Seed Co was precipitated by the increasing complexity of seed production, rapidly increasing seed volumes, changing economic conditions and the need to compete with global players. What started in 1940 as a relatively insignificant grouping of farsighted maize growers has evolved over 60 years to the largest seed company in the region.

In order to bridge the gap for resource poor farmers, between research and field application of appropriate technology, linkages must be created and nurtured. **The combined resources of CGIAR Centres, NARS, NGO's, donors and the private sector will need to be focused specifically at deploying this technology with the sole aim of raising productivity at smallholder level.**

The Urgent Need for Advanced Technology

As indicated above, smallholder farmers rapidly adopted the technology provided in hybrid seeds. While yields rapidly increased at that time, further increases have not been sustained. A succession of droughts, abnormally wet years, and the advent of gray leaf spot may be partly responsible.

The area planted to maize in the USA and the whole of sub-Saharan Africa are of similar orders of magnitude (Table 1). However, yields in the USA are almost six times greater than those in Africa. Furthermore, it is projected that the population in the region will approximately double in 20 years. We have no option but to reach sustainable yields of 2.5–3.0 t/ha by 2020!

How to Double Smallholder Yields in Twenty Years

Significantly increasing yields on smallholder farms is no small task. The development, deployment, and adoption of affordable and sustainable technology is the key to raising productivity of resource poor farmers. There is a long list of issues that need to be grappled with. It may, however, be appropriate only to mention a few that may likely be addressed by a group of agriculturalists:

- water conservation technologies;
- improved agronomic practices (planting dates, weed control, etc.);
- soil management;
- insect resistance;
- cultivars better adapted to low soil fertility; and
- cultivars more tolerant to frequent droughts.

There is need to develop appropriate "best practices" for different ecologies and economic situations. These practices need to be understood and supported by all who address the needs of smallholder farmers. Based on these best practices, breeders should use appropriate breeding and biotechnological techniques to develop cultivars that will be more productive in drought prone areas.

Table 1. Comparison of USA and sub-Saharan Africa maize production

	USA	Sub-Saharan Africa
Hectares planted (million)	32,6	24.54
Yield (t/ha)	8,06	1.39
Production (million tons)	262,76	33.99
Estimated exports %	21	-

USA - Adapted from Soyabean Digest, January 1998
Africa - From Wold Maize Facts and Trends 1997/98

Improving Drought Tolerance

In recent years, CIMMYT has demonstrated several breeding strategies that can produce maize cultivars with both higher and more stable yields in water-stressed environments; approaches include the selection of maize under carefully managed drought stress and selection of secondary traits that have been demonstrated to be associated with higher levels of drought tolerance

These approaches are now in the process of adoption by public and private breeding programmes, which should result in the release of improved cultivars for use by farmers. Although CIMMYT has already developed more drought tolerant germplasm, other breeders have only recently committed themselves to applying these breeding strategies. The impact of these screening techniques should become evident during the next five years, as a critical mass of breeding effort becomes targeted to drought.

Another very powerful tool, molecular markers, also appears to be on the verge of making a considerable impact. Molecular marker techniques have been used to identify quantitative trait loci (QTLs) associated with drought tolerant germplasm. Such techniques can be used to promote the transfer of traits associated with drought tolerance to locally adapted elite lines. By identifying the QTLs of interest, CIMMYT may be in a position to ensure that the technology and useful germplasm is accessible to developing countries at a minimal cost.

Biotechnology Cost Implications

Transgenic maize, soybeans, and cotton cultivars have been rapidly adopted by farmers in the USA and now constitute approximately half of the areas planted to these crops. These technologies offer the world's most competitive farmers a number of benefits, including

- improved weed control;
- decreased losses from insects, particularly stalk borer and cotton boll worms;
- improved nutritional quality; and
- decreased losses to fungal and viral diseases.

After three to four years of marketing this technology in the USA, some knowledge of the cost structure is beginning to emerge.

It appears that a 3:1 philosophy may be influencing price policy at present. In other words, for every dollar charged for the new transgenic variety, the farmer could expect an additional net return of three dollars. However, in all likelihood, like all new technologies, when it becomes more freely available from more competitive sources, the the price will decline. It goes without saying that farmers are only going to purchase this technology if they believe they will generate higher revenues. This principle would apply equally in Africa, except that the capacity of smallholder farmers to pay significantly more upfront for their seed is limited.

Bt Maize

The argument developed here on Bt maize is somewhat theoretical because little research has been

conducted in Africa and specific cost benefit analysis has not yet been conducted. This scenario is developed to support the contention that there is a real need for public sector work in biotechnology in order to make it available to the poorest farmers.

In 1996, Bt maize sold at a premium of approximately US$25 for an 80,000 kernel unit, almost equivalent to US$25/ha. By 1998, this figure had declined (from some seed companies) to US$15/ha. Yield gains of 10% (worth US $110/ha) when the targeted pests are present have been widely reported. Meanwhile, a single control application costs US $50/ha. American farmers are so convinced of the technology that seed companies cannot keep up with the demand.

In the African context, stalk borer is a major pest of maize, causing economic losses in most of sub-Saharan Africa. Since little research work in this area has yet been conducted in Africa, it may only be speculated that 10% more harvestable yield may result from the adoption of Bt (or similar trait) maize on smallholder farms. At a grain price of US $120/t and a seed premium of US $15/ha, we anticipate the returns presented in Table 2.

Clearly significant benefits may result to smallholder farmers, even at relatively low yields. The impact of the technology cost on the selling price of seed remains an issue that will need careful analysis. However, in order to justify the additional upfront costs to farmers, it may be necessary to achieve an additional US $3 return for every dollar invested. Based on this model, such a return may only be achieved at a yield level close to 4 t/ha. It is hoped that technology owners will, however, adopt affordable marketing strategies in seeking to raise productivity among the world's poorest farmers.

Seed of SC501, which is a popular hybrid with Zimbabwean smallholder farmers will cost US $15.80/ha. If the technology cost were $15 /ha, the SC501 seed price would almost double (95%), SC709, a high potential hybrid used by commercial farmers, would be less affected (34%), while the American hybrid would be affected even less (15%). However, it is likely that smallholder farmers in Africa will achieve in excess of 10% yield gains in many situations, depending on the technology applied. The aim of the CIMMYT drought project is to release maize cultivars that have 25% more yield under drought stress conditions.

Conditions of Licensing for Farmers in the USA

Licensing agreements have been put in place to ensure that the rights of the technology owner are recognised, to ensure a constant flow of licence fees, and to ensure that biosafety regulations are adhered to.

The grower agrees to

- comply with pest resistance management protocols designated by the EPA and vendor.
- comply with the one-time use of seed restriction.
- accept a non-compliance penalty.
- Return all unused seed to the dealer.
- allow field inspection for three years.
- herbicide usage that may be restricted to that of the technology owner.

In exchange the vendor will agrees to

- issue a licence for the use of the technology.
- provide the same-season replanting without an additional licence fee.
- provide grower training for optimal use of the enhanced seed.
- monitor the resistance control programme.

The legitimate right of technology developers to derive income is acknowledged. However, many of

Table 2. Possible return per dollar invested in Bt maize hybrids at different maize yields

Farm yield (t/ha)	Grain value (US$/ha)	Yield gain (US$/ha)	Return per dollar invested
1	120	12	0.8
2	240	24	1.6
3	360	36	2.4
4	480	48	3.2
8	960	96	6.4

Table 3. Comparison of possible Bt seed premiums on two Zimbabwean and one USA maize hybrid

Hybrid	Hybrid use	Seed cost US $/t	Seed cost US $/ha	Bt premium %
SC 501	Low potential smallholder	630	15.8	95
SC 709	High potential commercial	1740	43.5	34
USA	High potential commercial	4000	100	15

these conditions may make access to this technology extremely difficult in developing country environments.

Linkages, Costs, and Farmers in Developing Countries

The position of the CGIAR Centres in relation to the development of germplasm, farming systems, and so forth is clear. The fruit of such publicly funded research rests essentially in the public domain and should be available to all at negligible cost.

At both public and private breeding institutions, the costs of traditional cultivar development are known and can usually be managed using locally available resources. However, on entering the realm of biotechnology, the resources rise to a level far beyond the means of most developing world organisations—irrespective of perceived size and profitability.

In this context, Monsanto is reported to have invested US $500 million over 10 years in the development of Roundup Ready soybeans, and as a result has a legitimate need to recover this investment (Fede 1996). Companies recognise long-term (in the seed context) opportunities for business development and as a result invest considerable resources in research. Failure to recognise this could dampen commitment to future research and may make foreign technology more difficult to acquire, particularly in the developing world.

In conclusion, as we accept the urgency of sustainably raising smallholder productivity and the role that biotechnology may play in this process, at the same time we note a number of key questions that demand answers:

- What is appropriate technology?
- How will it be promoted?
- Who will bridge the research to farmer gaps?
- What costs are involved?
- Who will pay these costs?

In the legitimate interests of advancing science, many R&D agreements have been signed. Such science may have a massive impact on smallholder productivity, but we need to do more thinking and planning in the area of deployment and costs.

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Breeding for Drought Tolerance in Tropical Maize–Conventional Approaches and Challenges to Molecular Approaches

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Summary

Most maize in the developing world is grown under rainfed conditions and annually an estimated 24 million tons of maize are lost to drought. This paper gives a brief overview of the conventional breeding approach taken by CIMMYT in developing drought tolerant maize germplasm. Typically, progenies are evaluated in replicated trials under managed drought stress, and index selection that considers primary and secondary traits is used to identify superior germplasm. Estimates of progress of selection have been calculated. They averaged around 100 kg ha^{-1} year^{-1} of improvement. Selection gains carried over from open-pollinated varieties to hybrids and they proved to be consistent across drought environments. Improved performance under drought was largely the result of improved flowering synchronization, reduced barrenness, and an increase in harvest index; associated QTLs have been identified. Little change in water uptake and water use efficiency was found. Challenges to molecular approaches lay in (i) identifying the genes underlying known drought-adaptive traits; (ii) exploring additional traits that confer drought tolerance; and (iii) cost-effectively deploying molecular techniques that improve the drought tolerance in adapted germplasm.

Introduction

With most maize in the developing world being grown under rain-fed conditions and the proportion of maize grown in marginal areas increasing, breeding for tolerance to drought has become a major focus of CIMMYT's Maize Program. This investment seems justified as annually an estimated 24 million tons of maize are lost to drought, and yield increases in high-potential regions alone are unlikely to meet the projected increase in demand for maize over the next decades (Heisey and Edmeades 1999). Seed-based technologies are relatively easy to disseminate, and drought tolerant cultivars seem, therefore, key to future yield and productivity gains in marginal areas. Drought-tolerant cultivars increase and secure economic returns to labor, supplies, and land. They may therefore trigger farmers in those regions to invest in complementary improved agronomic practices, such as fertilizer applications and soil and water conservation techniques.

This presentation gives a brief overview of the selection approach taken by CIMMYT in developing drought-tolerant maize germplasm, the extent and background of progress made through conventional means, and resultant challenges to molecular breeding approaches.

Selection Approach

CIMMYT initiated breeding for drought tolerance in maize in 1975 and progress was such that selection for this trait has become routine for maize germplasm improved by the center Typically, progenies (early generation lines, inbreds, hybrids, testcrosses, OPVs) are evaluated in replicated trials at one or two drought stress levels during a rain-free period using irrigation. Drought is applied during flowering and grain filling such that average grain yield in these trials is reduced to 30–60% (intermediate stress level, grain-filling stress) or 15–30% (severe stress level, combined flowering and grain-filling stress), respectively, of unstressed

yields. The same progenies are additionally grown under well-watered conditions during the main season. Selection is for an index that seeks to maintain time from sowing to anthesis; maintain or increase grain yield under well-watered conditions; increase grain yield under drought; and decrease anthesis-silking interval (ASI), barrenness, the rate of leaf senescence, and leaf rolling under drought (Bolaños and Edmeades 1993a, 1993b; Bolaños et al. 1993; Byrne et al. 1995; Beck et al. 1996; Edmeades et al. 1999). Other breeding goals, such as yield potential, disease resistance, and grain quality, are also considered, based on observations made with the same progenies in trials grown during the main cropping season.

Evidence of Progress

Estimates of progress from this selection approach have been established using a wide range of germplasm (Bolaños and Edmeades 1993a, 1993b; Bolaños et al. 1993; Byrne et al. 1995; Chapman and Edmeades 1999; Edmeades et al. 1999). Depending on selection scheme and selection intensity used, yield increases of 59 to 233 kg/ha^{-1} cycle^{-1} of recurrent full-sib or S_1 selection were measured. Gains proved to be fairly similar across drought-stressed and well-watered conditions i.e., under conditions that produced average trial yields of less than 1 t/ha^{-1} to more than 10 t/ha^{-1} (Table 1). Although a considerable part of CIMMYT's drought tolerance research has focused on open-pollinated varieties (OPVs), evidence suggests that improvements carry over to lines and hybrids. Hybrids derived from drought-tolerant populations outyielded those derived from equivalent non-drought tolerant populations by 20%, at a mean drought-induced yield level of 1.6 t/ha^{-1} . The probability of obtaining a hybrid with a yield of 30-50% greater than this mean value was 3–5 times greater when lines were derived from drought-tolerant sources, compared with conventionally-selected source populations (Edmeades et al. 1997).

The majority of CIMMYT's breeding work for drought tolerance was done in Mexico. In 1996, lines selected for drought tolerance at CIMMYT Mexico were introduced to southern Africa and testcrossed to two non-drought tolerant local inbreds (CML202 and CML206) developed by CIMMYT-Zimbabwe. The resulting testcrosses were evaluated together with released commercial hybrids from southern Africa at four to five locations in Zimbabwe, one of them under managed drought stress. Tables 2 and 3 show the performance of the "drought-tolerant" testcrosses versus the local check hybrids for the highest-yielding site (Kadoma) where well-watered growing conditions can be assumed, and for the severely drought-stressed site (Chiredzi). Yields averaged around 9 to 10 t/ha under optimal conditions, and between 2 and 3 t/ha under drought-stressed conditions. Under optimal conditions, the commercial check hybrids performed, on average, better than the drought-tolerant testcrosses. Under drought-stressed conditions, the commercial hybrids yielded, on average, considerably less than the random set of drought-tolerant testcrosses from Mexico. Thus, even though the local check hybrids have

Table 1. Effects of selection for drought tolerance on gains per selection cycle in four maize populations when evaluated at 3-6 drought stressed (SS) sites, and at 5-8 well-watered (WW) sites. Locations were in Mexico (Mex.) or outside (Int.). *, **, ns: significant rate of change per selection cycle at *P*<0.01, *P*<0.05, or *P* >0.05 (Beck et al. 1996).

Population	Grain yield	
	Drought stressed	Well watered
	kg ha^{-1}	
Evaluation 1988/91		
Tuxpeño Seq. (Mex.)	100 **	125 **
Tuxpeño Seq. (Int.)	52 ns	101 **
Evaluation 1992/4		
La Posta Seq. (Mex.)	229 **	53 ns
Pool 26 Seq. (Mex.)	288 **	177 **
Tuxpeño Seq. (Mex.)	80 **	38 **
Pool 18 Seq. (Mex.)	146 **	126 **

Table 2. Results of 93 testcrosses of early-maturing drought tolerant lines from Mexico and 7 local check hybrids under optimal conditions (Kadoma; main season 1996/97) and under managed drought stress (Chiredzi; dry season 1997) in Zimbabwe.

Entry #	Pedigree	# Entries	Grain yield			
			Well-watered		Drought stressed	
			t ha^{-1}	Rank	t ha^{-1}	Rank
Mean drought-tolerant top crosses		**93**	**9.03**	**53**	**2.44**	**49**
Mean local checks		**7**	**10.68**	**19**	**1.95**	**71**
94	R201		11.68	3	1.37	94
95	R215		10.80	10	1.69	82
96	SC401		10.00	25	2.41	51
97	SC501		12.85	1	1.72	81
98	CG4141		9.90	28	1.79	78
99	PAN473		9.88	29	2.42	50
100	PAN 6363		9.66	37	2.27	58
Mean			**9.16**	**50**	**2.40**	**51**
LSD			**2.00**		**1.44**	
P			*******		*******	
Min			**5.83**	**1**	**0.68**	**1**
Max			**12.85**	**100**	**4.82**	**100**

been developed using multilocation testing that supposedly included results from randomly drought stressed sites, deliberate improvement for drought tolerance led to maize germplasm with much higher drought tolerance.

What Changed with Selecting for Drought Tolerance?

Passioura (1977) proposed the following framework for grain yield produced in a water-limited environment:

$$GY = W * WUE * HI$$

where: W is the water transpired by the crop; WUE is the water use efficiency, i.e., the amount of biomass produced per unit of water transpired; and HI is the harvest index.

In CIMMYT's drought breeding program, selection gains were largely the result of reduced barrenness under drought, and, using Passioura's framework, an associated increase in harvest index (Bolaños and Edmeades 1993a). The anthesis-silking interval became shorter under drought, and selection seemed to have led to significantly faster spikelet and ear growth at flowering, but also to a reduction in final spikelet number. Fewer spikelets were formed, grew more rapidly, and were ultimately more successful in forming grain, especially under conditions of drought at flowering (Edmeades et al. 1993). Except for one population, La Posta Sequía, total biomass production was unaffected by selection (Edmeades et al. 1999), and there was no change in any trait indicative of plant water status (e.g., predawn or noon water potential; osmotic adjustment; canopy temperature, water extraction profiles) in one population examined in detail (Bolaños et al. 1993). Gains under water deficits were at no cost to yield in unstressed environments.

Table 3. Results of 208 testcrosses of late-maturing drought tolerant lines from Mexico and 8 local check hybrids under optimal conditions (Kadoma; main season 1996/97) and under managed drought stress (Chiredzi; dry season 1997) in Zimbabwe.

Entry #	Pedigree	# Entries	Grain yield			
			Well-watered		Drought stressed	
			t ha^{-1}	Rank	t ha^{-1}	Rank
Mean drought-tolerant topcrosses		**208**	**10.06**	**111**	**2.84**	**108**
Mean local checks		**8**	**11.49**	**56**	**2.08**	**145**
209	SC701		10.55	80	0.75	217
210	SC707		10.30	98	0.51	218
211	SC709		14.00	1	1.24	214
212	ZS206		12.75	7	1.32	213
213	PAN695		9.95	114	3.22	68
214	CX5003		11.15	42	2.98	91
215	CX5005		13.10	4	2.64	124
216	CX5019		10.15	103	3.97	17
Mean			**10.12**	**109**	**2.81**	**109**
LSD			**2.46**		**1.56**	
P			*******		*******	
Min			**5.85**	**1**	**0.51**	**1**
Max			**14.00**	**218**	**5.27**	**218**

Bolaños and Edmeades (1996) determined genotypic correlations between a range of secondary traits and grain yield under drought stress for more than 3,500 S_1 progenies from several populations. Traits indicative of reproductive success (e.g., kernel number per plant, ears per plant and anthesis-silking interval) explained much more of the variation in grain yield than traits indicative of plant water status and water use efficiency (e.g., leaf extension rate, canopy temperature, leaf chlorophyll concentration, leaf erectness, leaf rolling, and leaf senescence) (Table 4).

Table 4. Broad sense heritabilities observed under severe drought stress, and genetic correlations between grain yield and selected traits under severe drought stress for S_1 progenies drawn from several maize populations. Heritability of grain yield under severe stress was 0.43 ± 0.10, and yields averaged 14% of well-watered plots. For details, see Bolaños and Edmeades (1996).

	No. trials	Heritability under stress	Genotypic correlation
Ears plant^{-1}	9	0.54 ± 0.08	0.90 ± 0.14
Kernels ear-1	8	0.39 ± 0.13	0.71 ± 0.22
Kernels plant^{-1}	8	0.47 ± 0.08	0.86 ± 0.15
Kernel weight	9	0.43 ± 0.14	0.14 ± 0.17
Days to anthesis	9	0.72 ± 0.08	-0.58 ±0.12
ASI	8	0.51 ± 0.12	-0.60 ± 0.24
Leaf rolling score	9	0.52 ± 0.09	-0.03 ± 0.15
Leaf erectness score	1	0.74 ± 0.07[a]	-0.28 ± 0.19[b]
Leaf senescence score	9	0.54 ± 0.08	0.14 ± 0.15
Canopy temperature	4	0.25 ± 0.05	-0.20 ±0.15
Tassel branch number	1	0.82 ± 0.04[a]	0.15[b]

[a] Trait observed under well-watered conditions
[b] Observed in S_2 or S_3 progenies under severe drought stress

The fact that we found little change in water uptake and water use efficiency during conventional selection may be a reflection of the restrictions imposed by our selection approach rather than the general usefulness of these traits. For a trait to be considered in our selection approach, it has to be fast and easy to measure under field conditions, have a

reasonably high heritability, and be positively correlated with production under drought. This strongly limits traits potentially useful for a drought breeding program. More recently, we initiated a divergent selection program for high/low root capacitance (Van Beem et al. 1998). After four cycles of recurrent full-sib selection, there is indication that selection for high root capacitance increased grain yield under drought through increases in biomass, and not through changes in ASI and harvest index (Mugo et al. 1999).

Challenges to Molecular Approaches

1. Conventional selection has led to maize germplasm with increased harvest index under drought, and QTL's have been identified for ASI and yield components using such germplasm (Ribaut et al. 1996, 1997). An immediate challenge to molecular approaches is to identify the genes underlying these QTLs, and to assess the response of maize to increased expression of those genes.
2. Conventional selection in tropical maize has not been successful in developing drought tolerance based on increased water uptake or water use efficiency, partly due to methodological constraints. Molecular approaches may offer different possibilities and these should be explored as to their effect on performance of maize under water-limited conditions.
3. CIMMYT has been successful in developing a selection method for improving the drought tolerance of maize through conventional means. However, the development and deployment of drought tolerant, adapted maize cultivars is slowed by funding constraints of National Maize Breeding Programs, and by low investments of the private seed sector in water-limited environments. Molecular approaches can offer exciting ways of shortening the time for product development. However, deployment of these and conventional methods by the public and private seed sectors in developing countries may be as much a limitation to the development of drought-tolerant adapted germplasm products as was the research that led to the development of these methods.

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Prospects of Using ABA in Selection for Drought Tolerance in Cereal Crops

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Summary

Abscisic acid is an integrative putative secondary trait that could be very useful because its genetic variance and mean value remain high under drought stress as genetic variance of grain yield falls. Variability of ABA concentration is low in maize seedlings, but leaf ABA concentration has been shown to decrease with selection for drought tolerance at the flowering stage. Heritability estimates and mode of gene action for leaf ABA concentration suggest use of either intra- or inter-population improvement procedures to decrease leaf ABA concentration in maize. Various QTLs for leaf ABA in cereals have been identified. Complexities in ABA gene expression induction, production, distribution, and physiological effects in genotypes, as well as technical and cost-related limitations in expression and ABA assays are discussed.

Introduction

Genetic variance for grain yield in crops decreases with drought stress. Secondary traits that are associated with grain yield and whose genetic variance remains high under stress could help identify drought tolerant genotypes. The phytohormone abscisic acid (ABA) is a stress-induced plant hormone and it has attracted much research attention as a potentially useful trait in selecting for drought tolerance in crops. This paper reviews briefly the relationships between ABA and drought tolerance in cereals with particular reference to maize, the current knowledge on ABA's major physiological effects, and classical and molecular level genetic control in crops under drought stress. Experiences from using ABA in improving the drought tolerance at seedling and flowering stage in maize; ABA induced gene expression, assays; and costs relative to other traits are discussed.

Physiological Effects of ABA in Drought Stressed Plants

ABA is ubiquitous in all flowering plants and is generally recognized as a stress hormone that regulates expression of many drought responses. When plants wilt, ABA levels typically rise as a result of an increase in the rate of synthesis (Taylor 1991). Increasing ABA concentration leads to many changes in development, physiology, and growth. Most importantly, ABA accumulation in higher plants in response to water deficit is thought to act as a signal for the initiation of processes involved in adaptation to drought and other environmental stresses (Hartung and Davies 1991; Bray 1993). The main developmental and morphological effects of ABA are in altering the plant in such a way that less water is lost through transpiration and roots obtain more water as reviewed by Setter (1997). Briefly ABA alters the relative growth rates of various plant parts such as increase in root:shoot dry weight, inhibition of leaf area development, enhancing root growth hence deep rooting (Sharp et al. 1994). Stomatal closure, the most important water-conserving response, involves a complex series of events triggered by ABA (Ward et. al. 1995). ABA improves water transport between plant parts by increasing the hydraulic conductance for water movement from roots to leaves (Zhang et. al. 1995). ABA is also involved in effecting cellular changes that confer an ability to maintain cell turgor and withstand the damaging forces associated with lowered water potential and desiccation. As examples, ABA stimulates osmotic adjustment, (Ober and Sharp 1994), induces the synthesis of protective proteins (the *LEA* and related proteins) (Bray 1993; Chandler and Robertson 1994).

ABA has been shown to induce expression of various water stress induced genes. Shinozaki and Yamaguchi-Shinozaki (1996) and Bray (1996) suggested existence of ABA-dependent and ABA-independent transduction cascades between the initial signal of drought stress and the expression of specific water-stress induced genes. Shinozaki and Yamaguchi-Shinozaki (1997) also showed existence of various pathways with protein synthesis is required or not required for ABA-inducible water-stress induced gene expression. These effects may explain in part the complex nature of ABA responses in drought tolerance in crops.

Genetics of ABA Control in Cereals

Genetic differences in accumulation of ABA in response to water-limited conditions have been demonstrated in numerous studies and for various plant parts such as leaves (Pekic and Quarrie 1987), stems (Abou-Mandour and Hartung 1980), roots (Sharp et al. 1994, Ribaut and Pilet 1991), grain tissues of cereals (Ober et al. 1991), and xylem sap (Tuberosa et al. 1994).

Leaf ABA concentration under seedling drought stress in maize is controlled by dominance gene action (Mugo 1999). Reciprocal recurrent selection or any selection involving tester crossing would be appropriate to reduce ABA concentrations. Inheritance studies on ABA at the flowering stage have indicated that additive effects are major factors, while additive-dominance interactions are also important but less so (Ivanovic et al. 1992; Mugo 1999). Broad sense heritability estimates range from 21% – 78% (Conti et al. 1994; Ivanovic et al. 1992; Tuberosa et al. 1994). Changes of ABA concentration at the flowering stage could, therefore, be achieved through either inter- or intra-population improvement procedures. Generation mean analysis has showed that a number of genes controlled leaf ABA in maize (Sanguineti et al. 1996).

Investigation of genetic control of ABA at the molecular level has been of interest recently. Quantitative trait loci (QTL) for leaf ABA have been identified in barley (Sanguineti et al. 1994), maize (Lebreton et al. 1995; Tuberosa et al. 1998), and wheat (Quarrie et al. 1994). Analysis of QTLs have indicated the complex control of leaf ABA in maize, though Tuberosa et al. (1998) identified one relatively more important QTL on chromosome 2 near csu133 which is under developmental control. The region near csu133 has previously been shown to control root pulling strength and grain yield in tropical maize (Lebreton et al. 1995). Single genes such as the *ABI1* and *ABI2* from arabidopsis have been cloned and demonstrated to function as negative regulators in ABA-dependent expression using maize protoplasts (Sheen 1996).

Experiences with ABA in Germplasm Improvement for Drought Tolerance

Seedling stage:

Bänziger et al. (1997) examined the feasibility of improving tropical maize for tolerance to post-emergence drought stress. They evaluated (1) progenies of a divergent S_1 recurrent selection program in the tropical maize population 'DTP1' for survival, biomass production, leaf rolling, and leaf ABA concentration under post-emergence drought stress, and (2) progress resulting from selection for survival and biomass production after two selection cycles.

Selection for improved survival and biomass production under post-emergence drought stress did not result in any significant differences compared with the original population, whereas selection for decreased survival and biomass production resulted in poorer survival. Significant phenotypic correlation coefficients were observed between leaf ABA concentration and seedling biomass (r=0.15*), but no significant genotypic correlation were observed between leaf ABA and any trait related to survival and production. Heritabilities for leaf rolling and leaf ABA concentration were higher than those for other traits, though no obvious relationships between these secondary traits and survival or biomass production were observed (Tables 1 to 3).

Neither leaf ABA concentration nor leaf rolling were of adaptive value for survival under post-emergence drought stress. Both leaf rolling and ABA production in the leaf are induced by reduced leaf turgor (Turner et al. 1986; Pierce and Raschke 1980), and genotypes with less turgor supposedly showed increases in both leaf ABA concentration and rolling. It was concluded that selection for improved survival and biomass production under post-emergence drought stress is difficult because environmental variation is high under field conditions and because natural selection may have exploited

positive genetic variation. Testing in a more controlled environment using an artificial rooting medium was suggested as an alternative to stress maize seedlings more uniformly and allow rooting characteristics to be examined.

Mugo (1999) determined the indirect responses of basal leaf ABA in seedlings to selection for drought at flowering and grain filling in selection cyles of four populations. Seedlings were evaluated under severe stress and well-watered conditions in controlled greenhouse environments. Leaf ABA concentration under severe stress linearly declined with cycles of selection in three populations (Table 4), indicating that basal leaf ABA accumulation of seedlings tended to decrease in response to increased drought tolerance at flowering and grain filling stage. However, as seedling weight was not correlated with leaf ABA (data not shown), this ABA alone seems unlikely to lead to improved drought tolerance at the seedling stage.

Table 1. Broad-sense heritabilities measured with S_1 lines of DTP1 SI, DTP1 SIBA and DTP1 SIWA under post-emergence drought stress at Tlaltizapan, Mexico between 1992 and 1995.

	Plant count			Leaf	Seed	Recovered	Leaf ABA
	initial	final	Biomass	rolling	weight	plants	concentration
DTP1 SI C_0 S_1	0.28	0.42	0.27	0.54	0.94	0.32	NA
DTP1 SIBA C_1 S_1	0.20	0.48	NA	NA	NA	NA	NA
DTP1 SIWA C_1 S_1	0.59	0.35	NA	NA	NA	NA	NA
DTP1 SIBA C_2 S_1	0.14	0.41	0.28	0.64	0.99	NA	0.56
Mean	0.30	0.42	0.28	0.59	0.96	0.32	0.56

NA = not available. *Source: Banziger* et al. *1997.*

Table 2. Phenotypic (above diagonal) and genotypic correlations (below diagonal) measured with 225 S_1 lines of DTP1 SIBA C_2 under post-emergence drought stress at Tlaltizapan, Mexico in 1994/95.

	Plant count			Leaf	Seed	Leaf ABA
	initial	final	Biomass	rolling	weight	concentration
Initial plant count		0.19*	0.29***	0.15*	-0.07	0.09
Final plant count	0.49		0.24**	-0.16*	0.06	0.02
Biomass	-0.26	0.52		-0.15*	0.04	0.15*
Leaf rolling	0.34	0.02	0.12		-0.05	-0.02
Seed weight	-0.11	0.12	0.22	0.16		-0.02
Leaf ABA concentration	-0.08	0.01	-0.10	0.18	0.15	

*, **, *** indicate significance at P £ 0.05, 0.01, 0.001 for phenotypic correlations. *Source: Banziger* et al. *1997.*

Table 3. Characteristics of germplasm selected for either good (DTP1 SIBA) or poor (DTP1 SIWA) survival under post-emergence drought stress, and of four experimental synthetics selected for specific traits from the original population DTP1 SI C_0. The entries were evaluated under post-emergence drought stress at Tlaltizapan, Mexico in 1994/95.

	Plant count				
	initial	final	Biomass	Leaf rolling	Leaf ABA concentration
	no. m^{-1}		g m^{-1}	score†	ng g^{-1}
DTP1 SIWA C_2	7.9	5.0	24	4.1	338
DTP1 SIWA C_1	8.4	5.7	24	3.7	337
DTP1 SI C_0	9.2	6.4	25	4.2	367
DTP1 SIBA C_1	8.5	6.2	19	3.7	373
DTP1 SIBA C_2	8.7	6.6	25	3.6	371
DTP1 SI C_0 high plant count	8.9	5.6	23	3.9	393
DTP1 SI C_0 unrolled leaves	8.3	5.9	19	3.2	398
DTP1 SI C_0 high biomass	8.4	5.6	22	3.7	348
DTP1 SI C_0 good recovery	9.4	6.6	18	4.1	333
Mean	8.6	5.9	22	3.7	362
$LSD_{0.05}$	1.3	1.0	7	0.5	49
Significance of entry effect	NS	**	NS	*	*

+, * indicates significance at P £ 0.10, 0.05, respectively. † 1 to 5 score, where 1 indicates unrolled and 5 indicates completely rolled leaves. *Source: Banziger* et al. *1997.*

Flowering stage

The same populations as used for the seedling study above was evaluated for indirect response of ABA to selection for drought tolerance at flowering and grain filling stage (Mugo 1999). Trials were grown under managed severe drought stress and well-watered field conditions at Tlaltizapan, Mexico, and pre-and post-flowering leaf ABA was measured. Leaf ABA concentration under severe stress linearly declined with cycles of selection in two populations (Table 5), even though this trait had not been part of the selection index. Pre-flowering leaf ABA and post-flowering leaf ABA concentrations were significantly correlated (phenotypic correlations) with grain yield under severe stress (r=-0.67** and r=-0.54*, respectively) and under well-watered conditions (r=-0.73** and r=-0.43*, respectively) (Table 6). Decreased pre-flowering leaf ABA may therefore indicate increased grain yields under drought and well-watered conditions. Pre-flowering leaf ABA measurement was less subject to down-regulation under drought stress in the field and is therefore preferred over post-

flowering measurement. Sampling at the pre-flowering stage would also allow selection of crossing parents before recombination, hence shortening breeding cycles.

Leaf ABA accumulation may have a positive role in the expression of the anthesis-silking interval (ASI). Anthesis-silking interval is also an indicator of relative partitioning of current photosynthates to the ear at flowering and a useful trait in selection for tolerance to drought occurring at flowering in maize (Bolanos and Edmeades 1996). As no trait appears to confer a high level of drought tolerance alone, a combination of multiple drought adaptive traits, such as ASI, ABA, and grain yield together in an index, could be appropriate for developing drought tolerance in lowland tropical maize.

Genetics, Technical, and Cost Considerations in Use of ABA for Selection

The utility of a secondary trait for selection programs is dependent not only on its genetics but also on the feasibility of creating the proper environment for expressing the trait, and the cost, speed, and ease of measurement. ABA concentration rises as a result of a decrease in turgor, which may depend on water use among genotypes. Hence, ABA is affected by genotype and test environment. In addition, mechanisms regulating ABA content and mode of action are complex. Abscisic acid production depends on the environment and stress occurrence. It has a nonuniform distribution in the plant, is highly mobile between xylem and phloem, and can penetrate membranes only in the protonated form, which depends on pH gradients. The complex nature of ABA involvement in drought tolerance will require the use of genetic and molecular analysis of water stress induced genes.

Measurement of ABA requires more time, labor, and facilities for sampling and assay as as compared to traits like leaf rolling, leaf senescence, and ASI. Assays generally fall into either physiochemical or immunological categories (Hedden 1993). Physiochemical assays involve

Table 4: Sums of squares from partial ANOVA for traits measured in seedlings of 20 maize varieties grown under a severe stress and under well-watered conditions in a greenhouse during summer 1996 and winter 1996/97.

		Severe Stress		Well-watered		Severe Stress		Severe Stress		Well-watered	
		Summer 1996	Winter 1996/97	Summer 1996	Winter 1996/97	Summer 1996	Winter 1996/97	Summer 1996	Winter 1996/97	Summer 1996	Winter 1996/97
Source	df	Leaf ABA (pmol cm^{-2})†				Leaf rolling (1-5 score)		Seedling Biomass (g)			
Rep	2	6.2	0.9	0.79	0.21	0.52	4.28**	28.53**	3.27**	3.55	135.80*
Variety	19	2711.0**	433.3	107.8**	7.01**	59.99**	16.06**	139.43**	28.29**	1454.26*	592.24*
Group#	6	926.8**	183.2**	26.8	3.12**	25.61**	6.34**	45.60**	10.38**	866.28	384.63*
Variety (Group)	13	1784.1**	250.1**	81.03	3.89**	42.30**	9.72*	93.83**	17.91**	578.98	207.61*
1. Among Tuxpeño Sequía	2	30.9*	0.1	8.7	0.30	9.06**	3.17*	30.75*	5.09*	232.66*	110.65*
Linear response††	1	(-)12.9*	0.1	5.5	0.14	(-)6.68**	(-)2.57**	(-)20.56*	(-)4.55**	(+)113.53*	2.99
2. Among La Posta Sequía	2	246.0**	5.3*	48.0	0.08	12.06**	0.88	7.53	6.04*	32.63	13.07
Linear response	1	(+)117.6**	0.4	(-)39.9*	0.01	0.38	0.88	1.57	(+)6.04**	22.32	6.44
3. Among Pool 26 Sequía	2	75.5**	80.1**	3.0*	2.74**	1.06	1.68	13.25**	2.06	109.46*	5.07
Linear response	1	(-)23.2**	(-)43.6**	(-)2.2*	(+)0.95**	1.05	0.04	0.59	(-)1.89*	101.20	4.18
4. Among Pool 18 Sequía	2	354.0**	0.5	2.3	0.51*	2.00	0.39	25.01	0.49**	8.25	0.58
Linear response	1	(-)39.3**	0.5	0.1	(+)0.51*	1.50	0.38	(+)17.66*	(+)0.08*	7.10	0.53
5. Among E.A. Composites	1	1015.2**	125.2**	5.9	0.00	5.04**	0.00	2.16	0.19	32.64	53.37
6. Among DTP1 Selections	2	5.4	33.9*	10.3*	0.13	9.72**	0.39	7.60	2.45**	46.61	22.36
C0 vs SIBA	1	5.1	7.0	7.7*	0.13	9.38**	0.38	2.18	1.12**	1.27	17.86
C0 vs SIBA & SIWA	1	0.3	27.0*	2.6	0.01	0.35	0.01	5.42	1.33**	45.34*	4.50
7. Among check populations	2	57.2**	5.0*	2.8	0.12	3.39*	3.21	7.53	1.58	125.72*	2.51
Pooled error	38	67.4	17.9	42.8	1.53	4.46	12.04	53.67	4.86	170.94	236.11

† Pre-flowering leaf-ABA concentration (pmol cm^{-2}).
‡ Post- flowering leaf-ABA concentration (pmol cm^{-2}).
§ Leaf senescence scores: 0=0% dry leaves, 10=100% dry leaves.
¶ Population groups (groups 1-4 consist of three cycles of selection, while groups 5-7 consist of genotypes of common origin or common trait of interest).
# Linear regression response. Linear responses in groups 1-4 refer to trends in the trait of interest among cycles of selection within the population group. Direction of change in the trait value with selection is indicated as an increase (+) or decrease (-).
*, ** Significant at the 5%

chromatographic separation of ABA from impurities followed by mass or gas spectrometry. Immunological assays involve the use of highly specific antibodies (Setter 1997). They are relatively cheaper and require less skills than physiochemical assays. More accurate estimates of ABA concentration may be obtained by assaying xylem sap instead of leaves (Lebreton et al. 1995; Tuberosa et al. 1994), however, at the expense of time, labor, skills, and facilities. Leaf ABA assays following the procedure by Ober et al. (1991) require some attention each day for at least three days, at an average cost of US $1 per sample. Recent improvements in ABA assay technology, however, may facilitate its use for the large numbers of samples required in a recurrent selection program (Setter 1997).

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Table 6: Correlation coefficients among leaf ABA cncentration, leaf rolling, ASI, and grain yield on 20 maize cultivars grown under a severe water stress (upper right diagonal) and a well-watered (lower left diagonal) regime over two years at Tlaltizapán, México (N=20).

Trait	Pref L-ABA† (pmol cm^{-2})	Postf L-ABA† (pmol cm^{-2})	Leaf rolling‡ Score (1-5)	ASI (days)¶	Grain yield (kg ha^{-1})
Pref L-ABA		0.41	0.11	0.42	-0.67**
Postf L-ABA	0.56*		0.61**	0.52*	-0.54*
Leaf rolling	-	-		0.43	-0.34
Anthesis-silking interval	0.05	0.17	-		-0.79**
Grain yield	-0.73**	-0.43	-	-0.11	

† Pre-flowering and post-flowering leaf ABA concentration (pmol cm^{-2}).
‡ Leaf rolling scores: 1=least rolled, 5=very rolled. Score takes into account extent of rolling of individual leaves and percentage of leaves rolled. Leaf rolling scored only on severe stress treatment.
§ Anthesis-silking interval
*, ** Significant at 5% and 1% level of probability, respectively and 1% levels of probability, respectively

Table 5: Sum of squares from partial ANOVA for leaf ABA concentration, ASI, and grain yield measured on 20 maize genotypes grown under severe stress and well watered conditions at Tlaltizapán, México, over two years.

		Severe Stress		Well-watered		Severe Stress		Well-watered	Severe Stress	Well-watered
Source	df	Pref-ABA (pmol cm^{-2})†	Postf-ABA (pmol cm^{-2})†	Pref-ABA (pmol cm^{-2})†	Postf-ABA (pmol cm^{-2})†	Leaf Rolling (1-5 score)	ASI (days	ASI (days)	YIELD (kg ha^{-1} x10^{-3})	YIELD (kg ha^{-1} x10^{-3})
Year	1	17065**	1645**	0	3871**	1.82*	2	632**	29931**	21641**
Rep (Year)	4	2428	703	149	307	5.02**	615*	1	160	4386
Group#	6	3279**	1024**	420**	357**	26.92**	2034**	146**	9,921**	58333**
Genotype (Group)	13	5102**	2564**	2987**	1751**	13.64**	2145**	99**	12,311**	14887**
1. Among Tuxpeño Sequía	2	7147	8225*	319	68	15.04	2349	49	1,097	1410
Linear response††	1	1506	552	17	61	0.49	(-)305*	(-)47*	3,586	1110
2. Among La Posta Sequía	2	536*	182**	194**	150	5.06**	538*	19	3,065**	1043
Linear response	1	(-)452**	(-)1**	(-)5**	23	0.44	(+)458*	19	(+)1543**	1043
3. Among Pool 26 Sequía	2	197	257	468**	8	4.40**	309	0	681	293
Linear response	1	1	60	(-)195**	0	1.20	38	0	679	102
4. Among Pool 18 Sequía	2	975	50*	1236	596	0.04	121**	13	3613**	2333
Linear response	1	(-)910*	(-)33**	459	217	0.01	(-)120**	11	(+)2791**	2336
5. Among E.A. Composites	1	96**	14**	138**	531**	1.84*	626	10**	307*	5518
6. Among DTP1 Selections	2	560	1226	610*	238	0.58	225	4	260	1502
C0 vs SIBA	1	51	764	508	237	0.24	4	3	19	11
C0 vs SIBA & SIWA	1	509	462	102*	0	0.34	220	1	240	1396
7. Among check populations	2	1233**	283	22**	160*		20	3	799*	2788**
Year x Group	6	2039**	4781**	858**	667**	4.81*	550	34**	4826**	16024**
Year x Genotype (Group)	13	5108**	344**	3630**	595**	10.23*	1800*	24*	6144**	6456**

† Pre-flowering leaf-ABA concentration (pmol cm^{-2}).
‡ Post- flowering leaf-ABA concentration (pmol cm^{-2}).
§ Leaf senescence scores: 0=0% dry leaves, 10=100% dry leaves.
¶ Population groups (groups 1-4 consist of three cycles of selection, while groups 5-7 consist of genotypes of common origin or common trait of interest).
# Linear regression response. Linear responses in groups 1-4 refer to trends in the trait of interest among cycles of selection within the population group. Direction of change in the trait value with selection is indicated as an increase (+) or decrease (-).
*, ** Significant at the 5%

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Genetic Control of Phosphorus Uptake and Utilization Efficiency in Maize and Sorghum under Marginal Soil Conditions

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Summary

Production constraints are more intense on acid soils, which cover 30% of the worlds land area. Toxic levels of Al associated with low availability and high fixation of P frequently occur in acid soils. Both Al toxicity and low P availability effect root development and interact with moisture stress in plant development, further complicating the negative effect of drought. Genetic resources tolerant to Al toxicity and more efficient in P uptake can reduce some of the negative effects of moisture stress in these marginal soils. This paper describes the effects Al toxicity and P stress on plant development and some plant responses to overcome these stresses. The identification of genes responsible for positive responses to these stresses and the stacking of these genes in improved cultivars can improve the productivity and/or reduce input requirements, making production more sustainable.

Marginal Soil Conditions

Acid soils are found on 3.95 billion ha, or 30% of the world's land area. The predominant acid soils in the tropical belt are Ultisols and Oxisols (von Uexkull and Mutert 1995). Marginal soils in this discussion will be limited to the acid soils of the southern tropical belt. Ultisols occupy 864 m ha and Oxisols cover 727 m ha; 22% and 18%, respectively, of the acid soils area in the world. Data from von Uexkull and Mutert (1995) show the extent of acid soils in various regions of the world (Table 1). Eswaran, et al. (1997), estimate that 28.8% of the African continent has acid surface soils and 19.6% has subsoil acidity problems (Table 2). Subsoil acidity in South America and South and East Asia comprise 50% and 15.4% of the area, respectively.

Table 1. Extent of acid soils in the world and selected regions[a]

Distribution Class	Global	Region						
		Central America	South America	Africa	Asia[c]	Australia/ New Zealand	North America	Europe
Acid Area ($x10^6$ ha)	3,950	37	917	659	532	239	662	391
Acid Area (%)[b]	30	35	14	22	76	30	30	37

[a] von Uexkull and Mutert (1995), [b]ice-free land area of the globe, [c]excluding South and East Asia

Table 2. Extent of acid soils in surface and subsurface soils by degree of acidity[a]

Distribution Class	Global	Region				
		South America	Africa	Asia	North America	Europe
Based on surface pH, % Area						
Slight (pH 5.5 - 6.5)	8.6	13.7	14.1	4.9	10.8	0.7
Moderate (pH 4.5 - 5.5)	10.6	24.8	10.7	5.5	15.7	11.5
High (pH 3.5 - 4.5)	6.7	20.4	3.9	9.1	4.7	28.2
Extreme (pH < 3.5)	0.1	7.2	0.1	0.2	0.0	2.3
Total	26.0	66.1	28.8	19.7	31.2	42.7
Based on Subsoil pH, % Area						
Slight (pH 5.5 - 6.5)	4.0	6.8	6.1	2.2	5.9	0.1
Moderate (pH 4.5 - 5.5)	9.5	23.3	9.5	4.3	14.8	25.1
High (pH 3.5 - 4.5)	6.5	20.1	3.9	8.7	4.7	2.1
Extreme (pH < 3.5)	0.1	0.1	0.1	0.2	0.0	0.0
Total	20.1	50.3	19.6	15.4	25.4	27.3

[a] Eswaran, et al. (1997). Asia represents South and East Asia. South America includes Central America.

Figure 1 shows the global distribution of soils with surface acidity problems. Periods of water deficiency compound the difficulty of sustainable production that frequently is associated with crop production on these marginal soils. For a more complete review of the distribution and characterization of acid soils in the world, the recent review by Baligar and Ahlrichs (1999) is recommended.

Oxisols are the most frequent soil type in the cerrado ecosystem in Brazil. They have excellent physical properties but are strongly weathered with low cation exchange capacity, frequently exhibiting major mineral element deficiencies and toxicities. Deficiencies of P, Ca, Mg, and Zn are common, toxic exchangeable Al is usually high, and the fixation of P by soil particles is extensive. Dry periods during the rainy summer growing season are common throughout the cerrado area. These soils have micro aggregates that may cluster into blocky structures. Oxisols have very low water holding capacity with 8 to 15 cm h^{-1} of hydraulic conductivity, which decreases rapidly with drying. The field research referred to in this discussion was conducted in the acid savanna or cerrado region of Brazil where Oxisols predominate.

Effect of Al Stress on Plants

The primary effect of Al on plant development is to inhibit root growth. This is rapid phenomenon; when exposed to toxic levels of Al in the soil solution, root hairs stop growing in minutes and roots stop growing in hours. Al stress affects the growing regions of roots, root hairs, the root apex, lateral root formation and nodule formation. Monomeric Al^{3+}, the dominant form of aluminum as the pH falls below 4.5, is toxic to plants. When Al complexes with organic matter, OH^- complexes, or P and S complexes, it is nontoxic. The visual effects and consequences of Al toxicity are stubby swollen roots, abscense of root hairs, poor nutrient uptake, poor mycorrhizal development, poor nodulation, poor crop growth and yield, and poor quality produce.

Responses of Plants to Al Stress

Two classes of mechanisms have been proposed for tolerance to Al toxicity; those that allow the plant to tolerate Al accumulation in the

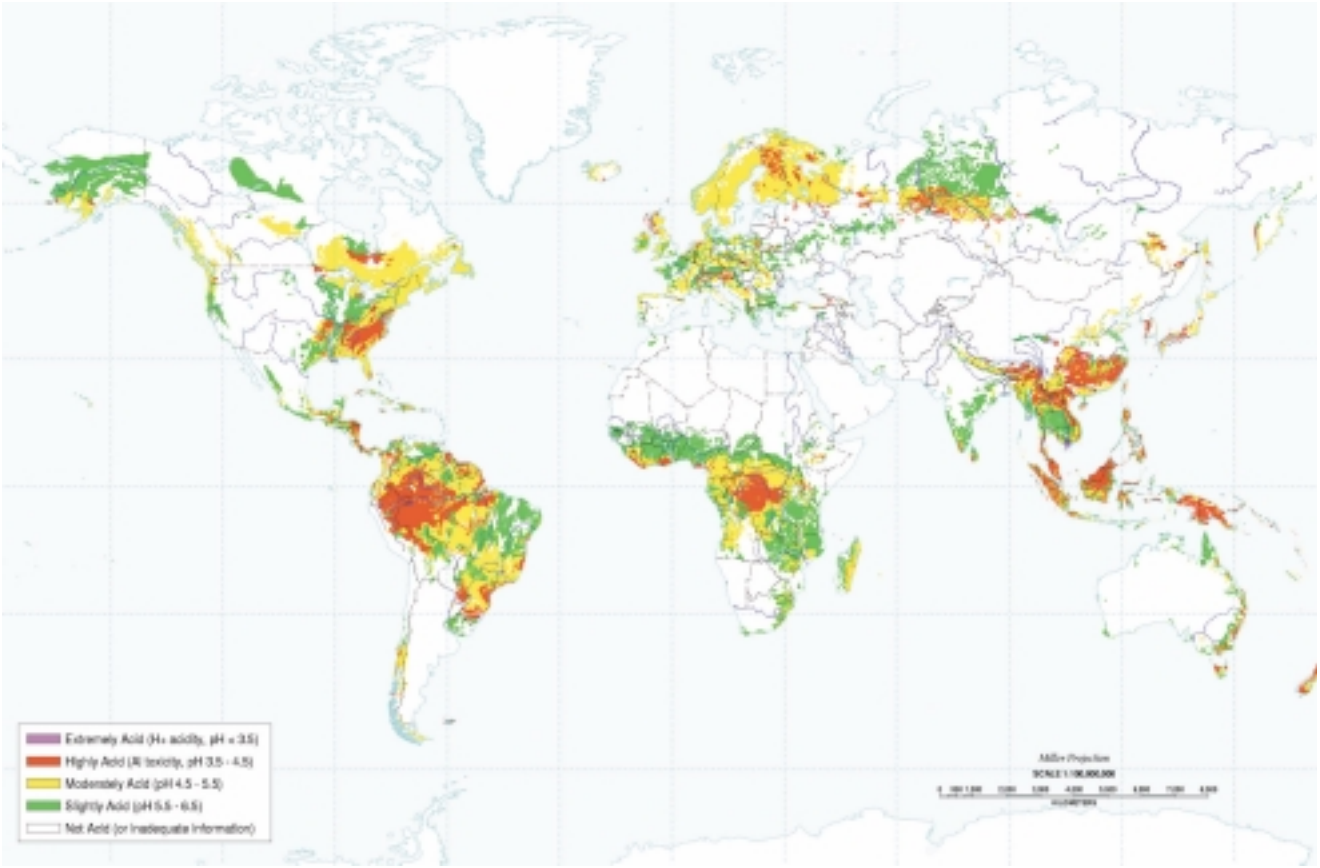

Figure 1. Global distribution of acid soils. (USDA, NRCS, World Soil Resources, Washington, D.C.)

symplasm (symplasmic tolerance), and those that exclude Al from the root apex (Al exclusion). Several hypotheses have been put forth in the literature regarding mechanisms of Al tolerance. For symplasmic tolerance, potential mechanisms include chelation of symplasmic Al with low molecular weight ligands or Al-binding proteins in the cytoplasm, and sequestration of Al within an internal compartment (e.g., the vacuole) (Kochian 1995; Taylor 1991). Mechanisms for Al exclusion include the release of low molecular weight, Al chelating ligands into the rhizosphere, root-induced increases in rhizosphere pH, binding of Al within the cell wall, decreased permeability of the plasma membrane to Al influx, and binding of Al within the mucigel associated with the root apex (Kochian 1995; Taylor 1991). For recent reviews on the physiology of aluminum toxicity and tolerance, the reader is referred to Kochian (1995) and Kochian and Jones (1997).

Recent studies from several labs have indicated that Al-induced organic acid released from the root apex plays a role in Al tolerance in different plant species; a major difference being the type of acid released. Malate release has been found in a number of different Al tolerant wheat cultivars (Ryan et al. 1995; Pellet et al. 1996), while work in maize indicates that citrate release is a response of Al stress and a mechanism of tolerance (Pellet et al. 1995). The organic acids are only released when Al is present at toxic levels (there is no continuous drain on resources). The excreted organic acid complexes with Al in the soil solution of the rhizosphere make it nontoxic. Luis Herrera-Estrella's Lab, (Fuente et al. 1999) has shown that aluminum tolerance in transgenic tobacco (*Nicotiana tabacum)* and papaya (*Carica papaya*) plants can be developed by engineering the overproduction of citrate.

Al Toxicity x Drought Stress

Acid subsoils frequently act as a chemical barrier to root growth. Crop species or cultivars susceptible to Al toxicity produced on soils with subsoil acidity will have shallow root systems and be more susceptible to drought stress during intervals between rains. Al tolerant species and cultivars produce larger and deeper root systems, making them more tolerant to intervals of drought stress during the growing season.

Response of Plants to P Stress

A persistently low level of available phosphorus in the soil solution has led to numerous morphological, physiological, biochemical, and molecular adaptations by plants to survive in the nature. Enhanced root growth under Pi starvation results in increased root surface area available for Pi acquisition (Lynch 1995). In addition to increased root growth, production and elongation of root hairs also increase under Pi deficiency. Root hairs play an important role in Pi acquisition under the nutrient deficiency conditions (Gahoonia and Nielsen 1998). Highly branched, actively growing root systems of some of the bean genotypes is positively correlated with phosphorus efficiency (Lynch and Beebe 1995). Proteoid root development in white lupins is a classical example of the plant adaptations to Pi deficiency. Proteoid roots are highly efficient in synthesis and secretion of organic acids to the rhizosphere (Dinkelaker et al. 1995). Secretion of organic acids enhances the release of Pi from Ca, Fe, and Al phosphate complexes. Increased secretion of organic acids may involve activation or synthesis of enzymes and anion channels to enhance secretory processes. The tropical legume crop, pigeon pea, excretes pisidic acid (p-hydroxybenzyl tartaric acid), a phenolic compound known to release Pi from iron complexes (Ae et al. 1990). A considerable amount (20 – 80%) of Pi in soil is found in the organic form (Jungk et al. 1993). Phosphatases induced and secreted under Pi starvation could mobilize phosphate from organic P sources. Production of phosphatases (extracellular and intracellular) increased during Pi starvation (Duff et al. 1994). The extracellular phosphatases may play a role in obtaining Pi from organic phosphorus compounds in the extracellular matrix. Phosphate deficiency in plants has also resulted in concurrent increases of phosphatases, phytase and RNases (Bosse and Kock 1998). The enzymes of "bypass reactions" that circumvent Pi and adenylate, required steps in glycolysis, are also activated under Pi deficiency (Plaxton and Carswell 1998).

Phosphate Deficiency Leads to Altered Gene Expression

Phosphate deficiency results in distinct changes in gene expression (Raghothama 1999). Some of these altered gene products may serve as molecular determinants of plant adaptations to Pi deficiency. The genes coding for proteins such as phosphatase (Patel et al. 1995), RNases (Green 1994), phytase, and phosphate transporters (Muchhal et

al. 1996) have a distinct role in Pi nutrition of plants. The number of genes known to be expressed under Pi starvation is increasing rapidly (Raghothama 1999). Some of the other genes induced under Pi deficiency are Ca^{2+} ATPase (Muchhal et al. 1997), PEPcase, vegetative storage protein, enolase, and pyruvate formate-lyase, -glucosidase and novel genes such as *TPSI1* and *Mt4* (Raghothama 1999). The evidence for an intricate gene regulation system in plants similar to the yeast *PHO* regulon is increasing. In addition the complexity of plant morphology and biochemistry point to the existence of other regulatory mechanisms that respond to changes in cellular Pi levels (Raghothama 1999).

Phosphate Transporters are Molecular Tools for Pi Acquisition

Phosphate transporters are membrane-associated proteins involved in Pi acquisition. The uptake is generally described as a two component mechanism involving a high affinity transporter operating at low (µM) concentration and a low affinity transporter functioning at high concentration (mM) of Pi. In the context of µM concentration of available Pi in soil solution, the high affinity Pi transport is considered as the primary mechanism of uptake for plants under natural conditions. An energy mediated co-transport process, driven by protons generated by a plasma membrane H^+ATPase, has been proposed for Pi uptake in plants. This process may be associated with movement of 2 to 4 H^+ across the plasmamembrane. The number of high affinity Pi transporter genes have been cloned from plants is increasing rapidly (Ragothama 1999). The Pi transporters are integral membrane proteins consisting of 12 membrane spanning regions a common feature shared by members the Major Facilitator Super family (Raghothama 1999). The deduced amino acid sequences of plant Pi transporters share a significant similarity with those of yeast (*Saccharomyces cerevisiae*), *Neurospora,* and the mycorrhizal fungi (*Glomus versiforme*) (Muchhal et al. 1996). The complex intra- and inter-cellular movement of Pi in plants points to the existence of additional Pi transporters.

Recent studies in Raghothama's lab have also demonstrated induction of phosphate transporter genes in Pi starved roots of maize and sorghum. For a more complete review of this topic, the recent review by Raghothama (1999) on phosphate acquisition in the Annual Review of Plant Physiology is recommended.

Selection for P Efficiency in Maize and Sorghum

The selection of maize and sorghum genotypes adapted to the conditions of the cerrado has been conducted by the plant improvement program at Embrapa/Milho e Sorgo since the early 1970s. Due to the complex nature of the limiting factors to crop production in the cerrado, the breeding strategy was to concentrate on specific factors limiting productivity. Tolerance to Al toxicity was the first characteristic chosen for improvement. Genetic resources were selected under both field conditions and nutrient solution (Magnavaca 1987). Genetic standards and commercial hybrids with tolerance to Al toxicity have been developed for both maize and sorghum. The maize variety, CMS 36, and sorghum line CMSXS 136 are genetic standards for Al tolerance. The hybrids BR 201 (maize) and BR700 (sorghum) were the first commercial hybrids released with tolerance to Al toxicity. Several other hybrids with tolerance to Al toxicity have been developed and released.

During the past five years, there has been more emphasis on developing maize and sorghum genetic standards and commercial hybrids that are more efficient in P uptake and utilization. Screening was initiated in 1993 for maize and 1995 for sorghum. Initially, genotypes were evaluated at two levels of P in the soil, the critical level (10 ppm P) and 50% of the critical level (5 ppm P). The evaluation of maize hybrids from diallel crosses and sorghum lines in these conditions have facilitated the identification of genetic standards for phosphorus uptake efficiency.

The maize lines L 36, L 723, and L11, and the lines L1143 and 1167 have been identified in several trials (Table 3) as being more efficient and less efficient in P utilization, respectively. These P efficient lines are not tolerant to Al toxicity and the P inefficient lines have been classified as tolerant to Al toxicity. The results of select hybrids evaluated in the field with 2 and 15 ppm P are shown in Table 4. The hybrids HT-16C and HS 20x723 have been classified as P efficient standards and the hybrids HS 20x22, HS 20x64, HS 64x724 and HS 16x22 have been classified as P inefficient

standards. The triple cross hybrid HT 16-C, highly productive at low P (2ppm P) and with a high relative yield of 91% (low P/high P * 100) was released in 1998 as BRS 3060.

Sorghum genotypes from the Embrapa sorghum improvement program have also been evaluated at two levels of P, 5 and 10 ppm. Representative results of 36 sorghum lines are presented in Figure 2. These sorghum lines have been classified into four groups: efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive. The sorghum line CMSXS 116 has been classified as P efficient and the sorghum line CMSXS 101 has been classified as P responsive. The line CMSXS 136 was not responsive to P. CMSXS 101 and 116 are susceptible to Al toxicity and CMSXS 136 is tolerant to Al toxicity. Selected lines tolerant to Al toxicity and responsive to P were derived from the cross of CMSXS 101 and CMSXS 136, indicating that tolerance to Al toxicity and responsiveness to P are independent traits.

Table 3. General Combining Capacity (GCC) for ear weight (kg/ha) of a diallele cross between 8 maize lines evaluated in two years and in four field environments (2 ppm P, 15 ppm P, 36% Al saturation and normal high fertility) and Net Seminal Root Growth (NSRG) and Relative Seminal Root Growth (RSRG) in nutrient solution with 6ppm Al (EMBRAPA-Milho e Sorgo, Sete Lagoas, 1997).

LINE	GCC 2 ppm P (kg/ha)	GCC 15 ppm P (kg/ha)	GCC 36% Al. (kg/ha)			GCC Fertile Soil (kg/ha)			GCC RSRG (%)	NSRG "per se" (cm)
	96/97	96/97	94/95	96/97	Ave.	94/95	96/97	Ave.		
L 11	1049	953	72	648	360	757	1206	981	- 8.8	12.9 ± 4.9
L 36	236	952	518	976	747	695	368	531	-10.6	11.5± 4.8
L 723	943	453	318	142	230	538	92	315	-13.5	24.0 ± 10.7
L 1143	-1026	-1159	- 834	-1213	-1023	- 373	216	-79	17.4	58.8 ± 18.0
L 1167	-968	-463	- 322.	-469	-369	- 742	1052	155	3.9	38.8 ± 14.5

Source: Parentoni et al., 1998.

Table 4. Ear weight (t/ha) and relative yield of select maize hybrids evaluated in soils with two level of P, 2 and 15 ppm. (Embrapa Milho e Sorgo, 1998)

Hybrid	Phosphorus level 2 ppm P (A)	15 ppm P (B)	A/B*100	Classification
HT-16 C	12.00 (1)*	13.17 (3)	91	P Efficient
HS-20 x 723	9.87 (4)	11.91 (10)	83	P Efficient
HS 64 x 36	8.87 (17)	10.48 (32)	85	P Efficient
BR 201	7.95 (32)	8.58 (57)	93	P Efficient
HS 20 x 64	7.34 (51)	10.45 (34)	70	P Inefficient
HS 20 x 22	7.22 (53)	12.47 (5)	58	P Inefficient
HS 64 x 724	7.03 (55)	12.13 (7)	58	P Inefficient
HS 16 x 22	6.42 (60)	9.63 (41)	67	P Inefficient
Trial Average (64 hybrids)	8127	10474	77	

* The ranking of each hybrid is in the trial of 64 hybrids is in parenthesis.

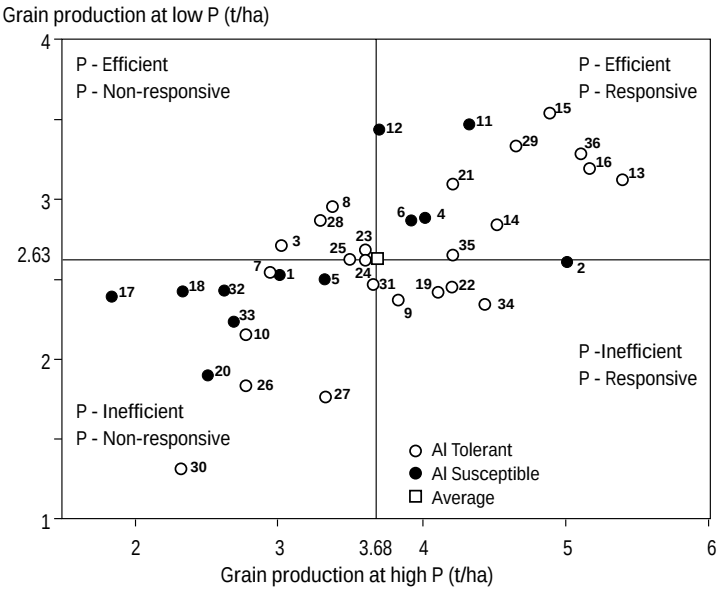

Figure 2. Graphic representation of grain yield of 55 sorghum lines at two levels of P.

The first commercial sorghum hybrid released by Embrapa, BR300, was a cross between CMSXS 101 and CMSXS 116; these lines are now recognized as being more P responsive and more efficient in P utilization, respectively. The first maize hybrid released by Embrapa, BR 201, has been recently recognized as having improved P utilization efficiency. Tolerance to Al toxicity comes from one of the single cross parents and improved P efficiency comes from the other single cross parent in this double cross hybrid. Both of these commercial hybrids were selected for productivity and yield stability at several sites across the cerrado and it is possible that indirect selection for more efficient P uptake occurred. These genetic standards are currently being used to study mechanisms of improved P efficiency and to develop new hybrids that are more efficient in P uptake and more productive with stable yields in the cerrado.

These recent evaluations are demonstrating that some of Embrapa's elite germplasm developed over the past 30 years is more efficient in P uptake. Our program is now working to incorporate the genes that regulate improved P uptake efficiency into the elite breeding material.

Interaction of Moisture Stress and P Acquisition in Acid Soils

Phosphorus uptake by plants is often reduced during water stress, as the P concentration in the soil is directly proportional to the water content of the soil (Novais et al. 1990). This is of particular concern in the acid soils and subsoils of the tropical regions of Africa, the Americas, Asia, and the Appalachian Region, the southern portion of the Great Plains, and the Southeast of the United States, where intervals of moisture stress are common throughout the growing season. Plant cultivars that initiate phosphate uptake more rapidly after a rain following a dry spell should have a greater production potential. The availability of P also directly effects the supply of N to maize (Magalhães et al. 1996).

General Conclusions

The results of our research indicate that plant adaptation to acid soils in the cerrado or acid savannas is intimately linked to better development of the plant's root system. Tolerant materials are able to grow in the layer of acid soil immediately below the 15 to 20 cm top layer, usually corrected by liming. In addition to a better developed root system, adapted materials also have the ability to rapidly absorb phosphorus from these soils when soil moisture is high. It is possible to conclude that adaptation to Al stress, low P availability, and short periods of drought may be controlled by mechanisms that are common as a whole or at least in some parts (pathways). Cultivars tolerant to Al toxicity and more efficient in P uptake have greater yield stability and better average agronomic performance over many growing seasons.

The strategy of eliminating the production constraints of the acid savannas with both liming practices and corrective applications of P is limited technically with respect to correcting the acidity of the subsoil, and economically with the high rates of P fertilizer required due to the high P fixing capacity of the soil. A combination of soil management practices, liming in association with corrective levels of P, and the use of crop cultivars developed for these low pH conditions is a solution for obtaining sustainable maize production in the cerrado.

The goal of the maize and sorghum improvement programs is to pyramid the genes that control the various mechanisms regulating plant adaptation to acid soils, including; tolerance to Al stress, tolerance to P stress and improved efficiency in P uptake and utilization, tolerance to water-deficit stress, greater efficiency in ammonium utilization, and a more developed root system. Cultivars with multiple stress resistances are expected to have higher yields and greater yield stability across environments.

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Assessing the Contribution of Glycinebetaine to Environmental Stress Tolerance in Sorghum

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Summary

Sorghum genotypes differ in the amount of glycinebetaine, a compatible osmotic solute, they accumulate under osmotic stress. To examine the contribution of glycinebetaine to osmotic stress tolerance, a recombinant inbred population has been developed from parents that contrast for glycinebetaine accumulation. Analysis of this population indicates that a single major gene conditions glycinebetaine accumulation and that modifiers affect the final level of this compound. This population is being used to map the genes responsible for variation in glycinebetaine content. Near isogenic lines have been developed that either accumulate or lack glycinebetaine. Analysis of these lines, as well as the segregating population, is in progress to evaluate the impact of this solute on tolerance of sorghum to a number of environmental stresses.

Introduction

Cellular dehydration is a general consequence of osmotic stresses, including water deficit and salinity. In response to this condition, many organisms accumulate solutes that help retain water within cells. Some of these solutes may also protect cellular components from injury caused by dehydration. Organic compounds that function as solutes include amino acids such as proline, sugar alcohols such as mannitol, and quaternary ammonium compounds such as glycinebetaine (GB). The positive effects of GB on growth of microbial species under osmotic stress are well-documented (Csonka and Hanson 1991). However, the role of GB in osmotic stress tolerance in plants is less clear. Sorghum is an important grain crop that exhibits excellent drought tolerance compared to other grasses. One of the proposed mechanisms that might contribute to this tolerance is the accumulation of high concentrations of GB. The objective of this research is to evaluate the effect of GB on tolerance of sorghum to a number of environmental stresses.

Many in vitro studies provide evidence for the role of GB in osmotic stress tolerance in plant cells. Water deficit and salinity can lead to denaturation of proteins and disruption of membrane structures. Glycinebetaine has been shown to maintain the activity of enzymes under a variety of unfavorable conditions including high temperature, extremes of pH, and high salt concentrations. For example, GB reduced the PEG-induced precipitation of barley glutamine synthetase (Paleg et al. 1984), prevented inactivation of *Aphanothece halophytica* ribulose-1,5-bisphosphate carboxylase activity due to salt, heat or cold (Incharoensakdi et al. 1986), and attenuated the salt-inhibition of phosphoenolpyruvate kinase activity by salt in both monocot and dicot species (Manetas et al. 1986).

Maintaining integrity of a plant's photosynthetic machinery is important for sustaining growth under environmental stress. Glycinebetaine protects the photosystem II (PSII) complex by stabilizing the association of the extrinsic PSII complex proteins in the presence of salt (Murata et al. 1992). PSII complexes that lack their extrinsic proteins are protected from inactivation by GB under extremes of temperature or pH (Mohanty et al.

1993), but not from the inhibitory effects of Na^+ ions on oxygen evolution (Papageorgiou et al. 1991). Glycinebetaine also provides protection against heat destabilization of membranes (Jolivet et al. 1982).

At the whole plant level, accumulation of GB has been correlated with growth under stress in some species (Colmer et al. 1995), with freezing tolerance in barley (Kishitani et al. 1994), and with maintained nitrogen fixation under osmotic stress (Riou and Le Rudulier 1990). Glycinebetaine may not be beneficial under all conditions however, as GB accumulation has been associated with increased incidence of some insect pests such as aphids (Araya et al. 1991) and microbial diseases such as *Fusarium* (Pearce et al. 1976).

Glycinebetaine Biosynthesis in Plants

Glycinebetaine is synthesized in plants from serine via ethanolamine, choline, and betaine aldehyde (Hanson and Scott 1980), with S-adenosyl methionine serving as the methyl donor (Bowman and Rohringer 1984). Although other pathways may exist (such as direct N-methylation of glycine), the pathway from choline to GB (Figure 1) has been identified in all GB-accumulating species to date (Weretilnyk et al. 1989).

Choline monooxygenase catalyzes the oxidation of choline to betaine aldehyde and the gene encoding this enzyme has been cloned from spinach (Rathinasabapathi et al. 1997), where it is localized in the chloroplast (Lerma et al. 1988). Choline monooxygenase activity appears to be the rate-limiting step for GB synthesis in spinach and expression of this gene is induced under osmotic stress (Hanson et al. 1985; Rathinasabapathi et al. 1997). Betaine aldehyde dehydrogenase (BADH) oxidizes betaine aldehyde to GB and is found primarily in the chloroplast of chenopods, although some BADH activity is present in the cytosol (Weigel et al. 1986). In chenopods, BADH is encoded by a single nuclear gene while the functional enzyme is a dimer.

Glycinebetaine Accumulation in Sorghum

Many cereal crops accumulate GB although some do not, notably rice. The concentrations of GB observed in species that use GB as a compatible osmotic solute are variable. The levels of GB found in sorghum are as much as ten-fold higher than those observed in maize. However, GB-deficient genotypes of both sorghum and maize have been identified. In a screen of sorghum landraces, approximately 3% were GB-deficient (Yang 1990). Genetic analysis of this trait in several crosses between GB-deficient and -accumulating lines indicated that a single nuclear gene was responsible for GB deficiency (Grote et al. 1994). Similar results have been obtained in studies on the genetics of GB accumulation in maize (Rhodes and Rich 1988), in which biochemical studies indicate that GB deficiency results from an inability to convert choline to betaine aldehyde, the first committed step in the synthesis of GB (Lerma et al. 1991).

Near isogenic lines of maize that accumulate GB have been shown to be more salt tolerant than GB-deficient isolines (Saneoka et al. 1995). Lines that accumulate GB suffer less membrane injury and less disruption to the photochemical reactions of photosystem II relatuve to their near-isogenic GB-deficient sister lines high temperature conditions (Yang et al. 1996). We are interested in understanding the contribution of GB to environmental stress tolerance in sorghum, in which the level of GB is much higher than in maize. To this end, we have developed GB-deficient and –accumulating near-isogenic lines of sorghum as described below.

Development of Near Isogenic Lines Differing in Glycinebetaine Accumulation

A recombinant inbred (RI) population was developed from a cross between IS2319, a naturally-occurring GB deficient genotype, and P932296, a high-GB genotype (Grote et al. 1994). One hundred and fifty F_2 lines were advanced by single seed descent to the F_8 generation, at which stage >99% of the loci should be homozygous in each line. Figure 2 shows the distribution of GB accumulators to nonaccumulators in a screen of the F_8 generation. The

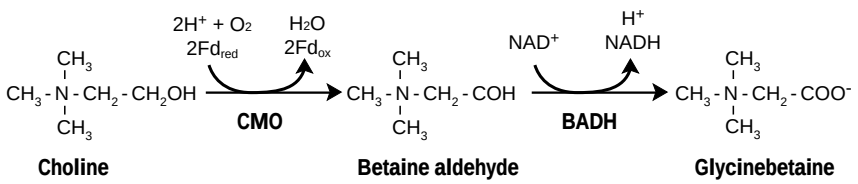

Figure 1. Glycinebetaine biosynthetic pathway in plants.

frequency of GB-deficient lines was 39%, significantly less than the expected 50%, suggesting that GB accumulation may contribute some selective advantage under the conditions used to develop the RI population. In lines that accumulated GB, there was significant variation in the levels of GB, ranging from 4 to 40 mmol gFW^{-1}. This variation suggests that other genes modify the level of GB in accumulating lines. We are in the process of mapping both the major gene that conditions GB accumulation in this population as well as the modifier genes.

A number of methods were used to identify lines in the F_8 generation that were still segregating for GB accumulation. From these, we developed pairs of near isogenic lines that either accumulate or lack GB. We have obtained three pairs of lines so far (Figure 3), and at least two more segregating lines are still being evaluated. The GB-accumulating lines show a five- to six-fold increase in GB under saline conditions and a two- to three-fold increase in GB under water-deficit conditions.

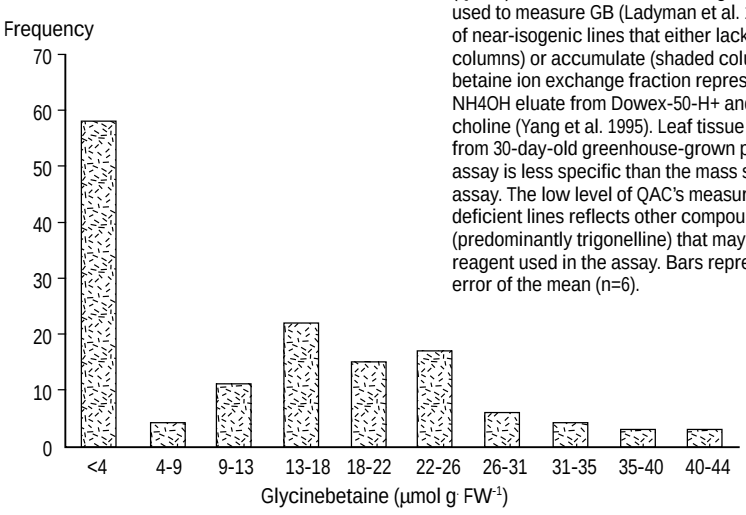

Figure 2. Variation for glycinebetaine (GB) in a recombinant inbred population.
Glycinebetaine concentrations were measured in leaves of F_8 plants of a recombinant inbred population derived from IS2319 (GB-deficient) and P932296 (GB-accumulating). 14-day-old seedlings were salinized with 100 mM NaCl for 14 days and GB was measured by PD-mass spectroscopy (Yang et al. 1995).

Preliminary experiments have been conducted on the parents of the RI population used to develop the near isogenic lines. The GB-accumulating parental line (P932296) maintained higher rates of net CO_2 assimilation under conditions of salinized soil (Figure 4). In addition, chlorophyll fluorescence decreased to a lesser degree in the GB-accumulating line (P932296) when exposed to high temperatures (48° for four hours) over the GB-deficient line (IS2319) (Figure 5). This reflects a greater stabilization of the photosynthetic apparatus in GB-accumulating lines over nonaccumulating lines under heat stress. However, there are likely many genetic differences between these lines. Consequently, the near isogenic lines will be evaluated under conditions of water deficit, salinity, and extreme temperatures to determine the impact of GB on tolerance to these stresses.

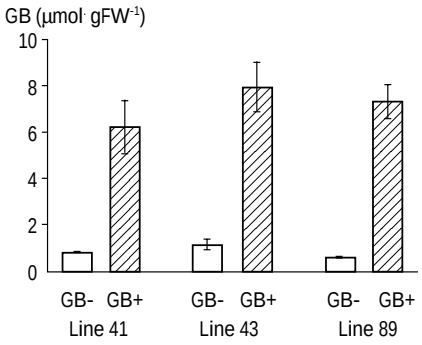

Figure 3. Near-isogenic lines differ in glycinebetaine (GB) accumulation.
A spectrophotometric periodide colorimetric assay that detects total quaternary ammonium compounds (QAC's) in the betaine ion exchange fraction was used to measure GB (Ladyman et al. 1983) in 3 pairs of near-isogenic lines that either lack (open columns) or accumulate (shaded columns) GB. The betaine ion exchange fraction represents the 6M NH4OH eluate from Dowex-50-H+ and is free of choline (Yang et al. 1995). Leaf tissue was harvested from 30-day-old greenhouse-grown plants. This assay is less specific than the mass spectroscopy assay. The low level of QAC's measured in the deficient lines reflects other compounds (predominantly trigonelline) that may react with the reagent used in the assay. Bars represent standard error of the mean (n=6).

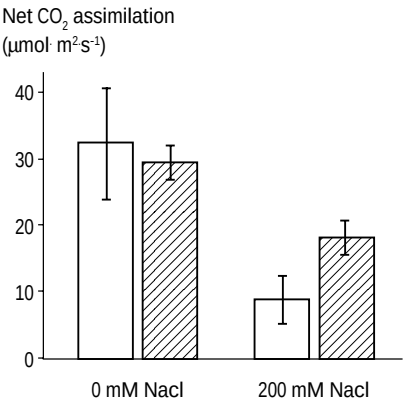

Figure 4. Glycinebetaine concentration is associated with less severe inhibition of photosynthetic rate under salt stress.
Net CO_2 assimilation of IS2319 (GB-deficient, open columns) and P932296 (GB-accumulating, shaded columns) grown under control (0 mM NaCl) or salinized (200 mM NaCl) conditions. Bars represent standard error of the mean (n=5).

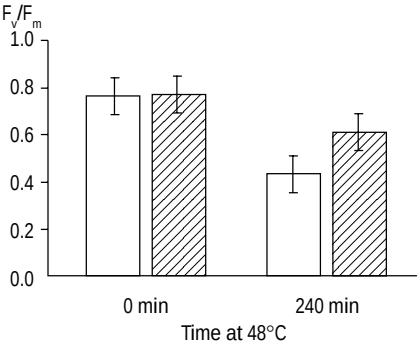

Figure 5. Glycinebetaine concentration is associated with a less severe decline in chlorophyll fluorescence under heat stress.
Chlorophyll fluorescence of IS2319 (GB-deficient, open columns) and P932296 (GB-accumulating, shaded columns) grown under greenhouse conditions (0 min) or grown under the same conditions and then placed in a 48°C growth chamber for 4 hours (240 min). Chlorophyll fluorescence was measured on both sets of plants 4 hours after removing the heat-treated plants from the growth chamber. Bars represent standard error of the mean (n=5).

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Probing the Vitality of Plants by the JIP-Test, A Novel Non-Invasive Phenotypic Screening Technique for Performance under Water-Limited Conditions

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Molecular breeding and transgenic strategies for genetic improvement of crop plants for water-limited conditions are often faced with the limitation of accurate, dependable, and rapid phenotyping methodologies. It becomes a particularly severe problem when plants are to be phenotyped for field drought tolerance, as it is an extremely complex trait and influenced by a diverse array of environmental variables. Furthermore, correlations between drought tolerance and yield potential become even more difficult when one has to do parallel measurements on productivity trait expressions and drought tolerance.

A potential new tool that can be used to calculate several structural and functional parameters is analysis using the JIP-test of the chlorophyll *a* fluorescence rise in intact plant leaves. The constellation of these parameters will reflect both the status and function of the photosynthetic apparatus and the water potential of the leaves. In principle, the polyphasic chlorophyll *a* fluorescence rise, denoted as OJIP, giving a fair indication of photosynthetic rates and, therefore, productivity. Several parameters of photosystem II can be examined simultaneously. Stress-induced changes in the OJIP fluorescence induction kinetics can be obtained simultaneously with other physiological tests, such as osmotic adjustment or water use efficiency, using the same leaves. With appropriate controls and a very large sampling capacity, the chlorophyll *a* fluorescence methods can help in defining an experiment system (i.e., tissues, organs, whole plant) in terms of vitality, productivity, and sensitivity/resistance to a given stress. The measurements are rapid—less than few seconds are needed for each measurement—and inexpensive. The JIP-test is being used extensively in stress physiology in a range of plant species, using the same type of instrument. This methodology can be easily adapted to field conditions. The digitized data from thousands of samples can be stored and transmitted anywhere in the world. More importantly, these data, in conjunction with the available databanks of physiological traits and crops, will help us in interpreting drought tolerance and, eventually, in establishing an easy and rapid diagnostic test for drought tolerance in target crops such as rice.

The JIP-test experiments with tobacco cultivars with known levels of drought tolerance could differentiate between tolerant and sensitive cultivars. JIP-test data on salt-induced changes have been used to define the tolerance/sensitivity levels in Spirulina. Preliminary experiments with detached leaves of rice also suggest its utility as a phenotyping tool for vitality and performance during the stress and recovery phases. One major advantage of this method is that repeated measurements, even on a single leaf of the test plants at defined time points, can be made during prolonged drought periods followed by recovery in the field. Transgenic plants, often limited in number, can be screened effectively by this method at seedling and

mature plant stages, both in the laboratory and in the field. The details of this method and its possible use in drought tolerance research will be discussed. It is worth noting that the JIP-test is undergoing continuous development; the obtainable data range from empirical expressions to biophysically well-defined parameters, while the higher complexity of the system can be described and quantified by numerical simulations. Due to these advantages, the JIP-test can be used for two main applications: (a) the bioenergetic description of a single cultivar under normal or stress situation and its comparison with data banks; (b) the vitality and stress mapping of many cultivars or mapping populations in greenhouses or fields, which can reveal their behavior with respect to the local microclimate or stress factors.

Impressive advances in molecular biology have made access to the genome and structure of the photosynthetic organisms feasible; the JIP-test can help access the function and, thus, promote an understanding of the Structure-Function relation. In practical terms, the JIP-test can be a handy tool in molecular breeding and transgenic strategies for crop improvement for stress tolerance. As it can screen this function at an early stage, i.e., at the seedling stage, with a few green cells, or regenerating calli etc., it saves time in providing feedback and is thus cost effective.

Towards a Comparative Genomics of Drought Tolerance in Cereals: Lessons from a QTL Analysis in Barley

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Summary

In this paper, we will present some data collected in our research group on drought tolerance in barley, with an emphasis on genetic comparison with other similar works done in cereals. We will discuss the physiological significance of criteria used for QTL analysis, the difficulties but usefulness of comparative mapping, and open perspectives in functional genomics for drought tolerance in cereals.

Introduction

Drought tolerance improvement is probably one of the most difficult tasks for cereal breeders. The difficulty comes from the diversity and unpredictability of drought conditions in the field, and from the diversity of drought tolerance strategies developed by the plants that are targeted and subjected to selection criteria. However, substantial progress has been made during recent years on different plant species, in terms of the physiology, genetics, and molecular biology of drought tolerance. Gathering these pieces of the puzzle together into a single scheme through functional genomics would certainly help better our understanding of drought tolerance control and help us define strategies for crop improvement.

In several cereal species, genetic maps have allowed the identification of chromosomal regions that control some traits related to drought stress response. Different segregating populations of maize, sorghum, rice, wheat, and barley have been studied for diverse quantitative characteristics or criteria such as phenology, root characteristics, plant architecture and growth, abscisic acid accumulation, photosynthesis parameters, chlorophyll quantity or "stay-green" character, water-use efficiency and carbon isotope discrimination, water status and osmotic adjustment parameters (This et al. 1999). In the various published works, several stress intensities have been used and the genetic comparison was done either with the same soil water status or at the same plant water potential. How can we then compare all of these studies? Our experience in QTL mapping in barley for drought tolerance gives some evidence that comparing works done in other cereal species is of great interest and provides new ideas for functional genomics studies.

Material and Methods

A first set of five barley genotypes (Tadmor, ER/APM, LM2887, Plaisant, Express) were assessed for their behavior in irrigated and water-stressed conditions. A segregating population of barley F8 recombinant inbred lines (RILs) derived from the cross between Tadmor (characterised by a high yield stability) and Er/Apm was then used for the genetic analysis.

An experimental design was conduced in controlled conditions at an early stage of growth in order to identify QTLs for osmotic adjustment traits. The methodology was described in Teulat et al. (1998): relative water content (RWC) and osmotic potential ($\psi\pi$) were measured at a soil moisture corresponding to 100% and 14% of field capacity, allowing the calculation of osmotic potential at full turgor and osmotic adjustment (OA), using Ludlow et al.'s (1983) method. Several yield components, as well as

carbon isotope composition ($\delta^{13}C$), were measured in the field in Granada, Spain in 1996.

The map construction, out of 167 RILs, was initially described in Teulat et al. (1998). To fill the gaps, 15 RFLP, 4 RAPD, one microsatellite, and 77 AFLP markers were added to the basic map. The marker trait associations were done by interval mapping and single marker ANOVA analysis. The putative QTLs were declared significant when the LODscore ≥ 2.0 and probability P<0.005.

Results

Evaluation of criteria and quantitative parameters decomposing drought tolerance in barley

Our objective was to decompose plant behaviors under different water-stress conditions in several criteria that are easy to measure in a large population and related to drought tolerance characters. Plant architecture, tillering, and root characteristics differed among the contrasted barley lines studied, but it was difficult to define a single ideotype for drought tolerance (Teulat 1997). Stomatal conductance and xylemic ABA accumulation were also measured, but differences were mainly due to precocity (Borel et al. 1997). Photosynthesis was affected differentially by water stress in several barley lines (Arnau et al. 1997), however no simple criteria could be found to study it in a large population. OA and leaf RWC maintenance measured in controlled conditions at an early stage and at the anthesis stage was however shown to be related to drought tolerance; OA was also related to water soluble carbohydrate accumulation in our material (Teulat et al. 1997a). $\delta^{13}C$, measured in grains under field conditions, was also a good indicator of yield capacity (Teulat 1997).

Development of a barley map from the cross Tadmor x ER/APM

Of a total of 209 markers scored on the segregating population, only 118 (38 AFLPs, 1 SSR, 1 morphological marker, 2 RAPDs and 76 RFLPs) were used on 167 RILs for the new map construction (Figure 1). A candidate gene mapping strategy was developed through RFLP or PCR (STS, SSR, PCR-RFLP). The mapped candidate loci concerned genes of known function in stress response like dehydrins, sucrose synthase, and rubisco activase.

QTL analysis for a few drought tolerance criteria

The first data, obtained with a preliminary map, on QTL controlling traits related to osmotic adjustment, were presented in Teulat et al (1997b, 1998). In order to analyze all the RILs with 5 replicates per line and per water treatment, 9 experiments (blocks) were necessary in an

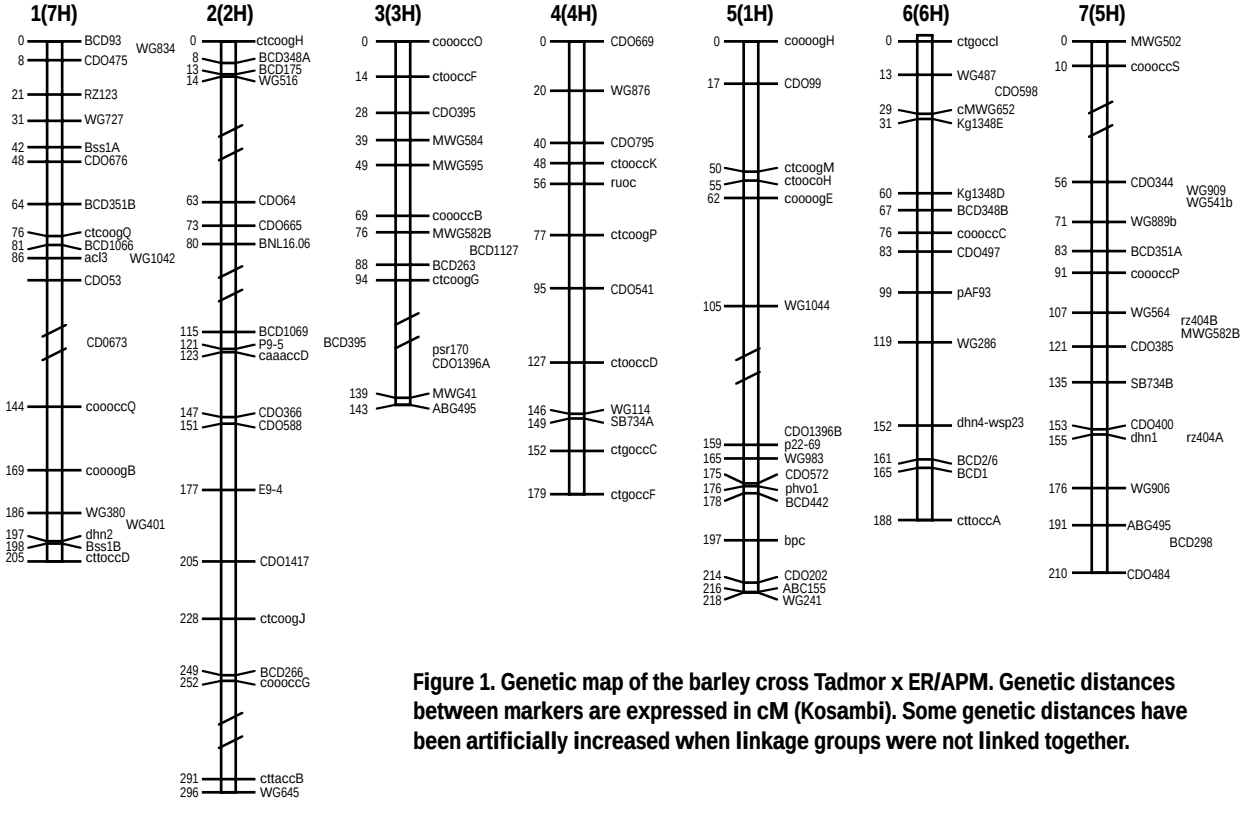

Figure 1. Genetic map of the barley cross Tadmor x ER/APM. Genetic distances between markers are expressed in cM (Kosambi). Some genetic distances have been artificially increased when linkage groups were not linked together.

incomplete-block random design, with 5 different lines per pot. Adjusted means, obtained by regression on block and pot within-block effects were used for the QTL analysis. This allowed us to identify several loci potentially involved in the variation of several parameters acting in OA calculation. Two main loci were emphasized, because several QTL were colocalized in the same regions on chromosome 7H and 6H. Those data have been revisited with the new map and by analyzing the phenotypic data directly from each block separately. Soluble sugar content also has been taken as a new criterion. The previous QTL regions were confirmed using adjusted means, and some significant new QTL have been obtained.

Some other traits like chlorophyll content, tillering, growth, yield parameters, and $\delta^{13}C$ have been studied in several other experiments and are still under way. $\delta^{13}C$ measurements allowed us to identify a significant QTL controlling this trait on chromosome 2H, colocated with some other QTLs for water-soluble sugar content, chlorophyll quantity, and RWC control.

Intra- and interspecific QTL comparison

Some collaborative research work has been initiated in order to compare our data with similar studies done on different genetic backgrounds; specifically in either the *Hordeum* genus in an INCO program started in 1999 (Forster et al. 1999), or in some other cereal species: durum wheat in collaboration with ICARDA and Cornell University for OA traits and $\delta^{13}C$; and rice and sorghum for tillering control, in collaboration with CIRAD, Montpellier. However a bibliographic compilation of QTL works undertaken in other laboratories for cereals, as far as genetic loci can be compared directly or indirectly, also serves as a very useful tool.

One of the target loci on chromosome 7H, where QTLs for ψπ, RWC and some other traits have been colocated in our work, was shown to be colinear with a region of rice chromosome 8, where a QTL for OA at 70% of RWC was found by Lilley et al. (1996) (Teulat et al. 1998) (Figure 2). Morgan and Pan (1996) also identified a major gene called *or* controlling osmoregulation on the same homeologous arm in wheat (chromosome 7A). However this gene is probably at a more distal position, corresponding by synteny to a rice chromosome 6 segment. The anchor probe CDO99 is mapped at the same homologous region of chromosome 8 of rice and chromosome 7 of wheat (with another locus on chromosome 1), but only on chromosome 1H of barley. However, in our cross, the CDO99 locus is defining several QTL for RWC and ψπ. The occurrence of a duplication corresponding to another QTL for OA traits on chromosome 1H of barley is therefore not excluded. A preliminary analysis in durum wheat suggested that QTLs for yp located on the triticeae group 7 may be also conserved with barley (Rekika, unpublished data).

Carbon isotope discrimination, measured in the same durum wheat segregating population on leaves and grains, seems to be controlled by at least two loci, potentially on groups 2 and 4, eventually conserved in barley (Merah 1999). Furthermore, data banks suggest the location of candidate genes within those regions.

Comparative mapping of tillering ability in barley, sorghum, and rice has not been very successful. However, the previous conserved region, which could be extended to chromosome A of sorghum, shared several QTLs related to tillering variation in rice, and the number of leaves in the main stem for sorghum and barley (This et al., unpublished data).

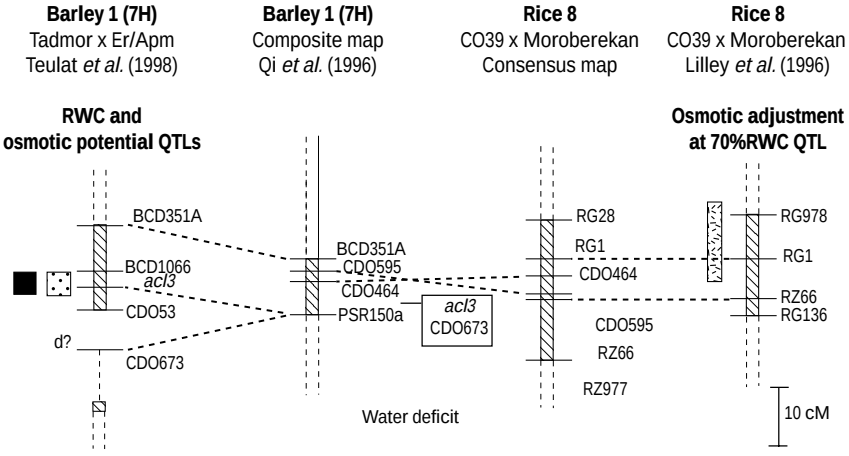

Figure 2. Compared maps between a portion of the short arm of the barley chromosome 1(7H) and chromosome 8 of rice involved in osmotic adjustment variation under water deficit. RWC: relative water content.

Discussion

Criteria and their physiological significance

Any criterion defined to assess drought tolerance in controlled conditions is necessarily reduced to a quantitative parameter that may be compared in many plants submitted to the same level of water stress. Should we take into account the stress perceived by the plant (i.e., the water potential of one leaf), or the soil water status? No simple response can be given to this question. What then is the physiological mechanism under this quantitative difference? One should also find a compromise between the number of repetitions for each physiological parameter and each segregating line (necessary to evaluate environmental effects) and the minimum number of segregating individuals necessary for QTL significance. No QTL study for drought tolerance is therefore undeniably exact, but each one certainly contributes to a general comprehension of this character if we are able to compare them. For instance, even if our criteria for OA control are not exactly similar to other studies, the fact that a conserved QTL cluster is found in different genetic backgrounds suggests a common physiological process controlled by this region.

Difficulties and usefulness of comparative mapping

Some comparison between genetic maps is possible when common markers are used or the same orthologous genes are mapped. Then, synteny existing among cereals allows us in some cases to compare homologous regions between different species or different crosses. However, our experience in comparing barley, rice, and sorghum for tillering ability showed us that synteny is much more complicated than expected, with many exceptions, and therefore it is not easy to compare QTL map locations, because confidence intervals usually cover several homologous regions. Microsynteny analysis for similar QTL regions and the identification of orthologous genes within them should complete the picture.

When similar QTLs or even different ones are colocated, then the region probably corresponds to a key region in drought tolerance with global implications. This is certainly the case for the region identified on barley chromosome 7H and rice chromosome 8. In maize, RZ66 is found on chromosome 4 and CDO595 on chromosome 1, those two markers being in the rice target region. Comparison with QTL studies done on maize is therefore very promising. For those key regions, much intensive research could now be undertaken by using the tools of functional genomics.

Prospects for functional genomics studies

Our next objective is to improve our knowledge of the region of the short arm of chromosome 7H (and some others), as part of our final goal of identifying genes or regulatory sequences directly involved through their allelic variation with drought tolerance, and validation through marker-assisted selection or transformation. Several questions remain: Is the chromosomal region characterized by the action of several genes and influenced by the environmental effects or one gene acting by pleiotropy? Is the region stable? And, what physiological mechanism controls exactly the identified locus? Also, which gene or genes underlie the QTLs? Those questions are probably the same for the other cereal species in which this region was emphasized, and some comparative responses should emerge from joint research.

As the region shows good synteny to the rice genome, rice BAC clones could be used for physical mapping. Sequence information provided by the international rice sequencing project will be soon available for ORF identification, and transfer to barley could then be accomplished.

The candidate gene approach could also be followed in the different cereal species by locating genes in this particular region that generate polymorphism within or in regulatory sequences of the genes, mapping them, and then analyzing allelic variation. The mapping of other candidate genes will go on in our group, mainly with genes related to carbon metabolism. Differences in the expression of the candidate genes will be measured through Northern blots and by analyzing enzymatic activities. On the other hand, microarray technology permits expression monitoring of thousands of genes at the same time. This methodology is quite expensive, but it should be possible, in collaboration with other research groups, to use high-density filters to gather genes that are responsive to water stress, or more generally, rice ESTs on DNA

chips, and to analyze gene expression on our material and other cereal models. This would allow the identification of the functions of a maximum number of genes specifying barley and cereals agronomic performance traits.

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Genetic Variation in Performance under Reproductive-Stage Water Deficit in a Doubled Haploid Rice Population in Upland Fields

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Summary

Water deficit at the flowering stage causes dramatic reductions in rice yield. Screening for tolerance to water stress at this stage is complicated by differences in flowering dates among lines and technical problems of imposing uniform, repeatable experimental conditions. The objectives of this work were to document genetic variation in tolerance to water deficit during flowering and grain filling among doubled haploid rice lines (DHL) from a japonica-by-indica cross, to identify genetic markers associated with performance, and to identify secondary traits that cosegregate with yield or yield components. In one season, staggered planting dates were used to synchronize flowering. In a second season, rice was grown using furrow irrigation and two periods of moderate water deficit were imposed in order to stress all entries at the most sensitive stage. In both seasons, stress treatments affected the percentage of sterile spikelets and the weight per grain. Quantitative trait loci (QTLs) were identified for yield components in each season and water level. A few QTLs had consistent effects across environments and/or seasons, but others were specific to the measurement environment. Across years, the QTLs identified for yield components under stress were not identified in the control, indicating that spill-over effects are not adequate to exploit the genetic potential for yield under stress that is present in this population. On the other hand, the interval containing the sd1 gene appears to have a major effect on sterility, yield and harvest index under varied conditions. Some QTLs for yield components under stress co-segregated with reported QTLs for drought-adaptive traits such as root depth and thickness.

Introduction

Rice is particularly susceptible to water deficit at the reproductive stage. The most susceptible period is thought to be from the stage of pollen meiosis, which occurs about 10 days before anthesis, through flowering. While stress at flowering is also harmful to other crops such as maize, the magnitude of the effect is even greater in rice. Because of the comparatively shallow root system of rice and its apparent inability to extract water from depth when the surface soil is dry, rice experiences water stress sooner after irrigation than other crops.

The difficulty of accurately timing and managing water stress means that secondary traits and genetic markers are very attractive selection criteria for tolerance to reproductive stage stress. A number of publications document efforts to relate traits such as leaf rolling or drying, constitutive root morphology and canopy temperature to performance under drought. Genetic markers have been identified for many of these traits.

The objective of this work was to document genetic variation in tolerance to water deficit during flowering and grain filling among doubled haploid rice lines (DHL) from a *japonica*-by-*indica* cross, to identify genetic markers associated with performance, and to identify secondary traits that cosegregate with yield or yield components.

Methods

In 1995, the upland *japonica* cultivar 'Azucena' and the lowland *indica* cultivar 'IR64' were sown along with 106 DHLs from the Azucena/IR64 mapping population (Huang et al. 1994). The lines were divided into four groups based on expected flowering dates. Groups were sown at four-day intervals with the

intention of having all entries flower around the same date. The entries were replicated twice under stress and twice in the well-watered control. The stress treatment consisted of no irrigation for 10 days with the stress starting at anthesis of most of the lines (70 DAS), followed by one hour of irrigation every three days until the end of the season. The control was irrigated one hour by sprinkler every day (application of approximately 10 mm daily). The plots consisted of three 3m long rows at 0.25 m spacing. Plants were spaced at 10 cm intervals along the row.

In 1998, the two parents were sown along with 82 DHLs. The DHLs were selected from the total population on the basis of flowering date; 54 lines were common to the two experiments. Plots were 3.0 m by 0.9 m in area, but the plants were concentrated on the tops of the beds in three rows spaced only 0.19 m apart. This arrangement was used in order to allow furrow irrigation of the crop after an initial 30-day period of sprinkler irrigation. Seed was dibbled in the rows at a rate of 80 kg/ha. Irrigation was applied twice a week except for two periods near flowering (52-64 and 70-84 DAS) in the stress treatment. The water was allowed to infiltrate to the centers of the beds at each irrigation, bringing the soil to field capacity to a depth of 30cm (as indicated by tensiometers installed in the field). Some plots had poor plant stands; in these, yield component samples were harvested from fully bordered plants, but yield and biomass were not measured.

Except for the irrigation schedules, trials were managed according to IRRI standard practice for upland rice experiments. Table 1 lists data collected in each experiment. Data were analyzed separately for the two experiments. Means for each entry were used to map quantitative trait loci (QTLs) for each trait using QTLMapper 1.0 (Wang et al. 1999). The QTLs identified were compared with those found when the population was examined under lowland conditions and under vegetative stress (Courtois et al. 1995; Courtois et al., In press), and with other published data on rice QTLs for leaf traits (Champoux et al. 1995; Price et al. 1997) and root penetration (Ray et al. 1996). They were also compared to QTLs for osmotic adjustment identified in other rice populations (Zhang et al. 1998).

Results

Sprinkler irrigation with staggered sowing dates, 1995 dry season

The objective of the stress was to affect grain filling. The staggered sowing was not completely effective in inducing synchronous flowering. Some of the lines started to flower well into the stress period. All lines that flowered more than 15 days after the start of the stress were fully sterile, as were some of those that flowered after 10 days. After eliminating lines that flowered late, the final population used in the analysis comprised 85 DH lines. Genetic differences were observed for most traits (Table 1). As intended, the stress primarily affected sterility, yield, biomass after stress, and harvest index. Significant interactions between genotype and water treatment existed for these traits, as well as for thousand grain weight. Yield and flowering date were strongly negatively correlated (r=-0.81 in control; r=-0.59 in the stressed plot). This partly reflects the increase in VPD with time that generally occurs in Los Banos during the dry season, and seems to show that both water levels were stressed, though at different levels of intensity. However, in a fully irrigated experiment conducted in 94DS the correlation was also high (r=–0.49; Courtois et al. 1995). There may be a genetic relationship between yield and flowering date in this population.

Furrow irrigation, 1998 dry season

The objective of having two stress periods was to ensure that all lines would experience stress during the sensitive period. Any line that flowered after day 70 and before day 95 should have experienced stress during the sensitive pollen formation or anthesis stages. Only 12 of the lines used flowered after day 95 in the control plots, but almost all plants were late in the stress plots. The average flowering delay was 12 days. The influence of stress on flowering date makes it difficult to assess if all lines experienced stress during the sensitive period. Examination of yield components indicates that water deficit had little effect on tiller number or spikelets per panicle (Table 1). Water deficit primarily affected the sterility of panicles and spikelets and final grain weight. Genetic differences were detected for these yield components and for most other traits. In contrast to the 1995 experiment, significant line-by-environment interactions were not detected for yield or most yield components.

The combination of very high plant density and hot, dry weather led to high levels of spikelet sterility in both control and stress treatments. The number of tillers per square meter

(excluding the area of the furrows) and leaf area index were large for upland rice. This resulted in large numbers of spikelets per square meter, even though the number of spikelets per panicle was low. Few of these spikelets could be filled, leading to high sterility and low harvest index in both water levels. Water deficit did, nonetheless, have a significant effect on yield and yield components. A high and variable percentage of the panicles were totally sterile. Inspection on plants indicated that this was primarily the result of stemborer damage. Panicle sterility was not correlated with stemborer score, which estimated the extent of leaf and tiller loss due to stemborers (deadhearts).

Across experiments

The 1995 experiment targeted the period from anthesis through grainfilling, and the primary effect was on sterility and TGW. In 1998, the stress occurred earlier, and affected the number of sterile panicles and spikelet sterility. Thousand grain weight was also affected even though the plot was fully irrigated throughout grainfilling for most lines. The phenotypic correlations across experiments were computed for the 54 common lines. They were high and positive across the control plots except for final biomass. They were low and positive across the stressed plots for most traits except for duration (as high as in the control plots), and for yield and harvest index (negative). However in the two

Table 1. Traits measured in upland rice field experiments in 1995 dry season (85 entries) and 1998 dry season (84 entries) grown under fully irrigation and with water stress around flowering. The probablility of significant differences among entries is indicated for each trait, along with broad-sense heritability. Significance of the genotype x water interaction is shown for traits measured across water levels.

	Control				Stress				Genotype X Water Level
			Gen. Effect				Gen. Effect		
Trait	Mean	Range	P	h^2	Mean	Range	P	h^2	P
1995DS									
Flowering (d)	98.9	84-116	0.0001	0.96	99.2	84-116	0.0001	0.88	ns
Diff in flow date c vs s (d)					-0.7				
Height (cm)	84.2	53-122	0.0001	0.92	-	-	-	-	-
Panicle length (cm)	24.2	17.1-31.3	0.0001	0.89	-	-	-	-	-
Exsertion (cm)	-1.5	-7.0-+6.6	0.0001	0.90	-	-	-	-	-
Leaf length (cm)	39.4	21.6-63.7	0.0001	0.88	-	-	-	-	-
Leaf width (mm)	14.3	9.6-20.0	0.0001	0.89	-	-	-	-	-
Panicle/m2	318	224-416	0.0096	0.42	299	196-489	0.0001	0.60	ns
Spikelets/panicle	103	47-154	0.0001	0.72	111	58-158	0.0001	0.66	ns
Sterility	44.7	11.5-84.9	0.0001	0.80	61.6	23.0-99.7	0.0002	0.55	0.0139
Thousand grain weight (g)	23.1	15.3-32.8	0.0001	0.94	21.9	14.9-32.3	0.0001	0.62	0.0457
Yield (g/m2)	114	12-299	0.0001	0.75	50.1	1-149	ns	0.20	0.0095
Harvest index (%)	15.2	0.5-37.0	0.0001	0.73	8.5	0.6-27.8	0.0265	0.35	0.0493
Biomass before stress (g/m2)	527	292-807	0.0101	0.41	468.4	255-729	0.0289	0.35	ns
Biomass after stress (g/m2)	726	473-1147	0.0151	0.39	568.7	300-860	ns	0.24	ns
1998DS									
Flowering (d)	91.8	70-113	0.0001	0.98	102.9	71-125	0.0010	0.94	0.0010
Flowering delay					11.9	3.0-23.0	0.0002	0.57	-
Height (cm)	94.8	68-123	0.0001	0.87	78.3	55-105	0.0001	0.85	0.0001
Panicle length, cm	24.7	18-37	0.0001	0.64	21.9	15-38	0.0001	0.69	ns
LAI	8	3.4-13.4	0.0001	0.68	4.8	1.6-8.8	0.0002	0.54	0.0159
Tillers/m2	391	183-697	0.0001	0.62	360.0	160-602	0.0055	0.50	0.0517
Panicles/m2	280	27-531	ns	0.18	224.0	69-463	ns	0.53	ns
%sterile panicles	30	2-92	0.0001	0.62	50.0	6-96	0.0050	0.64	ns
Spikelets/panicle	54.9	23-108	0.0001	0.70	62.0	30-167	0.0003	0.60	0.0075
Sterility	69	35-98	0.0001	0.79	82.0	61-99	0.0001	0.64	ns
Thousand grain weight (g)	23.9	16.7-34.6	0.0001	0.53	20.8	8.5-32.8	0.0001	0.67	ns
Yield, kg/ha	76.3	4-240	0.0040	0.49	29.3	2-96	ns	0.20	ns
Harvest index	9	0-27	0.0001	0.61	5.0	0-14	ns	0.24	ns
Biomass at harvest	837	187-1352	0.0200	0.38	619	246-1085	ns	0.07	ns
RWC, %	90.5	76.4-95.3	0.0001	0.61	76.3	50.1-92.9	0.0002	0.54	0.0010
Fresh weight, %	68	59-74	0.0001	0.48	71.0	64.8-76.3	0.0001	0.78	0.0024
Root exudate, average					444.0	0-2150	0.0145	0.33	-
Canopy temp., C					36.6	33-41	ns	0.24	-
WLR, area	1.33	0.74-2.60	0.0001	0.58					-
SLA, m2/g	222	162-331	0.0013	0.50					-
Leaf rolling					2.4	0.8-4.5	0.0001	0.71	-
Stem borer score	1.8	0-4.5	0.0001	0.64	2.8	1.0-5.0	0.0001	0.83	0.0470

last cases, the sample was very small (28 lines) because of missing values for yield in 1998. Half of the best 10 common lines identified for yield in 1995 were also in the top 10 in 1998 in the control treatment, but only one was common across years in the stress treatment. This confirms that the experiments were quite different in the type of stress imposed.

Identification of quantitative trait loci

QTLs were identified for almost all traits (Table 2). Some of these were common between stress and control treatments, particularly in 1995. When common QTLs were observed, their effects were in the same direction in the two environments, indicating minimal cross-over interaction for these QTLs. For these genes, we can expect a spill-over effect from well-watered to stress situations. A number of the QTLs identified for yield components were also identified when the population was grown under fully irrigated lowland conditions (Courtois et al. 1996). On the other hand, some QTLs were not common across the stress and control plots. These loci represent variation that is water-treatment specific.

Table 2. QTL position, LOD score, effect and relative contributions of intervals identified (P<0.005) for traits evaluated in lines of the population IR64 x Azucena grown under upland irrigated conditions (control) and with water deficit during the reproductive stage (stress) in 1995 and 1998 dry seasons. Intervals in bold were identified in both environments within a year. Intervals in bold italics were identified in both years. The first column indicates other traits mapped to the interval.

Cosegregating			CONTROL 95				STRESS 95				CONTROL 98				STRESS 98			
traits*	Chr.	Trait/interval	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2
		PLANT HEIGHT (cm)																
**	1	RZ730-RZ801	0.14	12.14	-13.5	35.6					0.24	11.58	-9.9	51.9	0.20	9.30	-7.6	27.8
5,n	1	RG810-RG331	0.02	5.96	-7.3	10.4									0.02	3.55	-4.5	9.7
1,d,h,j	2	RG95-RG654	0.00	3.07	5.1	5.0												
4	3	CDO87-RG910	0.02	6.29	-7.1	9.9												
1,g	4	RZ675-RG163										2.90	-4.1	8.8				
	5	RG313-RZ556														1.85	-2.9	4.0
3,b,h	9	RZ206-RZ422														2.02	-2.7	3.4
j	10	G2155-RG134														1.84	-2.8	3.6
		FLOWERING																
2, 5, n	3	RG348-RZ329					0.00	2.27	-2.7	13.1	0.02	5.96	-5.2	23.0	0.02	3.63	-4.7	14.8
	3	RZ892-RG100	0.04	3.08	-3.2	15.9												
d	6	RZ398-RG213	0.22	3.12	-2.9	12.6												
3,5	6	CDO544-RG653									0.16	3.54	3.9	13.1	0.16	2.89	4.0	10.8
a	8	AC5-RG418B	0.00	2.56	-3.0	13.7												
c	10	RZ625-CDO93									0.00	5.53	-4.7	19.0	0.00	6.82	-6.6	29.2
		FLOWERING DELAY																
6	8	RG978-RG1	0.00	2.78	-1.4	17.8												
4,g	8	Amp2-CDO99									0.04	2.32	1.7	14.7				
	10	RZ625-CDO93									0.04	3.71	-1.8	17.5				
	12	RG958-RG181									0.10	1.84	-1.3	8.2				
		TILLER NUMBER																
	1	RG690-RZ730	0.12	11.01	35.5	30.0	0.10	3.68	21.9	12.0					0.00	3.45	35.5	19.8
e	1	RG810-RG331									0.00	6.43	40.8	30.5				
5,n	2	CDO686-Amy1AC					0.00	2.32	17.1	7.3								
c,e,h	3	RZ329-RZ892					0.06	4.20	26.4	17.5								
	3	RZ337A-RZ448	0.00	1.87	11.2	3.0												
	4	RG214-RG143	0.00	3.78	17.5	7.3												
	5	RG313-RZ556					0.04	2.73	18.4	8.5								
3,5	6	CDO544-RG653	0.00	3.12	16.2	6.2	0.00	2.64	17.7	7.9					0.08	2.71	33.7	17.9
6	8	RG1-Amy3	0.10	3.36	-21.7	11.2												
g	8	Amp2-CDO99	0.04	6.04	25.7	15.6												
j,k	9	RG667-RG451					0.02	2.64	18.3	8.3								
c	10	RG257-RG241	0.20	2.41	16.4	6.4												
c	11	Adh1-RG1094	0.08	2.11	-13.6	4.4												
		SPIKELETS/PANICLE																
n	3	RZ574-RZ284					0.30	5.21	-13.7	28.7								
	3	RG910-RG418A													0.20	1.83	8.9	14.0
f	4	RG788-RZ565									0.02	2.52	-5.0	13.4				
	4	RG214-RG143	0.00	2.78	-7.8	14.2	0.00	3.60	-9.7	14.2								
2,5,c,e,h,m	5	RZ649-RZ67									0.26	2.42	-4.2	9.2				
	6	Pgi2-pRD10B	0.08	3.12	8.6	17.3												
	8	A10K-AG8Aro									0.06	2.30	5.7	16.9				

Continued...

Table 2. continued.

Cosegregating			CONTROL 95				STRESS 95				CONTROL 98				STRESS 98			
traits*	Chr.	Trait/interval	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2	Pos.	LOD	A	r^2
		STERILITY (%)																
	1	***RZ730-RZ801***	***0.16***	***2.37***	***-5.6***	***11.7***					***0.04***	***4.32***	***-8.4***	***37.0***				
5,c	1	RG810-RG331													0.06	3.62	-4.6	16.1
c,d,h,j	2	Amy1AC-RG95													0.00	1.94	3.5	9.3
	3	CDO87-RG910													0.06	1.94	-4.0	12.0
4	5	RG313-RZ556	0.00	3.66	-7.0	18.1	0.00	2.81	-8.8	16.0								
5	6	RG172-CDO544					0.10	2.26	7.2	10.7								
1,2	7	RZ337B-CDO497					0.00	2.11	-6.7	9.2								
a	8	RZ66-AC5	0.08	2.48	-6.1	13.8												
		CHANGE IN STERILITY																
1,2	3	RG104-RG348									0.00	2.47	4.2	14.6				
3,6,h,m	5	CDO105-RZ649									0.00	1.92	3.4	9.6				
		THOUSAND GRAIN WEIGHT																
	1	RZ730-RZ801					0.40	1.89	-0.9	5.2								
3	1	RZ19-RG690									0.00	1.99	-1.0	9.2				
1,2	2	PaII-RZ58													0.16	4.15	-1.8	17.2
c,e,h,j	2	CDO686-Amy1AC													0.02	3.40	-1.6	13.1
	3	RG179-CDO337					0.18	5.92	2.1	25.9								
3	3	Pgi1-CDO87					0.00	4.69	-1.9	21.0								
4,5,j,k	9	RZ12-RG677																
c	***10***	***RG257-RG241***	***0.20***	***2.46***	***2.0***	***18.2***	***0.28***	***2.97***	***1.3***	***10.7***	***0.18***	***2.93***	***1.4***	***20.7***				
m	12	RG463-RG901													0.00	2.45	-1.2	7.5
		BIOMASS AT HARVEST																
	1	RG381-RZ19					0.00	2.70	49.8	16.7								
	7	RG773-RG769	0.24	3.34	-68.3	22.5												
		YIELD (g/m2)																
	1	***RZ730-RZ801***	***0.08***	***2.70***	***22.9***	***11.1***					***0.10***	***6.24***	***28.8***	***29.4***				
1,d,h,j	2	RG95-RG654									0.02	2.30	-15.0	7.9				
n	3	RG348-RZ329									0.00	1.93	13.4	6.4				
	3	RZ337-RZ448									0.22	1.90	13.0	6.0				
	6	Pgi2-pRD10b									0.32	2.38	16.7	9.9				
	6	RG648-RG424	0.11	1.83	17.4	6.4	0.10	2.82	17.2	21.5								
5	6	RG172-CDO544					0.04	3.44	-18.2	24.1								
a	8	RZ66-AC5	0.06	3.88	30.8	20.2												
c	11	RZ536-Npb186	0.00	3.54	-24.6	12.8												
		HARVEST INDEX (%)																
c	1	U01-RG532									0.00	2.09	0.0	8.5				
	1	RG690-RZ730									0.14	6.27	0.0	30.6				
	3	RZ337A-RZ441									0.22	2.07	0.0	9.3				
n	6	RG648-RG424					0.10	3.36	3.0	23.7								
5	6	RG172-CDO544					0.04	3.66	-3.2	27.0								
a	8	RZ66-AC5	0.02	2.66	3.4	19.2												

* Published QTLs identified in IR64XAzucena population: 1=root penetration, 2=root thickness, 3=root weight, 4=maximum root length, 5=leaf traits (rolling, drying or RWC). Traits 1-2 from Zhang et al., 1999; traits 2-4 from Yadav et al., 1997; trait 5 from Courtois et al., 1999.
Published QTLs identified across several rice populations: 6=osmotic adjustment or lethal osmotic potential, from Zhang et al., 1998.
Cosegregating traits observed in 1998 experiment: a=leaf area index in control, b=leaf area index in stress, c=stem borer score in stress or control, d=relative water content in control, e=relative water content in stress, f=canopy temperature in stress, g=root exudate, h=water loss rate from excised leaves (g/g dry weight/hour), j=leaf drying score, k=change in relative water content, m=turgid leaf water content in stress, n=whiteheads in control.
** Cosegregating traits are not listed for the RZ730-RZ801 interval because of the large number of traits reported.

Across the two experiments, common QTLs were identified for plant height, panicle length, tillering, and sterility. These were located in the region of chromosome 1 that contains the *sd1* locus and confers the semidwarf habit. s*d1*, which we assume to be in the RZ19-RG690-RZ730-RZ801 broad interval, also had a strong effect on yield components. Effects of *sd1* on traits that are pleiotropically linked to height, such as leaf length, panicle length, and panicle exsertion are mentioned in the literature, along with effects on tillering, thousand grain weight, biomass, and yield. The same interval has also been shown in the past to be associated with root parameters and leaf field scores of leaf rolling and drying under water stress. It is not clear whether this is due to the direct effect of *sd-1* on plant height and biomass or through other genes located nearby.

There were several areas where QTLs were associated in repulsion phase (e.g., for thousand grain weight under stress on chromosome 3; for yield under stress on chromosome 6).

QTLs for some secondary traits were found to cosegregate with intervals associated with yield or yield components. These are identified in Table 2. For many traits, alleles with positive effects were contributed by both parents.

Epistasis seemed to explain a non-negligible part of the variability for most traits. Significant epistatic effects were observed for some traits for which no main-effect QTLs were identified. Common marker pairs were not identified across stress levels or across experiments. Most of the markers involved in the interactions were not involved in the main effect for the trait. The small number of lines evaluated makes further interpretation of the interaction effects difficult.

The data set was reduced to the 54 common lines and main-effect QTLs were identified for the two years within each stress level (data not shown). Of the 37 QTLs identified in the control, only 5 had not been identified in either year. Of the 25 QTLs identified under stress, 9 had not been identified in either year. Significant QTL x environment interactions (QxE) were not detected across experiments for the highly heritable traits. However QxE were observed for tillering, biomass, yield, and harvest index across the control plots, and tillering, sterility, thousand grain weight and biomass across the stress plots.

In the combined analysis of 54 lines, the effect of the IR64 allele in irrigated experiments was to decrease sterility and increase yield and harvest index while decreasing total biomass. Under stress, the effects of the IR64 alleles on biomass and sterility were positive for one region and negative for another. In the case of biomass under stress, the QTLs were associated in repulsion phase on chromosome 1.

Conclusions

Significant genetic variation exists in this population for performance under both fully irrigated upland conditions and under reproductive stage stress. Stress imposed before flowering on high-density plantings generally identified different superior lines, and thus different important QTLs, than stress imposed by water deficit after planting in spaced plants. Only a few QTLs had consistent effects across years and water levels — most were specific to one environment. Across years, the QTLs identified for yield components under stress were not identified in the control, indicating that spill-over effects are not adequate to exploit the genetic potential for yield under stress that is present in this population. On the other hand, the interval containing the *sd1* gene appears to have a major effect on sterility, yield, and harvest index under varied conditions. As far as we know, these are the first results reported on QTLs for yield components in water-stressed rice. Further studies under other drought conditions and with other populations are needed to confirm these conclusions. If they are confirmed, it should be possible to identify QTLs for performance under stress that are not linked with biomass, and introduce them into varieties with good yield potential through marker-aided selection.

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Development of Near Isogenic Introgression Line (NIIL) Sets for QTLs Associated with Drought Tolerance in Rice

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Summary

Two mechanisms are believed to contribute to drought tolerance (DT) in rice. A deep and thick root system in the upland rice lines (japonicas) is largely responsible for their tolerance to drought. Alternatively, a better developed osmotic adjustment (OA) capacity in indica cultivars is considered to be a reliable mechanism to maintain the cell turgor under diminishing leaf water potential, potentially contributing to DT. To test the hypothesis of whether the two different DT mechanisms can be combined through QTL pyramiding, four QTLs (located on chromosomes 1, 2, 7, and 9) identified in a rice DH population were transferred into an indica variety, IR64, using a marker-assisted backcross program. Thirty BC_3F_3 near isogenic lines with 1-2 introgressed QTLs were obtained and evaluated in a replicated greenhouse experiment for the target root traits and two nontarget traits—number of tillers per plant and plant height. Three of the 4 introgressed QTLs (targets 1, 7, and 9), which were detected with greater LOD previously, were associated with the expected root phenotype, while the other (target 2) was not. The introgressed root QTLs were associated with either reduced tiller number or increased height, but this association was inconsistent, indicating that the observed genetic drag was more likely due to linkage rather than to pleiotropy. The concept and procedure of a new approach — the molecular backcross breeding strategy — were proposed that allow simultaneous identification, transfer, and allelic diversity discovery of desirable QTLs. This approach should be able to overcome all the limitations of MAS for QTLs and has all the advantages of the AB-QTL analysis. However, the effectiveness of phenotypic selection for DT in the BC progenies remains the key to success for this approach

Introduction

The rice crop is sensitive to drought at different developmental stages, particularly during the reproductive stage when varied degrees of sterility can arise under drought stress (Widawsky and O'Toole 1990). In the rainfed lowland and upland ecosystems, there are frequent periods of drought. Tolerance of rice plants to drought is both genetically and physiologically complex. Rice genotypes vary significantly in their tolerance to drought. It is also known that drought tolerance (DT) mechanisms in rice cultivars in upland and lowland conditions are different, as a result of their adaptations to different environmental and soil factors present in the two ecosystems (O'Toole 1982; Lilley and Fukai 1994; Fukai and Cooper 1995). Thus, development of DT rice cultivars has not been very successful despite the tremendous efforts made by breeders. Recently, several studies have been undertaken to map quantitative trait loci (QTLs) for root traits that are presumably associated with DT in rice (Champoux et al. 1995; Ray et al. 1996; Price and Tomos 1997; Yadav et al. 1997). One of the major objectives in these QTL mapping studies was to identify DT QTLs that could be used for genetic improvement of DT of rice cultivars by marker-assisted selection (MAS). In this paper, we present two strategies for the development of near

isogenic introgression lines (NIILs) of rice for DT QTLs. The first approach used MAS to transfer four previously identified root trait QTLs into an elite rice variety, IR64. The second strategy is a new approach for massive introgression of large numbers of genes/QTLs associated with DT into IR64 by molecular backcross breeding procedures, phenotypic selection, and marker-aided pyramiding or recurrent selection.

Development of the Ir64 NIILs by Mas

Materials and methods

Four QTLs associated with rice root traits and their linked DNA markers on rice chromosomes 1, 2, 7, and 9 (designated as targets 1, 2, 7, and 9) identified in our previous mapping results, were selected as the targets for MAS (Fig. 1). The donor parents were 4 doubled haploid lines that had the desirable (Azucena) alleles at the target QTLs and > 50% of the recipient (IR64) genome. The recipient was IR64, an indica variety, which has been widely grown in South and Southeast Asia. In the marker-aided BC, 120 plants were genotyped for the markers flanking the target QTLs in each of BC generations up to BC_3F_1; those BC progenies with desired genotype profile were selected as the female parents for further crossing with IR64. The BC_3F_1 plants were selfed to produce the BC_3F_2, which were screened for plants having homozygous Azucena allele(s) at the target QTLs. A total of 58 such BC_3F_2 plants were selected. These were genotyped with 35 well distributed microsatellite markers to estimate the Azucena genome in each of the BC_3F_2 plants.

The 58 selected BC_3F_2 plants were also evaluated for their phenotypic similarity to IR64 under the field conditions. Phenotypic characters recorded included maturity, plant height, tiller number per plant, and total grain weight per plant. Based on both genotypic and phenotypic data, 35 BC_3F_2 plants, which carried the donor QTL allele(s) at the target QTLs and were similar to IR64 phenotypically, were allowed to produce BC_3F_3 lines. These BC_3F_3 were then evaluated for the target root traits in a replicated experiment. The phenotyping experiment had six replications for each of the NIILs arranged in 5 random blocks with IR64 as the check in each of the blocks. The traits measured on the BC_3F_3 lines included several target root traits—maximum root length (MRL), shallow root weight (SDW), total root weight (TRW), deep root weight (DRW). Four nontarget traits were also measured on the NIILs, including plant height (PH), maturity (MAT), tiller number per plant (NBT), and grain yield per plant (GY, data missing).

Results

Phenotypic changes for the target root traits of the NIILs

Table 1 shows the results of the differences between the NIILs and the recurrent parent, IR64, for the target root traits and nontarget traits. Of the three target-1 NIILs, only one had significantly improved target root traits as compared to IR64. None of the target-2 NIILs differed significantly from IR64 for the target root traits. For the 12 target-7 NIILs, 5 showed significant improvements for the root traits, but 2 had significantly reduced values for the root traits. Two of the 4 NIILs with targets 1 and 7 had one or more improved root traits. None of six target-9 NIILs had significantly increased TRW and DRW, but four of them were associated with increased MRL.

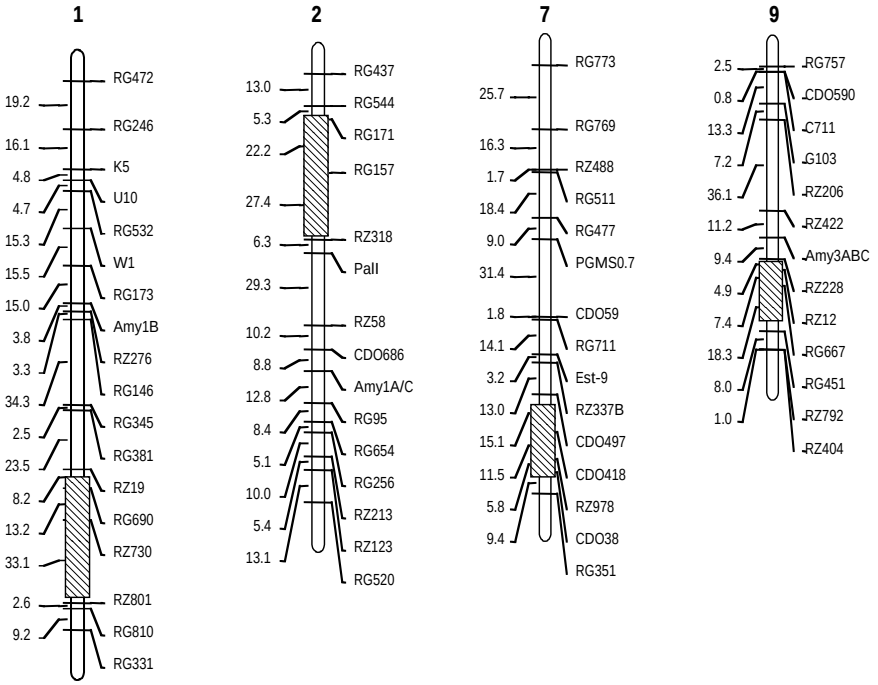

Figure 1. Target genomic regions introgressed for QTLs affecting root traits from Azucena.

Association of introgressed donor segments with the nontarget traits

Table 1 also shows the association of the NIILs with some nontarget traits. For instance, increased height and reduced tiller number per plant were detected for two of the three target-1 NIILs. Three of the five target-2 NIILs had increased height and reduced tiller number. Most target-7 NIILs had significantly increased height; some of them had either more or less tillers. All target-9 NIILs had significantly reduced tiller number. Three of the four NIILs with introgressed targets 1and 7 QTLs were significantly taller than IR64.

Development of Ir64 NIILs for Drought Tolerance QTLs by Molecular BC Breeding

To develop rice cultivars with significantly improved DT, a new strategy called the molecular BC breeding approach is being used at IRRI for development of NIILs.

Materials and methods

IR64 (indica) and a new plant type (NPT) line are being used as the recurrent parents. Approximately 200 diverse rice germplasm from around the world are being used as donor parents. These include about 70 elite commercial cultivars grown in major global rice target environments and ~130 landraces or breeding lines from IRRI's worldwide rice collection. Together, these lines are expected to have a tremendous diversity at loci for drought tolerance and traits of agronomic importance and to represent a significant breadth of the total diversity in the primary gene pool of *O. sativa*. Of the donor lines, there is a dominant mutant for male sterility (DMS), which will be used in the phase III for marker-aided pyramiding of multiple DT QTLs by recurrent selection. The procedure contains three phases, described as follows:

Phase I - molecular characterization of the parents:

Briefly, the 200 donors and the two recurrent parents are being genotyped using 500 well distributed anchor DNA markers (STSs, RAPDs, microsatellites, known genes, RFLPs, and AFLPs). In the meantime, massive introgression of DT genes/QTLs from the 200 donors into the recurrent parents are being carried out using backcrossing and phenotypic selection (Fig. 2). Specifically, 400 BC populations are being created between the recurrent parents and the donors (Fig. 2). Backcrossing will be repeated 3–5 times. Selection for improved DT under the stress conditions will be practiced in both BCF1, BCF2, or BCF3 bulks. At the end of the BC breeding, 2 sets of NIILs (IR64 and NPT) will be produced. Members of each NIIL set are genetically identical except that each contains unique identifiable introgressed genomic fragments associated with improved DT from known donor parents. In addition, a dominant male sterility mutant gene is also being transferred into the recurrent parents.

Table 1. IR64 NIILs from MAS showing significant associations with target root traits.

Pedigree	Target QTL	PH	NBT	MAT	MRL	SDW	RW3060	TRW	DRW	SDW_T
IR64		75.8	34.0	117	69.1	11.3	0.159	1.392	0.175	0.33
IR74392-108-6	1	75.4	34.1		75.9	12.1	0.129	1.699*	0.146	0.35
IR74392-118-4	**1**	**89.4****	29.9	118	73.5	12.0	**0.245***	**1.645***	**0.279***	**0.41****
IR74392-135-1	1	75.7	38.8	117	70.7	12.5	0.212	1.669*	0.233	0.32
IR74392-201-14	2	84.0**	24.9**		72.1	11.1	0.136	1.230	0.154	0.44**
IR74399-204-10	2	83.1**	21.2**		65.0	9.7	0.108	1.173	0.117**	0.45**
IR74401-215-5	2	76.7	25.7*	116	70.4	9.1*	0.116	1.333	0.127**	0.35
IR74401-215-18	2	75.8	31.4	115	73.2	11.4	0.203	1.424	0.228	0.37
IR74401-216-7	2	79.3	30.6	115	72.6	12.2	0.176	1.656*	0.194	0.39
IR74405-711-1	7	78.9*	36.8	112*	64.3	12.2	0.100	1.510	0.116	0.33
IR74405-720-7	**7**	**85.0****	**32.3**	**115**	**70.8**	**14.2****	**0.248***	**1.593***	**0.278***	**0.45****
IR74405-720-12	**7**	**86.0****	**33.4**		**78.5***	**14.5****	**0.239***	**1.572**	**0.260***	**0.43****
IR74409-730-8	7	72.1*	24.9*		67.9	8.2*	0.091*	0.988*	0.100**	0.32
IR74409-730-9	7	74.1	23.3**		65.5	8.9*	0.095	1.288	0.103**	0.37
IR74409-730-10	**7**	**74.1**	**44.6****	**118**	**68.2**	**11.9**	**0.245***	**1.897****	**0.265***	**0.28***
IR74409-734-4	7	75.8	30.9		65.6	10.5	0.079*	1.471	0.082**	0.33
IR74409-735-2	7	81.1**	32.1	116	66.2	12.0	0.202	1.411	0.222	0.38*
IR74409-735-12	7	80.0**	28.1*	114*	71.6	10.9	0.171	1.365	0.185	0.41**
IR74409-736-11	**7**	**86.5****	30.2	**119***	74.9	**13.4***	**0.233***	**1.807****	**0.263***	**0.44****
IR74409-737-12	**7**	**82.1****	37.5	118	69.7	11.4	**0.270****	**1.685***	**0.302****	0.33
IR74409-738-11	7	83.9**	27.8*	118	66.8	10.9	0.143	1.353	0.150	0.39*
IR74418-910-2	9	78.1	27.4*	116	**88.7****	10.5	0.196	1.585	0.225	0.38*
IR74418-910-3	9	75.6	26.6**		71.5	8.2**	0.171	1.271	0.187	0.30
IR74418-910-12	9	77.2	27.9*		72.8	10.3	0.121	1.212	0.143	0.37
IR74418-913-7	9	76.1	28.3*		**79.7***	8.1**	0.131	1.160	0.148	0.30
IR74419-921-1	9	78.7	27.7*		**77.9***	9.7	0.128	1.430	0.153	0.36
IR74419-921-8	9	75.1	24.5**		**88.1****	7.5**	0.163	1.306	0.187	0.31
IR74409-737-5	**1+7**	**81.4****	34.7	**121****	75.6	12.1	**0.286****	**1.616**	**0.308***	**0.34**
IR74409-737-12	**1+7**	**80.5****	36.3	118	70.0	**13.4***	**0.242***	**1.726***	**0.257**	0.38*
IR74409-739-4	1+7	81.3**	32.0	127**	67.9	10.1	0.126	1.467	0.135	0.31
IR74409-739-7	1+7	77.8	30.8	120**	68.5	10.4	0.102	1.581	0.106*	0.33

Phase II - QTL analyses using improved NIILs:
In this phase, all selected NIILs will be evaluated genetically using selected DNA markers (polymorphic between the recurrent and the donor parents) to identify introgressed donor DNA fragment(s) associated with improved DT traits and residual genetic drag(s) in each of the NIILs. The NIILs will also be evaluated phenotypically for improved DT traits in replicated experiments.

Phase III - Development of DT IR64, NPT, and near isogenic line (NIL) sets using marker-aided recurrent selection and NIILs:
At the end of the Project when large numbers of NIILs for DT QTLs are developed, many crosses between sister NIILs carrying nonallelic DT QTLs will be made with the help the DMS IR64 and NPT lines. This will be accomplished through a combination of marker-aided pyramiding of nonallelic desirable QTLs using recurrent selection with the help of the DMS IR64 and DMS NPT lines (as the female parents) obtained in Phase II. It is anticipated that we will take ***n*** generations to combine 2^n QTLs in the IR64 and NPT genetic backgrounds, from which the DT IR64, DT NPT, and the corresponding near isogenic line (NIL) sets for DT genes/QTL will be developed.

Expected results
The following results are expected from the molecular BC breeding program: (1) massive introgression of large numbers of DT QTLs from the diverse donors into elite genetic backgrounds; (2) identification of large numbers of genes/QTLs associated with DT traits; and (3) development of DT IR64 and NPT, and NILs for DT genes/QTLs.

Discussion
Two mechanisms are believed to contribute to DT in rice (Nguyen et al. 1997). A deep and thick root system in the upland rice lines (japonicas) is largely responsible for their tolerance to drought. Alternatively, a better developed osmotic adjustment (OA) capacity in indica cultivars is considered to be a reliable mechanism to maintain the cell turgor under diminishing leaf water potential, thus potentially contributing to DT. The scenario of developing the NIILs for DT here was to test the hypothesis of whether two different DT mechanisms can be combined through QTL pyramiding. Genetically, two potential problems associated with MAS of QTLs are genetic background effects, or epistasis, and genetic drag, though both theories and strategies for QTL introgression have been proposed (Tanksley and Nelson 1995; Tuinstra et al. 1997; Visscher et al. 1996a; Hospital and Charcosset 1997). In our MAS experiment, both problems were observed. We noted that only two of the introgressed QTLs (targets 1 and 7) that were detected with greater LOD previously were associated with the expected target phenotype, even though the QTLs were introgressed into the genetic background where there were originally identified. Nevertheless, it remains to be seen whether and how much the introgressed root-trait QTLs can contribute to DT under real stress conditions. Second, we noted that the association of the introgressed QTL regions with reduced tiller number and increased height was inconsistent, indicating that the observed genetic drag was more likely due to linkage rather than to pleiotry.

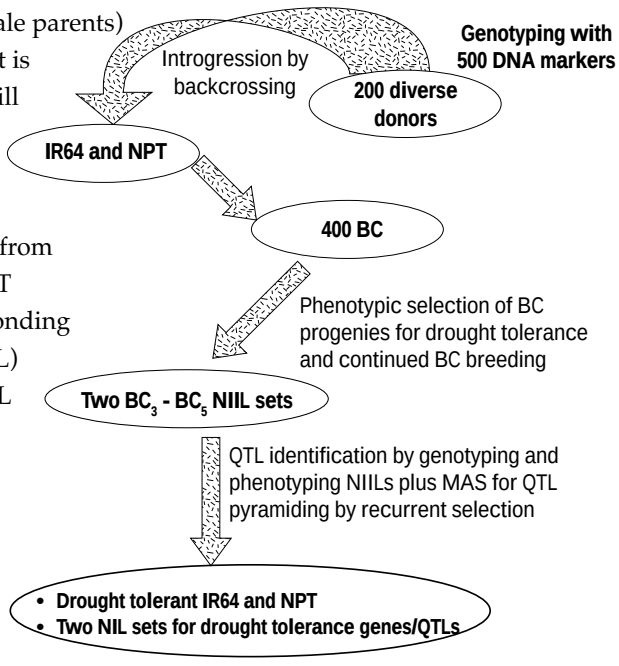

Figure 2. BC procedure at each of national programs.

The molecular BC breeding approach being adapted should be able to overcome all the limitations of MAS for QTLs mentioned above. This approach also has all the advantages of the AB-QTL analysis proposed by Tanksley and Nelson (1995), plus it also allows discovery of allelic diversity of multiple DT QTLs from diverse sources, and thus has a better chance to succeed. However, the effectiveness of phenotypic selection for DT in the BC progenies remains the key to success for this approach.

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Identification and Utilisation of Quantitative Trait Loci to Improve Terminal Drought Tolerance in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Summary

Drought at the reproductive stage of pearl millet is one of the most important environmental factors reducing its yield and yield stability. We are using a QTL mapping approach to better understand the genetic and physiological basis of drought tolerance in this crop and to devise strategies to utilise the identified QTLs for improving drought tolerance and yield in water-limited environments. Test-crosses of two sets of mapping population progenies, derived from inbred pollinators and from seed parents differing in their response to drought, were evaluated in a range of managed terminal drought-stress environments to identify individual QTLs associated with drought tolerance. A number of QTLs associated with drought tolerance of grain yield and its agronomic and physiological components were identified. Some of the identified QTLs were common across water-stress environments and genetic backgrounds of the two mapping populations while others were specific to a particular water-stress environment or genetic background. Interestingly, all the identified QTLs contributed to increased drought tolerance either through their effect on increased maintenance of growth, or harvest-index, or both in terminal drought-stress environments. Marker-assisted backcross transfer of the identified QTLs into the elite parent of these mapping populations and their further use in the improvement of pearl millet productivity in water-limited environments is discussed.

Introduction

Pearl millet is an important cereal grain and fodder crop in the driest areas of South Asia and sub-Saharan Africa. In such areas, post-flowering drought stress (terminal drought) has been identified as one of the major environmental factors that reduces both the yield and yield stability of this crop. Improving the adaptation of pearl millet to terminal drought-stress environments is, therefore, a major objective in pearl millet breeding programs. Breeding crop varieties for increased drought tolerance has traditionally been slow, but efficiency could be improved if attributes that help maintain yield under water-limited conditions are identified and used as selection criteria (Ludlow and Muchow 1990). Recent advances in molecular marker technology provide opportunities not only to identify individual genetic factors and their functions in determining complex phenotypes such as drought tolerance (Prioul et al. 1997; Quarrie 1996), but also help breeders in guiding introgression and selection through the use of linked markers.

The objective of the research reported in this paper is to identify the individual genetic factors that help maintain grain yield of pearl millet in terminal drought stress environments and to devise strategies to deploy them in improving the drought tolerance of this crop.

Materials and Methods

We have advanced two sets of mapping population progenies — one from a cross between two elite inbred pollinators (H 77/833-2 and PRLT 2/89-33; referred to as MP 1) and the other from a cross between two elite inbred seed parents (ICMB 841 and 863B; referred to as MP 2). In both crosses, one of the parents (PRLT 2/89-33 in case of MP 1, and 863B in case of MP 2) were derived from the *Iniadi* landrace material from Togo and Ghana, which is known for its better grain filling ability under terminal drought stress conditions. The other parents used in each cross are elite inbred lines, the hybrids of which are widely grown by the farmers in pearl millet growing areas of northwestern India. RFLP-based genetic linkage maps with markers well distributed over all seven linkage groups, were constructed using approximately 150 individual F_2 plants derived from a single F_1 plant, in each cross. For phenotyping the response to drought, a subset of F_3 progenies (104 from MP 1 and 79 from MP 2) of the mapped F_2 plants were test-crossed onto unrelated inbred tester(s) to produce test-cross hybrids. Test-crosses were produced on an elite A- line (843A) for MP 1 and on two different elite inbred pollinator lines (H 77/833-2 and PPMI 301) for MP 2. MP 2 was evaluated using two testers to determine whether genetic background influences the expression of the identified QTLs.

The performance of test-crosses (TC) under terminal drought stress was characterised under field conditions at the ICRISAT-Patancheru (India) research farm using managed terminal stress environments created with controlled irrigation. They were evaluated in a number of stress environments (Table 1) using either RCB- or alpha-designs during the dry seasons of 1997 to 1999. The dry season at ICRISAT-Patancheru is usually rain-free, with a high mean air temperature and large vapour pressure deficit, which provides an excellent opportunity to expose plants to controlled but severe drought stress by adjusting the timing of irrigation (Bidinger et al. 1987a). Drought stress in different experiments began either at flowering stage or at early grain filling stage (Table 1) and was initiated by withholding irrigation. In addition to

Table 1. Mean and range of percentage reductions in growth and grain yield parameters of pearl millet mapping population test-crosses in stress environments as compared to paired irrigated control environments.

	Mapping population progeny set 1									Mapping population progeny set 2												
Terminal drought stress environments	Environment 1 (LS*, S97DN**)			Environment 2 (ES*, S98ROS**)			Environment 3 (ES, S98NROS**)			Environment 1 (ES, S98DN**)						Environment 2 (LS, S98DN)					
Tester background	843A tester			843A tester			843A tester			H 77/833-2 tester			PPMI 301 tester			H 77/833-2 tester			PPMI 301 tester		
Trait	Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		min	max		min	max		min	max		min	max		min	max		min	max		min	max
Grain yield (g m^{-2})	-26.9	-4	-54	-27.4	+1	-48	-60.5	-34	-77	-57.1	-42	-76	-53.1	-22	-68	-43.0	-12	-65	-38.3	-20	-54
100-seed weight (g)	-21.2	-8	-32	-22.1	-13	-32	-37.3	-21	-51	-43.8	-30	-56	-43.3	-22	-57	-33.5	-18	-49	-31.0	-16	-46
Panicle number m^{-2}	-3.5	+26	-35	+0.7	+38	-27	-20.3	+15	-40	-5.4	+23	-38	+4.7	+34	-19	+9.5	+39	-14	+15.2	+64	-16
Panicle grain number (x1000)	-2.3	+31	-39	-7.5	+20	-32	-21.8	+8	-41	-21.3	+3	-41	-22.2	+8	-43	-22.8	0	-38	-21.7	+5	-41
Stover yield (g m^{-2})	-7.4	+38	-43	-20.9	+27	-51	-55.0	-40	-71	-34.3	-20	-53	-31.1	-1	-51	-25.6	+3	-50	-26.4	+8	-48
Biomass yield (g m^{-2})	-14.9	+24	-36	-21.0	+11	-44	-53.4	-40	-67	-43.5	-31	-57	-40.2	-15	-56	-29.7	-5	-50	-30.0	-8	-45
Harvest-index (HI, %)	-13.6	+8	-29	-8.5	+16	-23	-17.0	+15	-36	-25.8	-13	-45	-22.1	+7	-42	-19.6	-3	-39	-12.1	+9	-29
Panicle harvest-index (PNHI, %)	-8.2	+38	-26	-8.5	+14	-18	-18.8	+4	-32	-16.0	-4	-35	-12.5	+5	-30	-15.8	-2	-32	-8.7	+7	-20

* LS = late-onset terminal drought stress commenced at early grain filling stage of crop growth; ES = early-onset terminal drought stress commenced at flowering stage of crop growth

** S97DN = 1997 summer season drought nursery; S98ROS = 1998 summer season rainout shelter; S98NROS = 1998 summer season without rainout shelter; S98DN = 1998 summer season drought nursery (all at ICRISAT-Patancheru, India)

the terminal stress environment, all the drought environments had a paired control environment that received irrigation throughout the growing season. Time to flowering was recorded as the days to stigma emergence in 50% of the main shoot panicles in a plot. At harvest, data were recorded on the number of plants and number of panicles per plot, dry stover yield, grain yield, and 100-seed weight. Grain yield, stover yield, total plant biomass yield at maturity, and panicle numbers were expressed on a per-m^2 basis. Panicle grain number [(100 × grain yield)/(panicle number × 100-seed weight)] was derived from these primary data. Harvest-index (HI=grain yield/biomass yield) and panicle harvest-index (PNHI=grain yield/panicle yield: Bidinger et al., this proceedings) were calculated on a plot basis. In addition to agronomic traits, the mapping population test-crosses were also evaluated for their expression of physiological responses to drought stress such as leaf rolling, leaf senescence, relative water content, and osmotic adjustment.

Analysis of variance was performed to determine the significance of genetic variation between test-crosses for all the traits measured in the irrigated control and in stress environments. Drought tolerance (i.e., expression maintenance under stress conditions) of grain yield and grain yield component traits was calculated either as a ratio of trait expression in each stress environment to trait expression in the paired irrigated control environment or by calculating drought response index (DRI; Bidinger et al. 1987b). When applicable, DRI was calculated to correct for the effect of drought escape and yield potential expression in the stress environment. QTL mapping was performed on these values using the method of interval mapping with the QTL mapping software package MAPMAKER/QTL 1.1 (Lander and Botstein 1989).

Results and Discussion

Growth and yield parameters of test-crosses in stress environments

The mean and range in the percent change in the values for grain yield, biomass yield, and their component traits under stress as compared to their paired irrigated control environments, are presented in Table 1. Terminal drought stress reduced mean grain yield from 26.9% to 60.5% in MP 1 and 38.3% to 57.1% in MP 2 in the different stress environments. Reductions in grain yield in all stress environments were due to reductions in three grain yield determining components: the number of effective tillers per m^2, grain size, and grain number per panicle. Collectively they explained 95% of the variation in reduction in grain yield in all stress environments.

Although terminal drought stress reduced both mean grain and biomass yield, individual component traits showed both increases as well as decreases amongst the test-crosses in all stress environments as compared to the irrigated control environment (Table 1). Increased performance of panicle number m^{-2}, panicle grain number, harvest-index, panicle harvest-index, and stover and biomass yield was particularly evident in some test-crosses in all the stress environments. Both increases and decreases in the performance of some of the component traits under stress indicated that different test-crosses adopted different growth and production strategies when exposed to drought. Increased expression of some component traits under stress resulted in a compensation effect; reduction in performance of one component was associated with increase in the performance of another component.

At the whole plant level, reduction in biomass yield explained more than 60% of the variation in reduction in grain yield while reduction in harvest-index explained more than 50% of the variation in reduction in grain yield. Collectively reduction in both biomass yield and harvest-index explained most of the variation (more than 95%) in reduction in grain yield in stress environments in both sets of mapping population progenies in different stress environments. Reductions in biomass yield and in harvest-index were also both positively and significantly correlated to the three grain yield determining component traits (effective panicle number m^{-2}, grain size, and panicle grain number). The observed relationship between these traits indicate that in these two mapping populations, both the accumulation and the partitioning of dry matter during the stress period were important mechanisms determining grain yield and its components.

QTLs associated with drought tolerance of grain yield

QTLs on linkage group 2

Using MP 1, a QTL associated with drought tolerance of grain yield was obtained on linkage group 2 in two of the three stress environments, explaining up to 23% of the variation in drought tolerance response of

grain yield (Table 2a). In the same interval on linkage group 2, QTLs associated with drought tolerance of 100-seed weight (three environments), HI (two environments), PNHI (two environments), and panicle number m^{-2} (one environment) also mapped. A QTL for maintenance of stover and biomass yield in the most severe stress environment (environment 3; MP 1) also mapped to this interval on linkage group 2. Interestingly, the PRLT 2/89-33 allele at this putative QTL was associated with increased drought tolerance of grain yield and all the component traits described above. Increased HI and biomass

Table 2. Quantitative trait loci (QTLs) associated with drought tolerance of growth and yield parameters in two sets of mapping population progenies.

a. Mapping population progeny set 1

Terminal drought stress environments	Stress environments											
	Environment 1 (LS*, S97DN**)				Environment 2 (ES*, S98ROS**)				Environment 3 (ES, S98NROS**)			
Tester background	843A tester				843A tester				843A tester			
Trait	Linkage group	LOD	R^2	Parent	Linkage group	LOD	R^2	Parent	Linkage group	LOD	R^2	Parent
Grain yield	1	2.61	11.2	PRLT[a]	2	2.30	13.5	PRLT	2	4.68	23.0	PRLT
100-seed weight	2	2.01	9.5	PRLT	2	2.49	11.8	PRLT	2	3.19	16.1	PRLT
					6	2.04	11.6	H 77				
Panicle number m^{-2}	6	2.59	10.9	H 77[b]					2	2.97	15.3	PRLT
Panicle grain number	1	2.41	10.2	PRLT								
Stover yield									2	2.21	12.8	PRLT
									6	2.99	17.9	PRLT
Biomass yield									2	3.27	16.2	PRLT
Harvest-index (HI)					2	1.92	10.1	PRLT	2	3.77	18.0	PRLT
Panicle harvest -index (PNHI)					2	2.20	12.7	PRLT	2	3.09	14.5	PRLT
					4	2.03	15.4	PRLT				

* LS = late-onset terminal drought stress commenced at early grain filling stage of crop growth; ES = early-onset terminal drought stress commenced at flowering stage of crop growth

** S97DN = 1997 summer season drought nursery; S98ROS = 1998 summer season rainout shelter; S98NROS = 1998 summer season without rainout shelter (all at ICRISAT-Patancheru, India)

[a] PRLT; PRLT 2/89-33 parent allele has positive effect

[b] H 77; H 77/833-2 parent allele has positive effect

b. Mapping population progeny set 2

Terminal drought stress environments	Stress environments															
	Environment 1 (ES*, S98DN**)								Environment 2 (LS*, S98DN)							
Tester background	H 77/833-2 tester				PPMI 301 tester				H 77/833-2 tester				PPMI 301 tester			
Trait	Linkage group	LOD	R^2	Parent	Linkage group	LOD	R^2	Parent	Linkage group	LOD	R^2	Parent	Linkage group	LOD	R^2	Parent
Grain yield	2	1.89	10.9	863B[c]	2	2.28	13.5	863B	6	2.30	27.0	863B	2	2.42	14.4	863B
	5	2.44	14.8	841B[d]												
100-seed weight	1	3.59	21.2	863B									2	2.40	13.9	863B
	5	1.86	10.9	841B												
Panicle number m^{-2}	2	2.35	14.7	863B												
	5	2.87	16.7	863B												
Panicle grain number	5	1.95	11.1	841B	2	3.16	20.7	863B	2	1.72	10.3	863B	5	2.00	12.9	863B
Stover yield	2	2.38	13.2	841B												
	5	2.67	18.0	841B												
	7	2.03	16.4	863B												
Biomass yield	2	2.26	12.7	863B												
	5	3.09	18.2	841B												
Harvest-index (HI)					2	3.31	18.7	863B	2	1.83	10.5	863B	2	2.59	15.9	863B
									6	2.58	22.8	863B	6	1.90	11.3	841B
Panicle harvest - Index (PNHI)	5	2.04	12.0	841B	2	2.63	15.6	863B	6	4.44	24.9	863B	2	2.91	16.2	863B
									7	2.17	40.1	863B				

* LS = late-onset terminal drought stress commenced at early grain filling stage of crop growth; ES = early-onset terminal drought stress commenced at flowering stage of crop growth

** S98DN = 1998 summer season drought nursery, ICRISAT-Patancheru, India

[c] 863B; 863B parent allele has positive effect

[d] 841B; ICMB 841 parent allele has positive effect

yield conferred by the PRLT 2/89-33 allele at this putative QTL suggested that increased drought tolerance conferred by this QTL on grain yield and its components may have been achieved by the effect of this QTL on both increased dry matter production and on increased partitioning of dry matter to the grain. Delayed leaf rolling was also mapped to this position on linkage group 2 which suggested that the ability to maintain water potential enabled continued growth and partitioning of assimilate in stress environments.

Using MP 2, the QTL on linkage group 2 was again observed to be associated with drought tolerance of grain yield in early stress environments using both testers. Its effect on drought tolerance of grain yield, however, was achieved differently depending on the tester used. In tester 1 (high tillering line H 77/833-2) background, it exerted its effect on grain yield through increased maintenance of biomass yield and effective panicle number m^{-2}, while in tester 2 (large panicle line PPMI 301) background it maintained grain yield via increases in panicle grain number and harvest-index. When stress was initiated at a later stage (early grain filling) on MP 2 test-crosses, the effect of this QTL on linkage group 2 was evident on grain yield for tester PPMI 301 but not for tester H 77/833-2. With tester PPMI 301, the 863B allele at this QTL achieved increased grain yield through its effect on increased 100-seed weight and harvest-index. With tester H 77/833-2, although the effect of the 863B allele at this QTL was positive on panicle grain number and harvest-index (Table 2b), its effect on grain yield was not detected.

QTLs on linkage group 5 and 6:
In MP 2, a QTL associated with drought tolerance of grain yield was also obtained on linkage group 5, which explained 14.8% of the variation in reduction in grain yield. This QTL was detected only for the H 77/833-2 tester in environment 1, and was not expressed in the background of the PPMI 301 tester. An allele from ICMB 841 at this interval increased the drought tolerance of grain yield and the yield of stover and biomass. Similarly, a QTL associated with tolerance of grain yield in late stress environment was obtained on linkage group 6. The effect of this QTL was again evident only in the genetic background of tester H 77/833-2. This QTL exerted its effect on increased maintenance of grain yield via its effect on maintenance of increased harvest-index and panicle harvest index (Table 2b). The 863B allele at this QTL was associated with the maintenance of both the grain yield and harvest-index.

QTLs associated with drought tolerance of grain-yield determining component traits:
In addition to the QTLs described above, we detected a number of additional QTLs that were associated with maintenance of grain yield-determining component traits but that were not associated with maintenance of grain yield itself. One on linkage group 1 was detected in one of the three stress environments for MP 1 and in only one genetic background in one of the stress environments for MP 2. This QTL was consistently linked to increased grain filling but was apparently pleiotropic to decreased panicle number so its effect on expression of grain yield in stress environments was not evident. Similarly, a QTL on linkage group 6 was linked with increased grain filling in two of the three stress environments for MP 1, but was pleiotropic to reduced panicle grain number. The effects conferred by these QTLs on increased maintenance of one or the other grain-yield determining traits thus were compensated for by a reduction in the expression of one or more other grain-yield component traits.

Utilisation of QTLs in hybrid parent breeding

A number of crop improvement strategies for improving yield in water-limited environments have been proposed (Blum 1988; Ceccarelli et al. 1998; Ludlow and Muchow 1990). We believe that grain yield of pearl millet in water-limited environments can be improved if specific traits and responses associated with drought tolerance can be identified and incorporated into otherwise high-yielding genotypes of appropriate crop duration analogous to disease resistance breeding (Bidinger et al. 1987b; Fussel et al. 1992; Yadav et al. 1999). The QTL mapping approach is ideal to meet such objectives as it not only can identify individual genetic factors associated with a specific response but also can monitor the incorporation of the identified factors in breeding programmes away from the target environment, even in programmes lacking an appropriate screening environment. In the present study, the selection of mapping population parents and the evaluation of sets of mapping population progenies for QTL identification were planned in such a way that the mapping results will directly find their way into applied pearl millet

cultivar improvement programmes. Using two sets of mapping population progenies, our objectives were to improve the drought tolerance of the inbred pollinator and the seed parents so that the popular hybrids produced on them will have greater tolerance to drought. From the initial evaluations, we are starting to understand how these QTLs will behave in the genetic backgrounds of different A- and/or R-lines. Using marker-assisted backcross approaches, we are currently incorporating the identified regions of the genome into the backgrounds of parental lines of interest. The sets of near isogenic lines so developed will also provide us with an ideal opportunity to further test the effects of the identified QTLs and to clarify the associated physiological mechanisms in finer detail.

In addition to QTLs associated with drought tolerance per se, the study has also led to the identification of individual QTLs associated with grain yield and grain yield determining component traits in both stress and irrigated control environments. Efforts are also underway to recombine desirable QTLs to produce both hybrid parental lines and topcross pollinator populations with increased yield in irrigated control environments as well as under stress. Molecular-marker supported genotypic information at the identified QTL has the potential for enabling the quick and accurate accumulation of desirable alleles. The marker-assisted breeding strategies we are currently conducting to improve the drought tolerance and yield of this crop is further discussed in a paper by Hash et al., in this proceedings.

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Marker-Assisted Backcrossing to Improve Terminal Drought Tolerance in Pearl Millet

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Summary

Several alternative marker-assisted backcrossing (MABC) procedures are described that can be used for transferring quantitative trait loci (QTLs) from a donor to an elite recurrent parent when these two lines have been used in forming the base mapping population. We describe ICRISAT's experience to date in using these methods in pearl millet (Pennisetum glaucum (L.) R. Br.). We are attempting to improve terminal drought tolerance of elite inbred pollinator H 77/833-2 using donor PRLT 2/89-33, and elite inbred seed parent maintainer line ICMB 841 using donor 863B. The advantages and disadvantages of the alternatives are discussed.

Key Words

Backcrossing, contiguous segment substitutions, hybrid parental lines, marker-assisted selection, RFLP

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the staple food and fodder crop of millions of poor rural families in the hottest and driest dryland agricultural environments of Asia and Africa. Although grain and stover of this crop are not commercially important commodities (FAO and ICRISAT 1996), as most is consumed in the homesteads where they are produced, crop losses are economically important. These losses can be attributed to biotic stresses (principally *Striga* sp., birds, diseases, and insects) and abiotic stresses (principally nutrient deficiencies, drought, and heat). Increased yield and yield stability of pearl millet grain and stover would contribute to improving living standards and food security of poor families living in these harsh agricultural production regions.

ICRISAT, in collaboration with researchers in the UK and India, and supported by the Plant Sciences Programme of the UK's Department for International Development (DFID), has made a considerable research investment targeted to the development and application of molecular genetic tools for improving the yield and yield stability of pearl millet hybrid cultivars. Such hybrid cultivars are currently sown on >5 m ha each year by small holders in India. They have contributed to the substantial increase in pearl millet grain yields (ca. 100%) and grain production that has occurred in India over the past five decades. It is noteworthy that this increase has occurred during a time when the area sown to this crop has not only decreased, but shifted to more marginal lands thereby freeing up better land for production of higher value crops. Identification of marker-flanked quantitative trait loci (QTLs) associated with superior grain yield performance under terminal drought stress conditions has been a major part of this research activity during the past five years (see Yadav et al., this proceedings).

This paper describes several alternative procedures that can be used in pearl millet, and perhaps other crops, for marker-assisted backcross transfer (MABCT) of QTLs from a donor to an elite recurrent parent when the donor and recurrent parent have been used in forming the base mapping population. Advantages and shortcomings of each alternative are discussed.

Materials and Methods

Mapping of drought tolerance QTLs in pearl millet (Yadav et al. 1999a, b) began as a secondary target trait in a project intended to identify QTLs for seedling thermotolerance in pearl millet (Howarth et al. 1997). This first pearl millet mapping population with drought tolerance as a target trait was based on the cross of thermotolerant, drought-sensitive elite inbred pollinator line "H 77/833-2" from Haryana Agricultural University and thermosensitive, drought-tolerant breeding line "PRLT 2/89-33" from ICRISAT (Hash and Witcombe 1994). Studies of this population were followed with development and evaluation of a second pearl millet mapping population having terminal drought tolerance as its primary target trait. In this case, the drought-sensitive parent was "ICMB 841" (Singh et al. 1990) and the drought-tolerant parent was "863B." Both ICMB 841 and 863B were bred at ICRISAT-Patancheru and are elite maintainer lines of hybrid seed parents that are extensively used in India. Both PRLT 2/89-33 and 863B are derived from the Iniadi landrace of pearl millet (Andrews and Anand Kumar 1996). Mapping population development was as described by Hash and Witcombe (1994), with RFLP skeleton mapping, trait phenotyping, and QTL mapping as described by Yadav et al. (1999a, b). The parental lines, skeleton maps, and skeleton-mapped progenies from these two mapping populations have been used by us as starting points in a series of marker-assisted backcrossing (MABC) programs, initiated before or after completion of QTL mapping of the target trait (terminal drought tolerance, and its components). These MABC programs are described in detail below.

Conventional MABC Programs

Conventionally, MABC programs begin only after QTL mapping has identified the map position and closely linked flanking markers for donor parent gene blocks that contribute substantially to target trait phenotypic variation in the mapping population. At that point the breeder selects one or more genotyped (and preferably phenotyped) progenies from the mapping population that combine(s), as a minimum, heterozygosity for donor parent markers in the vicinity of the target QTL with homozygosity for the recurrent parent marker genotype in most of the remainder of the mapped genome. There are then two broad avenues that can be pursued (along with many paths between these). The first of these makes extensive use of marker genotyping in nontarget regions of the genome to reduce the number of backcrosses required to recover a desirable segregant (Hospital et al. 1992, 1997). The other extreme is to marker genotype only at points immediately flanking (and inside) the target region, and use serial backcrossing to more rapidly recover the recurrent parent genotype in nontarget regions of the genome. Choice between these two extremes, and/or some intermediate path, will largely be determined by the type of molecular markers available and length of the vegetative phase of the crop life cycle. For species with a long juvenile phase in which microsatellite markers (SSRs) are available, extensive use of marker genotyping would make a lot of sense; however, for pearl millet this is not the case.

- ***Advantages*:** It is less likely that any MABC program that is started will have to be abandoned, since the marker polymorphism of the donor and recurrent parents is already characterized and the markers identified appear to be linked to substantial differences in phenotypic performance (i.e., significant QTLs of large effect have purportedly been found).
- ***Disadvantages:*** A long time is required before the MABC program can start. Further, this program is, of course, restricted to using as its starting point the best marker genotype segregant(s) present in the original mapping population (which is largely a function of genotyped mapping population size).
- ***ICRISAT experience:*** In pearl millet we have a crop with a short life cycle, and short juvenile phase that can be reduced further by artificially reducing day length to induce early flowering. Combined with RFLP markers as the only co-dominant marker system currently available, this has lead us to initiate a program of MABC based on two mapping progenies from the cross H 77/833-2 × PRLT 2/89-33 (Fig. 1). Both selections were homozygous

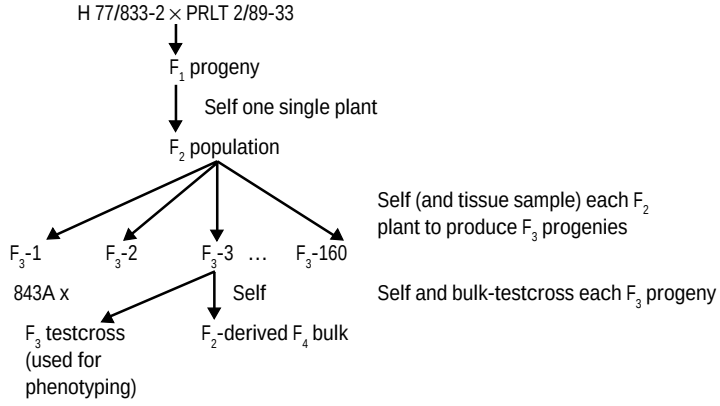
for two drought tolerance QTLs from linkage group 2 (LG2) and LG4 of PRLT 2/89-33, and at least heterozygous for the drought tolerance QTL on LG6 of H 77/833-2. These have been backcrossed to H 77/833-2, and the resulting BC_1F_1 progenies will be backcrossed again, yielding BC_2F_1 progenies segregating 1:1:1:1 for the two QTLs from PRLT 2/89-33. Individual plants from these progenies will then be genotyped at three markers flanking and centered over each of the three target drought tolerance QTLs.

Jump-started Marker-assisted Backcrossing

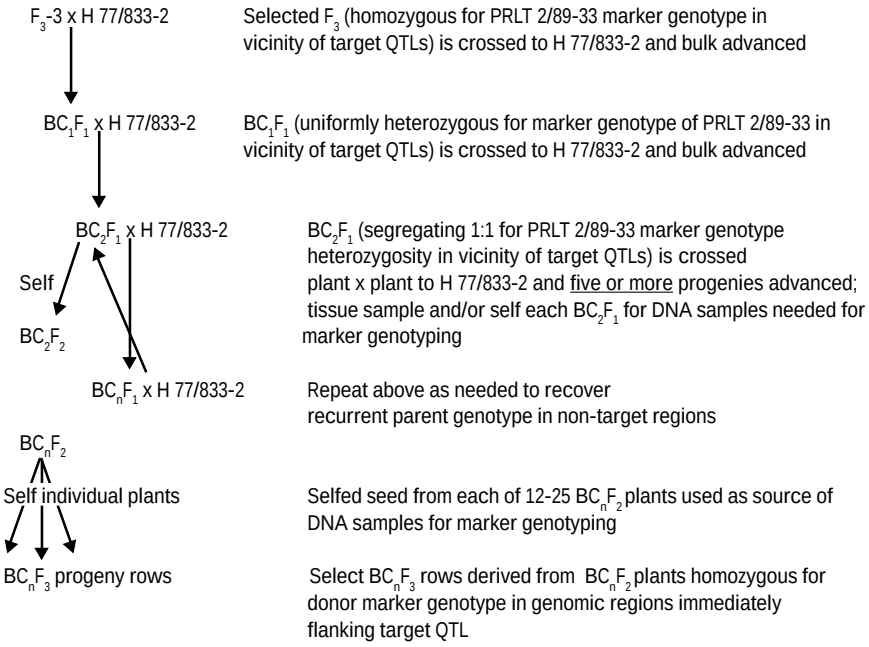
The procedure in this case begins during mapping population development itself, and perhaps even before marker polymorphism of the two parents has been fully characterized. The individual F_1 plant from which the mapping population will be derived (and itself the product of a cross between the trait donor and recurrent parent) is backcrossed to the parent weakest for the target trait. Alternatively, but less reliably, selfed progeny from the individual plant of the donor parent used in creating the mapping population can be used as the trait donor in the backcrossing program. This procedure uses probability theory (Sedcole 1977) to ensure that every possible QTL for the target trait is carried forward as rapidly as possible through the backcrossing generations. This continues until such time as markers become available, when a minimum of two markers per chromosome or linkage group arm can be used to identify segregants in which individual donor chromosome arms have been transferred into the recurrent parent genetic background. Once QTL mapping has succeeded in identifying flanking markers for QTLs of large effect, these can be used to rapidly bring the MABC program to its logical conclusion—one or more derivatives of the recurrent parent, each carrying a small segment of the donor genome consisting of a QTL for improved drought tolerance (or one of its components) and two flanking markers.

- ***Advantages:*** The major advantage of this procedure is early and rapid recovery of the recurrent parent genotype in nontarget regions. This is made possible by the early onset of the backcrossing program—even before QTL mapping, skeleton mapping, or in extreme cases even determination of parental line marker-polymorphism, have been completed.

Figure 1. Schematic for conventional marker-assisted backcross improvement of terminal drought tolerance in pearl millet inbred line H 77/833-2 based on quantitative trait loci from donor parent PRLT 2/89-33.



After F_2 skeleton mapping, F_3 testcross phenotyping, and QTL mapping, then use marker genotypes to select one or more F_2-derived F_4 bulks (or their F_2-derived F_3 progenitors) for use as drought tolerance donor for marker-assisted improvement of H 77/833-2, and proceed as below:



- ***Disadvantages:*** The down side of this procedure is that if the F_1 used as nonrecurrent parent does not have a marker and QTL genotype identical to that mapped, all of the efforts may go waste.
- ***ICRISAT experience:*** We have used this procedure to transfer the drought tolerance QTL identified on LG2 of PRLT 2/89-33 to H 77/833-2, advancing to generation BC_4F_1, where we have identified progenies likely to segregate for the target QTL and its flanking markers based on marker genotypes of the nonrecurrent parents used to produce them (Table 1). The nonrecurrent parent plants were visually very similar to the H 77/833-2 recurrent parent, so we have high hopes of quickly completing marker-assisted improvement of terminal drought tolerance of this elite male parent of several popular hybrid cultivars.

Contiguous Segmental Substitution Line Sets

A logical extension of the two procedures outlined above is the development of a contiguous segment substitution line ("contig line") set (Fig. 2).

- ***Advantages:*** This procedure will also permit detection of QTLs associated with smaller portions of the phenotypic variability for the target trait than can be detected by phenotyping modest-sized mapping populations. Further, it results in a small set (say 25-35) near isogenic homozygous lines that differ from each other by pairs of introgressed segments. For QTL mapping it will be much less expensive, and probably even more effective, to phenotype this small set of near isogenic substitution lines than a conventional mapping population. Finally, it will be possible to use the substitution line set to map QTLs for many traits that individually would not be worth the effort. An example of this is fertility restoration for the A_1 cytoplasmic-genetic male-sterility system in pearl millet, which we have mapped to LG3 while developing a contig line set of "ICMP 85410" substitutions in the background of elite maintainer line "843B" (Hash, Witcombe, and Kolesnikova-Allen, unpublished).

Figure 2. Graphical genotypes of linkage group 2 substitution line and three derived contiguous segment substitution lines (produced by backcrossing to recurrent parent and selfing out segmental substitution homozygotes).

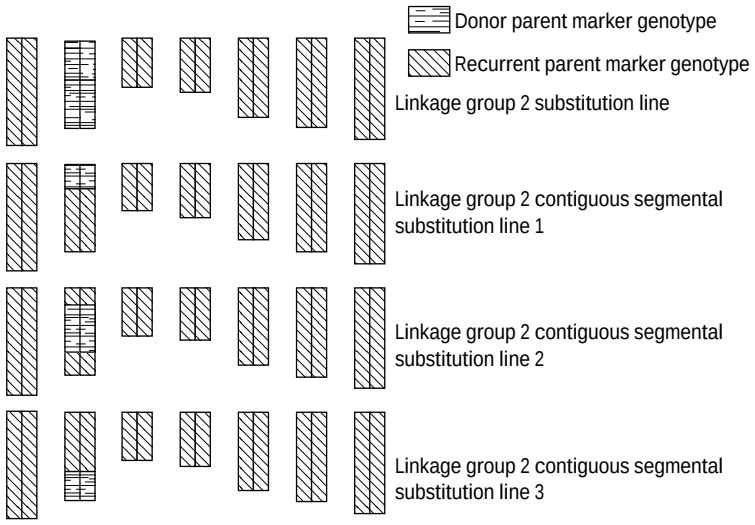


Table 1. Marker genotypes (A = donor allele homozygote; H = heterozygote; B = recurrent parent allele homozygote; - = missing data) of 25 seed parents of most recent generation of jump-started marker-assisted backcrossing program targeting transfer of improved downy mildew resistance (linkage groups 1 and 4) and terminal drought tolerance from PRLT 2/89-33 to elite pearl millet pollinator H 77/833-2. Plant numbers not "bolded" have marker genotypes indicative of crossing failure in the previous generation.

Linkage group	Probe	Enzyme	**BC3F1/**BC2F2 **1**	2	3	4	5	**6**	7	**8**	**9**	**10**	**11**	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
1	PSM757	*Eco*RV	H	A	A	B	H	H	A	H	B	B	H	A	B	B	B	B	B	B	H	H	H	B	B	H	H
	PSM565	*Hind*III	H	A	H	B	H	H	A	H	B	B	H	A	B	B	B	B	B	B	H	H	H	B	B	H	H
	PSM386	*Eco*RI	H	A	A	B	H	H	A	H	H	H	H	A	H	H	H	B	H	H	H	H	H	B	B	H	B
2	PSM322	*Eco*RI	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	PSM214	*Dra*I	B	H	A/H	H	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	PSM321	*Eco*RV	H	H	A/H	H	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
4	PSM716	*Hind*III	H	B	H	H	H	H	H	H?	H	H	H	B	B	B	B	B	B	B	H	H	H	H	H	H	B
	PSM416	*Hind*III/U	H	H	H	B	B	H	B	H	B	B	-	B	H	B	H	B	B	B	-	-	-	-	-	-	-
		*Hind*III/L	H	H	H	A	H	B	B	H	B	B	-	B	B	B	B	B	H	B	-	-	-	-	-	-	-
	PSM612	*Dra*I	H	H	H	-	H?	H	H	H	H	H	B	A	B	B	B	B	B	B	H	B	B	H	B	B	B

- ***Disadvantages:*** These substitution line sets are rather expensive (in terms of both human and operational resources) and time-consuming to produce. Therefore, they are probably not worthwhile unless several of the derived lines are expected to prove economically useful. This in turn will generally require multiple target traits and extremely diverse parents, at least one of which is extremely elite.
- ***ICRISAT experience:*** We have just initiated development of a (reciprocal) contiguous segment substitution line set based on the cross ICMB 841 × 863B (Table 2), and plan to use it for mapping drought tolerance QTLs of small effect.

Recommendation

In pearl millet, for most cost-effective MABCT of a small number of QTLs of large effect, we recommend advancing to BC_2F_2 and BC_3F_1 by advancing five random plants in each of five BC_2F_1 progenies (each derived from a single BC_1F_1 plant having a 50% probability of carrying any given marker or QTL). DNA restriction digests of the 25 advanced generation segregants (BC_2F_2/BC_3F_1 pairs), the donor and recurrent parent, and the "Tift 23DB" standard genotype will fit on a 30-well filter along with molecular weight markers on each end. This gives >90% probability of having advanced any target QTL, located anywhere in the donor parent genome, to BC_3F_1 in the recurrent parent genetic background before spending any resources on marker-genotyping the backcross progenies. Further, once the appropriate BC_3F_1 progeny has been identified for advancement, five plants from it can be randomly advanced to BC_4F_1, and five plants from each of these randomly advanced to BC_4F_2 and BC_5F_1 before the next round of marker genotyping is necessary. This should be followed by two generations of selfing and one more cycle of marker genotyping to produce the desired homozygous substitution lines. If target QTLs have been identified by the time the BC_3F_1 selection must be done, it is possible to get by with just 25 BC_4F_2/BC_5F_1 pairs (and 25 BC_5F_2 plants) per target QTL. If target QTLs have not yet been identified, then the amount of marker genotyping required in later generations will be much larger, and probably not economical except for high value traits of low heritability (Hospital et al. 1997) despite the potential time savings, unless development of a full or partial contiguous segment substitution line set is intended.

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Table 2. List of 19 F_3 progenies, from the (ICMB 841 × 863B)-derived pearl millet mapping population of 160 F_2 individuals, selected as possible starting points for development of a reciprocal set of contiguous segment substitution lines in pearl millet. Selection was based on marker genotype homozygosity for alleles of a given parent across the length of the indicated linkage groups. Terminal drought tolerance differences are expected among linkage group 2 substitution lines (Bold) .

	Target linkage groups													
F_3 progeny	841-1	841-2	841-3	841-4	841-5	841-6	841-7	863-1	863-2	863-3	863-4	863-5	863-6	863-7
F_3-4							X	X						
F_3-12										X	X			
F_3-13				X		X								
F_3-22		**X**						X		X	X	X		
F_3-34	X		X		X	X								
F_3-35			X		X									
F_3-47												X		X
F_3-57		**X**	X					X						
F_3-77					X		X			X				
F_3-85	X								**X**					
F_3-96		**X**				X								
F_3-97				X		X	X							
F_3-101	X											X		
F_3-107			X					X						
F_3-112			X					X						
F_3-122													X	X
F_3-127		**X**		X	X									
F_3-128						X			**X**			X		
F_3-148			X										X	
	Backcross these lines to 863B							Backcross these lines to ICMB 841						

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QTL Mapping Activities and Marker-Assisted Selection for Yield in the Germplasm Enhancement Program of the Australian Northern Wheat Improvement Program

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Summary

The target population of environments encountered for wheat breeding in the northern grains region of Australia is highly variable and large genotype-by-environment (G×E) interactions complicate any studies of the inheritance of quantitative traits. Theoretical considerations in combination with experimental investigations of QTL models for quantitative traits and computer simulation studies of Marker-Assisted Selection (MAS) have enabled the development of a quantitative framework for defining when advantages may be expected from MAS over the current phenotypic selection strategy used in the Germplasm Enhancement Program (GEP). An overview is given of the strategy that is currently being used to evaluate the scope for using MAS for improvement of the quantitative trait grain yield and associated drought resistance in the GEP. A QTL mapping study investigating the grain yield variation relevant to the GEP is underway. It is anticipated that with the currently available technologies applied to the mapping population we will be able to enhance our understanding of the genetic architecture of a number of important quantitative traits segregating in the populations being improved in the GEP. However, routine use of MAS for yield and drought resistance is not considered to be an efficient option for the GEP at this time. The reasons for this conclusion are discussed.

Introduction

The Germplasm Enhancement Program (GEP) is a modified S1 recurrent selection program with the objective of developing high yielding lines for use as parents by the pedigree programs that operate within the Australian Northern Wheat Improvement Program (NWIP) (Figure 1a). Improving yield for a range of water-limited and low-stress (yield potential) environment types is critical to the success of any breeding strategy in the northern grains region Target Population of Environments (TPE) (Cooper et al. 1995, 1997). Therefore, there has been a long-term research program investigating efficient designs for the conduct of multienvironment trials (METs) for all aspects of the NWIP. The GEP operates on a four-year cycle (Figure 1b). Years 1 and 2 are used for random intermating selection on single plant and S1 family-row bases for maturity, height, and rust resistance, and seed multiplication of between 500 and 1,000 S1 families. The METs for yield and quality evaluation of the S1 families are conducted in years 3 and 4, and selection is based on grain yield and grain protein concentration (Fabrizius et al. 1996; Podlich et al. 1999).

Inheritance studies conducted as part of the activities of the GEP focus on the quantitative traits, grain yield, and grain protein concentration. Parental genotypes for some traits and phenotypes for others indicate that a number of quantitative traits contributing to resistance/tolerance to important abiotic and biotic stresses in the TPE are expected to be segregating in the recurrent selection populations. The strategic inheritance work is undertaken to provide an information base that will assist in the design of selection strategies to improve on the current strategy. This paper will concentrate on studies

relevant to understanding the genetic control of variation for grain yield in relation to the reference populations that are currently being subjected to recurrent selection. These quantitative trait investigations combine traditional genetic experiments with a crop physiological framework as a background to any molecular-marker-based investigations of the architecture of grain yield variation. An additional feature is a strong emphasis on detailed characterisation of experimental environments to define the types of environment sampled in METs. The objective of this work is to characterise the environments in terms of the key abiotic and/or biotic variables influencing the expression of genetic variation and repeatable genotype-by-environment (G×E) interactions. Complementary work is underway to characterise the TPE within the northern grains region. Crop growth modelling and probe genotype methodologies are applied to historical meteorological and breeding trial data to characterise environment types sampled in METs. These characterisations can be summarised by pattern analysis and Geographical Information Systems (GIS) methodology. This process can be used to quantify the range of types of environments influencing expression of repeatable G×E interactions within the TPE, their frequencies of occurrence, and their spatial and temporal distributions (Cooper and Chapman 1996; Cooper and Hammer 1996).

Quantitative trait research is conducted in three areas: (1) theoretical development of genetic models for quantitative traits; (2) experimental work to define traits contributing to adaptation, estimate key genetic parameters for these traits and identify associated quantitative trait loci (QTL); and (3) computer simulation modelling of the experimental investigations and the implications of hypothesised genetic models for expected response to selection. Each of these areas of investigation is conducted to identify breeding strategies, or refinements to the current breeding strategy, that have potential to improve the effectiveness of the GEP. They are based on information relevant to the lines that were used to establish the two base populations subjected to recurrent selection. This ensures that the strategic QTL mapping research and evaluations of marker-assisted selection (MAS) are linked to the imperatives and objectives of the GEP. In contrast to most other groups, we have concentrated our efforts on developing a quantitative framework for deciding where benefits from MAS may be expected and have taken a more cautious approach towards developing molecular marker capability.

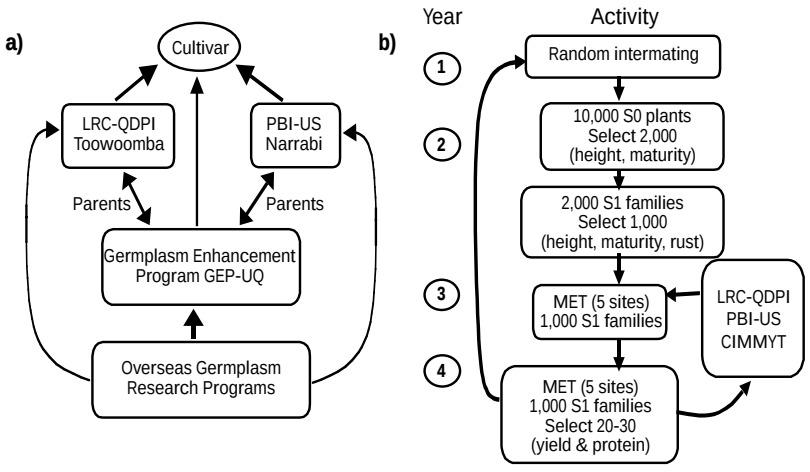

Figure 1 (a.) Components and pathways of germplasm transfer for yield improvement in the Australian Northern Wheat Improvement Program: LRC-QDPI represents the Queensland Department of Primary Industries pedigree breeding programs located in Toowoomba at the Leslie Research Centre; PBI-US represents the University of Sydney pedigree breeding programs located in Narrabri; GEP represents the University of Queensland Germplasm Enhancement Program. (b.) Phases of activity in the four-year cycle of the Germplasm Enhancement Program: MET represents Multi-Environment Trials for the S1 families.

Current Research Activities

Theory: Genetic models for quantitative traits

There is a growing body of information on the genetic architecture of quantitative traits. A long-term objective associated with much of this research is directed toward developing an understanding of the architecture of these traits that extends from the level of DNA base sequences to plant phenotypes within target environments. At present there is no single quantitative framework that uniquely and generally unites these levels of organisation within gene-environment systems. However, we have a series of working models that range from the classical polygenic extensions based on multiple Mendelian units to complex networks of interacting DNA sequences influencing gene expression and function within the context of growth and development and adaptation to biophysical

features of the environment. The *E(N:K)* model was developed to study this range of possible models for quantitative traits: *E* is a specification of the number of repeatable types of environments that exist in the TPE; *N* is the number of genes influencing the traits under investigation; and *K* is a measure of the level of epistatic interaction among the *N* genes. This is an extension of Kauffman's *NK* model, specified to incorporate G×E interaction effects in combination with epistasis. The familiar features of quantitative genetic models can be specified within this framework; e.g., additive and dominance effects for individual genes, linkage relationships among genes, and gene frequencies.

Genomics research, covering genome structure, functional and comparative genomics, and QTL mapping is starting to provide some insights into the range of complexity we may expect to encounter as we seek an understanding of the genetic basis of quantitative traits. It is clear that genes rarely, if ever, function independently from the rest of the genome and are more likely to form part of a network of genes that interact to influence the relative value of the alternative alleles for a gene. This feature of the architecture of quantitative traits has major implications for the transferability of genetic improvements among breeding programs that operate with different germplasm pools and reference populations. However, for a given sample of gene combinations available in the reference population(s) of genotypes of interest to a breeding program, not all of the genes in a network will be segregating. Therefore, within specific reference populations we may observe genes segregating that act as major genes determining trait variation. The allelic variation for these genes can be manipulated through selection and the value of the alleles quantified in terms of the traditional concepts of average effects of genes and average effects of gene substitution for the reference population. However, when other parts of the genetic network are allowed to segregate, the prior estimates of the value of the alleles may change due to influences detected as epistatic effects. Thus, genes detected as major QTLs in one reference population may not be detected as such in other populations. Because researchers generally work with only a few populations, the extent to which epistasis may limit the usefulness of detected QTLs has probably not been fully appreciated.

Another feature of gene function that requires consideration is that of G×E interactions. The relative value of alleles and allelic combinations can change with the biophysical conditions of the environment. The specific nature of these interactions is not well understood for most, if not all, of the genes contributing to the important quantitative traits manipulated in breeding programs. In view of these complexities in gene function, initial successes from MAS are more likely to come from working with QTLs that have some consistency of value across both environments and reference populations or QTLs associated with repeatable components of G×E interaction. However, this does not mean that the most significant long-term advances will necessarily come from these simple applications of QTL theory.

The traditional linear models that are used in quantitative genetic theory to ascribe value to the alternative alleles at the loci contributing to variation for quantitative traits have considerable practical value and merit. They will continue to provide general guidance on the expectations of applying plant breeding strategies. However, it is widely recognised that many assumptions are made when we apply these models (Kempthorne 1988). These assumptions are often questionable, particularly when we start to consider the growing body of evidence on the molecular structure of genomes and the complexities of gene function. In addition they tend to ignore many details that are relevant to specific situations encountered by individual breeding programs. The common assumptions of no epistasis and treating G×E interactions as a source of error in determining genotypic value are two that appear inappropriate for yield improvement of wheat in the genotype-environment system faced by the NWIP (e.g., Table 1; Cooper et al. 1997). We are using the *E(N:K)* model to examine the capacity of families of nonlinear genetic models of the inheritance of quantitative traits to deal with the process of relaxing or removing some of the common assumptions and capture important features of quantitative trait variation (Podlich and Cooper 1998). This avenue of research is linked with the computer simulation research, discussed further below, to enable evaluation of the implications of these quantitative trait models for the likelihood of breeding strategies realising potential responses from selection (Podlich and Cooper 1999).

Experimental: Phenotypic evaluation and QTL analysis

Phenotypic evaluation of experimental populations is based on two broad strategies. These are referred to here as the top-down (black-box) and ideotype approaches (Fischer 1981). In the top-down approach, yield variation among a sample of lines from a reference population is examined to identify the repeatability of the major components of genetic variation within the TPE. In the ideotype approach, specific traits or trait combinations are examined for their contribution to adaptation and performance within the TPE. Most of our work to date has concentrated on the top-down approach. However, we have commenced collaborations with the CSIRO group in Canberra to evaluate the significance of genetic variation in stomatal aperture and transpiration efficiency traits for yield improvement within our breeding populations.

From the results of the top-down investigations repeatable differences in the patterns of yield variation among lines have been identified. These have been examined in terms of the yield component configurations and biomass production and partitioning attributes of the lines (e.g., Cooper et al. 1994a,b). This process was used to identify possible parents for construction of QTL mapping populations. Candidate parents were screened for their levels of molecular marker polymorphism (Nadella et al. 1996). Four promising biparental crosses were identified; Hartog/Seri, Hartog/11IBWSN50, Hartog/Genaro, and Banks/Genaro (Seri is the CIMMYT line Seri M 82 and Genaro is the CIMMYT line Genaro T 81). Samples of recombinant inbred lines (RILs) were developed by single seed descent (SSD) from each cross. To assist in the analysis of the genetic component of variation for each cross a family structure, based on F_3-derived lines within F_2-derived families, was established in the SSD process to enable initial estimates of additive and epistatic (nonadditive) components of variance (Hanson and Weber 1961). Following evaluation of these and other RIL populations (e.g., Peake et al. 1996; Table 1) the Hartog/Seri population was identified for further development as the principal mapping population. The main criteria for selection of the Hartog/Seri cross were (1) Hartog is a major cultivar in the northern grains region of Australia; (2) Seri expressed a substantial yield advantage over Hartog following extensive testing in METs (ª 10% to 20% depending on the study); (3) the coancestry of Hartog and Seri was relatively low compared to other elite/elite crosses used in the NWIP (COP = 0.297; Fabrizius et al. 1996); (4) workable levels of molecular marker polymorphism were identified between Hartog and Seri (Nadella et al. 1996); (5) high yielding, good quality lines were identified among the RILs developed in the preliminary MET studies, making the cross relevant to the objectives of the NWIP; and (6) Hartog/Seri is a key cross contributing to the base populations subjected to recurrent selection in the GEP, and therefore any findings from the QTL investigations have a good chance of being applicable to the operation of the GEP.

Crop physiological investigations of the source of the yield difference between Hartog and Seri have been conducted and are still being pursued (Table 2). The higher yield of Seri relative to Hartog is attributed to both a larger number of grains and a larger grain weight. This increase in yield is also detectable in terms of a higher above-ground biomass and harvest index. The higher grain number does not appear to be the result of a difference in biomass or crop growth rate characteristics at around anthesis but is more likely a result of a change in the partitioning of biomass to set a larger number of grains per unit area. This larger yield sink is associated with a yield

Table 1. Estimates of additive (σ^2_A), additive-by-additive epistasis (σ^2_{AA}), additive-by-environment interaction ($\sigma^2_{A\cdot E}$) and additive-by-additive epistasis by environment interaction ($\sigma^2_{AA\cdot E}$) genetic components of variance and their approximate standard errors for grain yield for five experimental Recombinant Inbred Line populations evaluated in the northern grains region of Australia. The estimates of the components of variance were based on the Hanson and Weber (1961) Random Homozygous Line mating design with random F_2-derived families and F_3-derived lines within the F_2-derived families. Sources: Fabrizius et al. (1997) and A.S. Peake (Unpublished data).

Cross	Component of variance[1]			
	σ^2_A	σ^2_{AA}	$\sigma^2_{A\cdot E}$	$\sigma^2_{AA\cdot E}$
11IBWSN50/Vasco	-0.005±0.028	0.006±0.012	**0.146±0.042**	-0.068±0.017
Hartog/Vasco	-0.030±0.032	0.018±0.013	**0.192±0.052**	-0.060±0.019
Hartog/Seri	-0.019±0.037	**0.077±0.017**	**0.066±0.036**	**0.082±0.038**
Hartog/Genaro	0.044±0.037	0.000±0.011	-0.081±0.037	**0.189±0.036**
Hartog/11IBWSN50	0.021±0.018	0.013±0.009	-0.006±0.013	**0.035±0.008**

[1] Bold numbers highlight important components of variance detected.

component configuration based on fewer fertile tillers per plant and per unit area and a larger number of grains per fertile tiller for Seri relative to Hartog. The larger grain weight of Seri may be associated with higher levels of post-anthesis biomass production relative to Hartog. These hypotheses have yet to be tested using the RIL progeny developed for the QTL mapping work. A major difficulty in quantifying the potential contributions of genetic variation for biomass production and partitioning to genetic variation for yield is the large amount of experimental error associated with the biomass measurements in the breeding trials. The small size of the genetic and G×E interaction components of variance relative to that for error is indicated for the biomass measurements by the low gamma ratios (Table 2). Measurements of plant responses to water-deficit by either pressure bomb readings of leaf water potential or canopy to air temperature differentials normalised to a Crop Water Stress Index have failed to detect any consistent difference between Hartog and Seri. This is being re-evaluated as we improve our capacity to characterise the different types of drought environments sampled in the METs.

To enable reliable QTL investigations the size of the Hartog/Seri mapping population was expanded and inbred generations have been derived in four ways (Figure 2): (1) from the F_2 generation by SSD (686 lines, RIL-SSD); (2) from the F_1 generation by the maize-wheat cross doubled haploid procedure (300 lines, RIL-DHAP); (3) from the backcross 1 to Hartog generation by SSD (230 lines, BCRIL-SSD); and (4) from the backcross 1 to Hartog generation by the maize-wheat cross doubled haploid procedure (317 lines, BCRIL-DHAP). The common assumption of additive independent genes determining the variation for the quantitative traits is to be questioned prior to any QTL analysis. The multi-generation structure of the mapping population provides an experimental procedure to quantitatively test for the presence of epistasis and linkage for any traits measured on the lines. The results of these tests are used to

Table 2. Best Linear Unbiased Predictors (BLUPs) for grain yield, yield components and dry matter production and partitioning, nitrogen uptake and drought-response traits used to characterise the parents of the QTL mapping population Hartog/Seri within multi-environment trials conducted in the Australian northern grains region target population of environments from 1986 to 1989. The number of environments sampled in the studies used to compute the BLUPs and the range of the environment mean values for the traits are shown.

	Environments		Variance Components Gamma[1]		Parents[2]		
Trait	no	Range	σ^2_g	σ^2_{ge}	Hartog	Seri	SED[3]
Grain Yield (t ha^{-1})	31	0.70-5.32	0.45	0.69	**3.24**	**3.67**	**0.06**
Grain number (grains m^{-2})	31	1618-13290	0.67	0.59	**8790**	**9328**	**155**
Grain weight (mg grain^{-1})	31	29.8-48.6	3.47	0.99	**37.6**	**39.7**	**0.3**
Days to flower	30	74-107	3.55	1.56	**87**	**89**	**0.2**
Fertile tillers (tillers m^{-2})	30	100-429	0.44	0.11	**311**	**267**	**9**
Grains per tiller	30	17.5-35.6	0.65	0.22	**27.8**	**34.5**	**0.8**
Maturity dry weight (g m^{-2})	22	191-1448	0.02	0.14	**901**	**949**	**24**
Harvest Index	22	0.25-0.47	0.58	0.47	**0.37**	**0.40**	**0.006**
Anthesis dry weight (g m^{-2})	26	110-840	0.17	0.11	540	562	15
Anthesis Crop Growth Rate (g m^{-2} day^{-1})	26	2.2-27.0	0.01	0.13	13.2	13.1	0.6
Pre-anthesis Nitrogen uptake (g m^{-2})	20	1.0-15.5	0.02	0.05	8.6	8.4	0.3
Pre-anthesis Leaf Area Index	18	0.24-4.88	0.18	0.35	2.3	2.4	0.11
Pre-anthesis Radiation Interception (%)	23	22-98	0.04	0.12	72	71	1.2
Pre-anthesis Crop Water Stress Index	23	0.00-1.00	0.01	0.17	0.27	0.27	0.02
Pre-anthesis Leaf Water Potential (MPa)	24	-1.4 - -4.5	0.11	0.16	-2.2	-2.2	0.05
Post-anthesis dry weight Increase (g m^{-2})	22	87-787	0.01	0.10	***368***	***404***	22
Post-anthesis Distribution Ratio	22	0.6-1.9	0.04	0.02	1.1	1.2	0.1
Plant population (Plants m^{-2})	16	70-99	0.05	0.07	82	86	3

[1] The gamma ratios are the ratios of the genetic (σ^2_g) and genotype-by-environment interaction (σ^2_{ge}) components of variance on the residual components of variance. The estimates of the components of variance were derived from a set of lines that included Hartog and Seri.

[2] Differences between parents declared as significant are identified in bold and differences close to significance are identified in bold italics.

[3] SED = Standard error of the difference between line means.

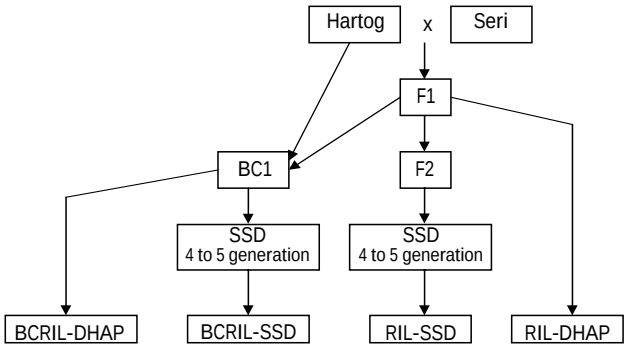

Figure 2 Schematic representation of the four mapping populations generated for the Hartog/Seri cross: BCRIL-DHAP is a recombinant inbred population derived by the doubled haploid procedure from the backross 1 generation; BCRIL-SSD is a recombinant inbred population derived by single seed descent from the backcross 1 generation; RIL-SSD is a recombinant inbred population derived by single seed descent from the F_2 generation; RIL-DHAP is a recombinant inbred population derived by the doubled haploid procedure from the F_1 generation.

provide some guidance on the appropriate QTL models to be evaluated in the molecular marker QTL linkage analyses of the phenotypic data generated from either the METs or specific biotic and abiotic stress screens.

At present we have developed a framework map for the population based on AFLPs (Nadella et al. 1996; Nadella 1999). From this initial study, a total of 407 polymorphic bands were identified between Hartog and Seri using 74 AFLP primers. A subset of 114 AFLP markers and 10 loci of known function were used to construct the framework map. Approximately 25% of the polymorphisms detected between Hartog and Seri were associated with the 1BS:1RS polymorphism on chromosome 1B (Nadella 1999). Seri is the donor of the 1BL/1RS translocation in the mapping population. We are now extending the map by adding the remaining AFLP markers and microsatellite markers. Based on the framework map there appears to be no obvious problem with clustering of the AFLP polymorphisms. Using the framework map, major QTLs for height and maturity have been identified. In addition, a putative QTL for tolerance to root lesion nematode (*Pratylenchus thornei*) was repeatedly detected in two environments where the nematode was considered to be a key environmental variable influencing variation for grain yield.

Simulation studies

The QU-GENE simulation platform was developed to investigate issues relevant to the inheritance of quantitative traits (Podlich and Cooper 1998). By developing application modules that represent plant breeding strategies the efficiency of modifications to the current strategies and alternative breeding strategies can be examined for a wide range of quantitative trait models. The studies to date have focussed on two areas: (1) evaluation of the implications of alternative QTL models suggested from the theoretical and experimental work, and (2) the relative effectiveness of alternative breeding and selection strategies, including MAS, for manipulation of quantitative traits. Associated with this work are evaluations of the power of genetic experiments, including molecular marker-based linkage analyses, to detect QTLs contributing to quantitative trait variation. There is particular interest in the impact of complexities to the genetic model such as epistasis and G×E interactions. Also of interest is the extent and effects of co-location of minor genes into gene families. We are investigating the influence of these complexities on the efficiency of MAS.

The relative efficiency of MAS strategies in comparison to phenotypic selection is being examined to identify the situations where an advantage from MAS may be expected. For example, Cooper et al. (1999) discussed the use of a QU-GENE application module that has been developed to examine procedures for optimising the selection index weights given to data obtained from both molecular marker polymorphisms among breeding lines and phenotypic data obtained from METs.

Discussion

At this time, we are a number of steps away from practical implementation of any MAS strategy to facilitate the genetic improvement of yield and drought resistance in the GEP of the Australian NWIP. The one piece of marker technology that we are in a position to use routinely in the GEP is the enzyme-linked immunoabsorbent assay (ELISA) test for the presence and absence of the 1BL/1RS translocation in wheat (Andrews et al. 1996). This test can be used to select against the presence of the 1BL/1RS translocation. The area where we have made substantial advances is that of defining the situations where advantages may be expected from MAS. In particular the simulation work conducted to date provides technology development benchmarks for determining where, when, and how the advantages may be realised as the QTL mapping work progresses.

Current situation and opportunities

The concentrated research effort that has centred on the GEP over the last ten years has examined the inheritance of yield and the efficiency of alternative breeding strategies for quantitative traits. Experimental evaluations of gain from selection suggest positive results from the GEP. This has enabled the design and implementation of a breeding strategy that is improving yield for a range of water-limited environments in a complex rainfed TPE. We are currently evaluating whether MAS can improve the efficiency of the incumbent breeding strategy and what levels of technology development are required to realise these advantages.

Questions

Prior to the conduct of any MAS in the GEP, extensive assessment of its feasibility and efficiency is required. There are a number of important questions to be addressed initially: (1) Can markers be used to detect repeatable QTLs within the Hartog/Seri mapping population across environments? (2) Can markers be identified that are linked to specific genes for resistance to biotic and abiotic stresses? and (3) Can markers be used to identify regions of the genome where allele frequencies are being influenced by selection across cycles in the GEP?

Needs

Should MAS be identified as a desirable modification to the GEP, a large amount of work has yet to be done on developing the necessary information management system prior to implementation of any MAS strategies in the GEP (Cooper et al., this volume). This is considered to be a critical need in the process of linking the data generation and analysis processes within the breeding program with the selection decision processes used by the breeders. We would also have to acquire or develop significant capability to generate sufficient marker data on the family units subjected to selection in the GEP. To enable real-time selection decisions feasible, marker profiles of up to approximately 1,000 S1 families would need to be generated in a two to three year time frame to enable computation and implementation of an optimised marker-phenotypic index. The first priority, however, is to demonstrate that molecular marker technology can enhance the efficiency of the GEP.

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Efficient Selection for Adaptation to the Environment through QTL Mapping and Manipulation in Maize

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Summary

Factors of adaptation of maize to northern latitudes include early flowering and the ability to germinate and grow under low temperatures (referred to as early vigor). Marker-assisted selection approaches were used to select for these traits. In two separate experiments, and thus independent segregating populations, QTL's were identified. In one experiment alleles responsible for early flowering were transferred from the early line to the high yielding later line through marker-assisted backcrossing. BC3S2-derived lines flowered five days earlier than the original recurrent parent while maintaining the original high yield potential. In the second experiment favorable alleles for early vigor and other agronomic traits were selected for in a recurrent selection scheme. Major improvements in early vigor, often beyond the best parent's performance, were observed with the derived lines.

Introduction

Selection for early flowering and the ability to germinate and grow under low temperatures (referred to as early vigor) has allowed the extension of the maize growing area to the North. Because improvements in grain or silage yield are often achieved through the use of late, southern germplasm, selection for early flowering and vigor remains necessary in today's attempts to develop superior maize hybrids for the northern markets.

Two strategies can be used to breed for these environmental adaptation traits. The first one consists of the improvement, for these specific traits, of elite germplasm through backcross conversion programs. The second one consists in the development of new germplasm through simultaneous selection for agronomic performance and adaptation to the environment.

Because of the great promise held by molecular markers for achieving higher levels of genetic gain per year we decided to investigate these two strategies using molecular markers. The results of two experiments will be presented here. First, a selection experiment aimed at developing an early flowering version of a high yielding line through marker-assisted backcrossing. Second, another selection experiment aimed at developing lines with good early vigor and elite agronomic performance through marker-assisted recurrent selection.

Marker-assisted Identification and Introgression of Flowering QTLs

An unselected F3 population of 235 plants was developed from a cross between NSE331 and NSE626, two proprietary lines. NSE331 is a late inbred with superior agronomic characteristics. NSE626 has shown average to low agronomic performance but flowers 8 to 12 days earlier than NSE331 in the Parisian basin. The 235 F3 individuals were genotyped at 83 polymorphic RFLP loci spread uniformly throughout the genome and phenotyped both per se (as F4's, one year, one location) and as testcross combinations with NSE101 (two years, two locations).

Four flowering QTL's were identified using SAS (SAS Institute 1989) and Mapmaker/QTL (Lander et al. 1987), where NSE626 contributed early alleles; on chromosomes 1, 3, 5, and 8. The cumulative effects for these QTLs were 7.7 and 2.3 days earlier flowering, respectively, and as testcross combinations with NSE101.

A backcross program was initiated to introgress these four early alleles from NSE626 into NSE331 using molecular markers both to select for the early alleles at the QTLs, and for recovery of the recurrent parent's genotype elsewhere. At the BC1, BC2, BC3, BC3S1, and BC3S2 generations, 150 plants were analyzed at the 83 marker loci tested in the mapping phase. At each generation plants were selected solely based on marker data. No phenotypic data were ever used for plant selection.

Several versions of NSE331 were obtained, including one containing the four regions from NSE626, and having recovered more than 90% of NSE331's genotype. The effects on flowering were more important than the estimates obtained in the QTL mapping phase. Lines with NSE331's background and NSE626 genotype at all four QTLs flowered 8.5 and 5.5 days earlier than NSE331, respectively, per se and as testcross combinations with NSE101. Yield trials conducted at several locations showed also that the agronomic potential of NSE331 had been maintained in its early versions (Figure 1). Early versions also showed levels of grain moisture about 4% lower than the original line.

Marker-assisted Identification of and Recurrent Selection for Early Vigor QTLs

An unselected segregating F3 population of 200 plants was developed from a cross between NSE986 and NSE002, two proprietary lines. Both lines are elite lines, parents of commercial hybrids. However, while somewhat limited in agronomic potential, NSE986 has a very good ability to germinate and grow under low temperatures. On the contrary, NSE002 has a very high agronomic potential but suffers from low early vigor. Phenotypic evaluations of the 200 F3's were conducted both per se (F4 progeny) or as testcross combinations (F4's crossed onto two different testers, NSE505 and NSE331) at several locations both in Europe and North America during one year. More than 30 traits were recorded on the population, some both on per se and testcross trials, and some at several locations. These traits included early vigor, plant health, flowering, grain yield and grain yield components, and grain moisture measurements. The 200 F3 individuals were genotyped at 82 polymorphic RFLP markers spread throughout the genome.

Mapping was performed using SAS (SAS Institute 1989), Mapmaker/QTL (Lander et al. 1987), and QTLcartographer (Basten et al. 1996). A total of 285 QTLs were identified for all traits, among which 81 for early vigor-related traits. Based on these results, marker-only selection indices were constructed and used to select plants in a recurrent selection scheme. First, F3 plants were selected based on their genotype. Selfed progeny of these selected F3's (referred to as cycle C0) was grown and genotyped for all markers flanking QTLs included in the index. Selected C0 plants were either intermated or selfed. This process was pursued until cycle C3 where all plants were selfed.

The first field trials of this marker-assisted recurrent selection program were conducted in the summer of 1998 and involved C0 and C1-derived material, which had undergone one generation of per se phenotypic selection and one or two generations of selfing. Some of the lines developed showed much better early vigor characteristics than either of the parents, as demonstrated by the emergence and the dry matter of the plantlets (Figure 2).

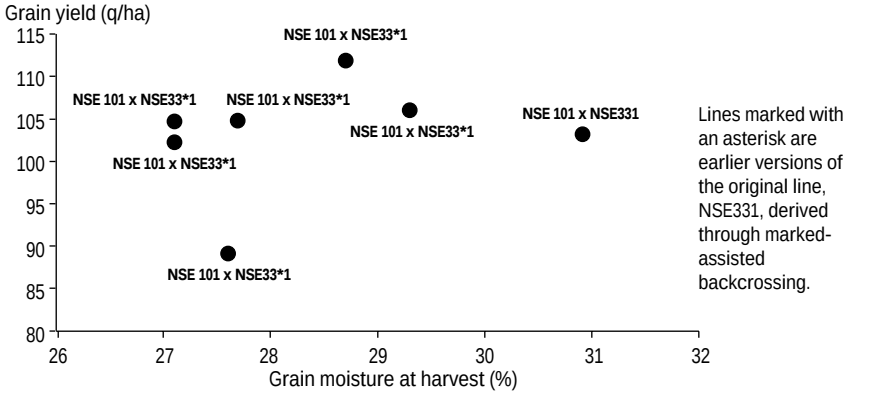

Figure 1. Grain yield and grain moisture of marker-assisted, backcross-derived versions of NSE331 evaluated as testcross combinations with NSE101 at three locations in France.

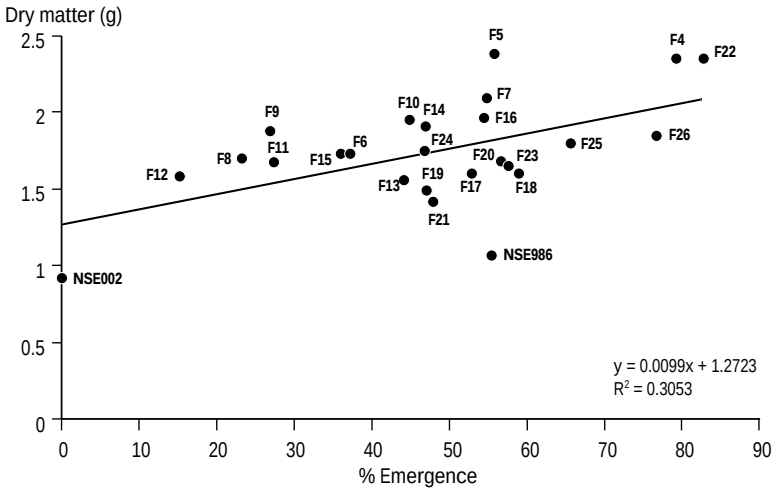

Figure 2. Dry matter (aerial parts of five plantlets) and emergence of C0 and C1-derived lines from the marker-assisted recurrent selection based on the segregating population NSE002/NSE986, together with the parental lines.

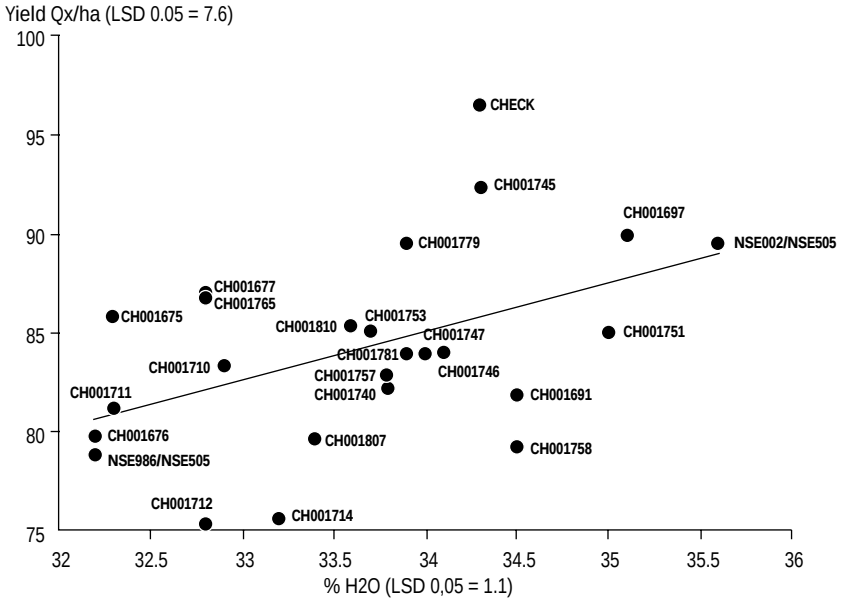

Figure 3. Grain yield and moisture of C0 and C1-derived testcross combinations from the marker-assisted recurrent selection based on the segregating population NSE002/NSE986.

Similarly the marker-assisted, selection-derived lines also showed very good agronomic performance, with a few examples of lines yielding more than the better of the two parents (Figure 3).

Material issued from further cycles of recurrent selection is being tested in the field during the summer of 1999.

Conclusion

Results from both of the above experiments show that adaptability to the environment can be effectively manipulated through marker-assisted selection. The choice of whether to use backcross or recurrent selection approaches will depend on the breeding needs and objectives.

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QTL Analyses, MAS Results, and Perspectives for Drought-Tolerance Improvement in Tropical Maize

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Summary

The objective of plant improvement can be defined as the accumulation in a plant genome of the maximum number of favorable alleles involved in the expression of target traits. This task can be achieved through several approaches and selection schemes, including phenotypic observations and/or DNA marker technology. The emergence of molecular genetics and associated technologies represents a major new breeding tool; the current challenge is to integrate this tool and the information it generates into breeding schemes to further the development of efficient marker-assisted selection (MAS) strategies. This challenge is addressed in this paper, which particularly considers plant improvement under limited water conditions. After the presentation of QTL identification for target traits involved in maize drought tolerance and a description of two successful MAS experiments for both line and population improvement, prospects for plant improvement through biotechnology are discussed.

Introduction

Improvement in drought tolerance in staple crops is a major objective for agriculture in developing countries (Edmeades et al. 1998). In nontemperate maize, drought and soil infertility represent the two major causes of grain yield reduction (Beck et al. 1997). Considering ongoing climatic changes caused principally by global warming (Curry et al. 1995), the pressure on food production in water-limited environments should increase in the near future.

Like most abiotic stresses, drought can induce major changes in the growth and development of cultivated crops through complex modifications in physiological pathways. Moreover, the global impact of water-limited conditions is not easy to evaluate, especially when drought is combined with insect or pathogen attack, or with less than optimal soil conditions. Although impressive progress has been achieved through conventional breeding (for review, see Heisey and Edmeades, 1999), the potential for genetic improvement of maize production under drought conditions is still large. Such improvements under drought should be accelerated, given the rapid increase in supply of new molecular tools, and the explosion in genetic information generated through genomic approaches.

Genetic Dissection of Drought Tolerance Traits and Parameters

A segregating population was developed by crossing a drought tolerant (P1, Ac7643S5) with a susceptible line (P2, Ac7729/TZSRWS5), where the lines showed considerable differences, especially for anthesis-silking interval (ASI) under drought. Using this population, the genetic dissection of several traits related to maize performance under drought—essentially the identification and characterization of the principal genomic regions involved in the expression of those traits—has been performed at two different inbreeding levels (Figure 1).

At the F2:3 (S2) level, flowering parameters, plant and ear height, leaf size and number, and yield components have been studied under three different water regimes: well-watered (WW), intermediate (IS), and severe water stress condition (SS). A genetic map was constructed with DNA extracted from F2 plants and the field evaluation was conducted on the corresponding F3 families. The most relevant results of this experiment have been presented in two papers (Ribaut et al. 1996; 1997a). From this QTL analysis, it was concluded that (1) a MAS experiment based only on the QTL involved in the expression of yield components would not be the most efficient approach, because only a few of the QTLs are stable across environments, and (2) a MAS experiment should consider the QTLs involved in the expression of secondary traits of interest that are correlated with yield under drought, such as ASI and plant height. The selected QTLs should be stable across environments and account for a large percentage of the phenotypic variance. Therefore, an efficient MAS strategy should take into account the most suitable QTLs from different traits as an index.

At the F6 (S5) level, physiological parameters such as relative water content, osmotic adjustment, root conductivity, and chlorophyll content have been measured, in addition to the morphological traits measured on the F2:3 material. In 1996, an initial set of field evaluations was conducted under three water regimes (SS, IS, and WW); in November 1998–May 1999, a second set was evaluated under the same field conditions. In addition, two ongoing collaborative projects are using the same RIL population to quantify the ABA content in the ear at the flowering stage (Tim Setter, Cornell University) and to evaluate root growth under hydroponics (Roberto Tuberosa, Bologna University). Although most of these physiological traits are not useful for routine screening purposes because they are too time-consuming (a typical example being the osmotic adjustment measurement), once DNA markers closely linked to the QTL involved in the expression of a physiological trait are identified, they can be used efficiently in a MAS experiment. Identification at the same genomic locations of QTL related to physiological and morphological traits should be expected, given that changes in physiological pathways have an impact on the plant phenotype and phenotypic correlations among these traits are often significant.

As an example from our first field evaluation, a QTL for chlorophyll concentration was identified on chromosome 2 close to a QTL for ASI (under IS and SS) and grain yield (under IS only). This QTL for chlorophyll content was consistent when measurements were conducted on the ear leaf and on a young leaf close to the tassel. On chromosome 6, a QTL for relative water content corresponds exactly to a QTL for ASI (under IS and SS) and grain yield (under IS and SS). At the same chromosomic region, the identification of a dehydrin gene (dhn1) has also been reported (Campbell and Close 1997). Since several physiological pathways involved in the drought tolerance are well known (e.g., ABA biosynthesis), the characterization of the gene(s) corresponding to identified QTL can be achieved. Thus the candidate gene approach appears to be an attractive option.

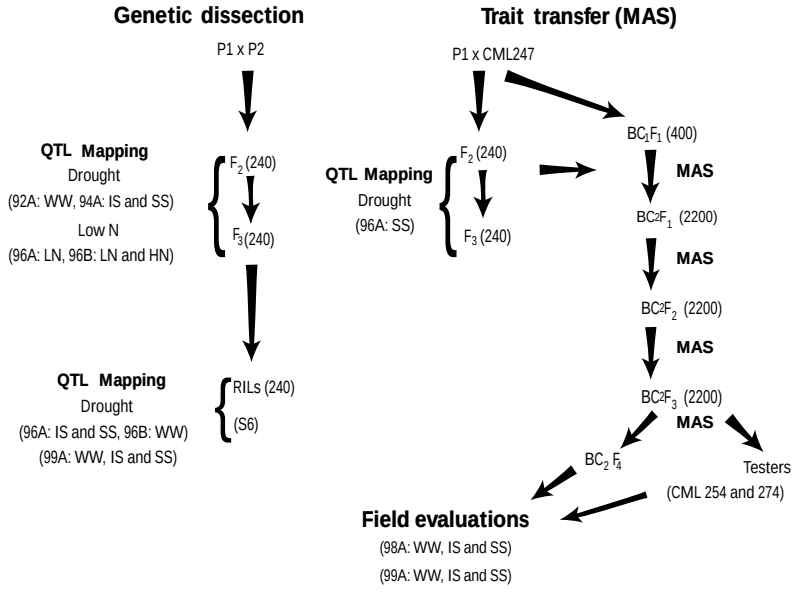

Figure 1: Summary of the different activities conducted at CIMMYT during the last 6 years to dissect the principal morphological and physiological traits involved in the drought tolerance response in tropical maize (P1 xP2, left part of the scheme), and the BC-MAS experiment to improve CML247 under drought using P1 as the donor line (right part of the scheme). The cycle and the nature of the stress (WW: well watered; IS: intermediate water stress; SS: severe water stress; LN: Low nitrogen; HN: high nitrogen) for each of the trials is indicated for both drought and the low nitrogen experiment. At each inbreeding level the number of genotypes considered is indicated in parens.

MAS for Maize Line and Population Improvement under Drought

CML247 is an elite tropical line with outstanding combining ability and good yield per se under well-watered conditions, but poor performance under drought, partially due to poor synchrony between male and female flowering. To improve CML247's performance under drought, a BC-MAS was initiated using P1 as the donor line. By combining genetic data from a segregating F_2 population derived from this cross and F_3 family evaluations in the field under different water regimes, QTL identification of drought-related traits t was conducted. Five genomic regions of interest were selected for transfer from the donor to the recipient line. The screening of large populations (about 2,000 plants) at each selection cycle was possible by taking advantage of the development of reliable PCR-based markers, used in our scheme as pre-selection tools. After 2 BCs and 2 self-pollinations, the best genotype was fixed for the five target regions (12% of the genome), as well as an extra 7% from the donor genome lying outside the QTL regions (Figure 2). Because of this level of donor genome contribution outside the target regions and the distribution of that contribution, 70 BC_2F_3 were identified and crossed with two CIMMYT testers, CML 254 and CML 274. Those hybrids, as well as the BC_2F_4 families derived from the selected BC_2F_3 plants, were evaluated in the 1997–1998 dry cycle (December 1997–May 1998) under different water regimes. Results from this first field evaluation indicated that the mean of the 70 selected genotypes performed better than the control (CML247 crossed with the same two testers). The best genotype among the 70 selected (BC_2F_3 x testers) performed 2X (x CML274) to 4X (x CML254) better than the control. It is important to mention that the water stress was quite severe due to unusually high temperatures, inducing about an 80% yield reduction in the control. No yield reduction was observed under well-watered conditions for the hybrids derived from the MAS genotypes. A second field evaluation was conducted during the most recent dry cycle (December 1998–May 1999) and results are being analyzed.

A second MAS project aimed at improving populations by exploiting changes in allelic frequency has been conducted. By comparing C0, C4, and C8 from a series of eight cycles of full-sib recurrent selection in the population "Tuxpeño Sequía" during the dry season in Mexico (Bolaños and Edmeades 1993), changes with selection in allelic frequencies at loci of known map position were quantified. It was postulated that these changes, observed by screening 120 genotypes of C0, C4, and C8 with about 40 RFLP probes, resulted from the selection pressure applied by breeders at each cycle, and that alleles increasing in frequency with selection favored drought tolerance.

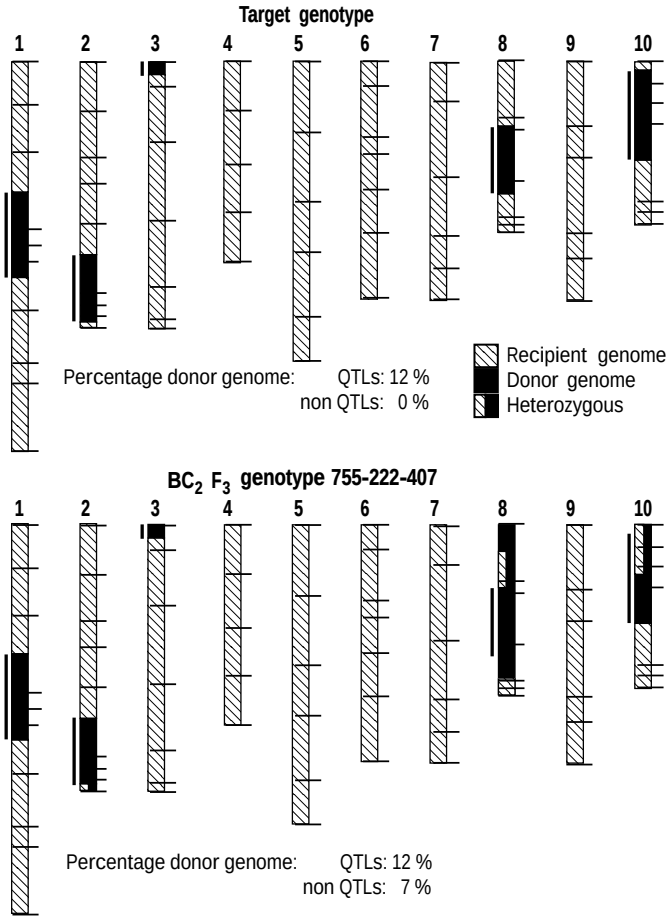

Figure 2: With five selected regions, the target genotype of the BC-MAS for drought improvement conducted at CIMMYT is presented at the top of the figure. Those five regions, at which fixed donor genome contribution is expected, represent 12% of the mapped genome (70 loci/RFLP probes) and are presented in black. At the bottom of the figure, the best genotype identified after 4 cycles of MAS (2 BCs and 2 self-pollinations) is presented. For this genotype, the remnant donor genome contribution outside the target regions represents about 7% of the mapped genome.

To test this hypothesis, 21 DNA markers were used to screen 400 plants from C_0 and C_4. Based on their allelic composition, the 40 "best" and "worst" genotypes from each of the two cycles were selected and evaluated under several water regimes (WW, IS, and SS) one year ago (November 1997–May 1998). Preliminary analysis of those results in this extremely dry season showed a significant difference of about 20% in yield performance under drought conditions between the two groups of genotypes from the same cycle but selected using markers. These results suggest that MAS can be used to improve open pollinated varieties and populations.

Perspectives for MAS

Geneticists working with maize are obviously in a privileged situation for a number of reasons: the diploid nature of the genome, high level of polymorphism, large number of publicly available DNA markers, numerous QTL studies already published, large number of genes already characterized, thousands of expressed sequence tags (EST) are publicly available, and possible collaboration with private sector entities leading the research in maize genomics. Considering this situation and the rapid increase in data available at the genomic level, various MAS strategies can be used to improve maize production. Today, the manipulation of genomic regions can be divided into two categories: gene and QTL introgression. An identified gene generally refers to one that has been cloned and sequenced. Thus DNA markers can be designed that amplify or hybridize to the target gene itself and can be directly used for MAS. For QTL introgression, on the other hand, a target genome segment, probably comprising many genes, lies between the two DNA markers that define the QTL.

MAS for QTL Manipulation

For approximately 10 years, genetic dissection of polygenic traits has been hailed as a promising application for DNA markers, resulting in extensive mapping experiments aimed toward the development of MAS (Lee 1995). However, because of the limited number of QTL identified per trait, the relatively small amount of phenotypic variance that they generally express individually, their interaction with the environment, and the difficulty of assessing epistasis, few concrete MAS results have been published that would justify the initial enthusiasm (Mohan et al. 1997; Ribaut and Hoisington 1998). Until recently, a clear technical limitation has been the restricted population sizes that can be handled, limiting the flexibility and the power of selection. With the development of reliable PCR-based markers, a substantial increase in segregating population size that can be screened is now feasible (Ribaut et al. 1997b). Other limitations are the reduced flexibility of the "good line by bad line" concept, and the transfer of genomic segments from a donor to a recipient elite genome through BC, which imposes constraints in time and cost. These limitations became apparent in our BC-MAS for drought tolerance. However, when this project was initiated five years ago, it appeared to be an attractive option, given the low amount of information available about elite line performance under drought.

Today, the situation has evolved at both the germplasm (Bänziger et al. 1999) and technology levels. Considering this progress, new MAS strategies should be considered for the improvement of drought tolerance; some of these strategies are already being employed at CIMMYT. If suitable germplasm is available, the pyramiding of favorable alleles through DNA markers in new germplasm by crossing two elite lines that perform well under the target environment conditions ("good by good") should open new doors for MAS (Ribaut and Betran 1999). We also underuse, at least at our Center, pedigree information that, when combined with fingerprinting molecular data and field evaluation of our germplasm, would help us trace favorable alleles identified and accumulated through conventional breeding. From our point of view, the BC approach for QTL introgression remains relevant if the recipient line is really exceptional and a key future line for an on-going breeding program, or if the target alleles are present only in germplasm with relatively poor agronomic performance.

MAS for Identified Genes

Through the recent development of genomic technologies that provide structural and functional information (Habben et al. 1999), gene characterization (i.e., the localization, sequence, and expression framework of a gene) has received a significant boost during the last couple of years. The questions now are how to prioritize the research aimed at characterizing the genes involved in the drought-tolerance process, and once those genes are characterized, how to identify and efficiently

manipulate the elite alleles at those target loci to improve a given variety. The first question must be addressed principally by the research groups conducting basic genomic research. Of course, establishing such priorities is more or less a function of the available resources and the research objectives of a group. The recent discovery of promoter regulatory elements, like DRE (dehydration-responsive element) or ABRE (ABA-responsive element) involved in both dehydration- and low-temperature induced gene expression in *Arabidopsis* (Shinozaki and Yamaguchi-Shinozaki 1997), as well as the identification of transcriptional factors interacting with those promoters (Liu et al. 1998), are exciting developments. The characterization of the genes involved in the initiation phase of the stress response (e.g., genes encoding for stress-induced transcription factors) should be a logical priority, since they represent the "up-stream keys" to global genomic responses that might involve hundreds of genes. Moreover, once they have been identified, expression of these key genes should serve as a "timing reference" to identify expression products from downstream genes involved in stress responses. This can be achieved using microarray technology as described by Chu (1998).

Once gene-level genetic dissection of the different components of the stress response is achieved, even partially, strategies to use this information for plant improvement can be developed. This second phase should consider two approaches: transformation and allelic introgression through MAS. Transformation might be attractive for intellectual property reasons, and to allow the control of gene expression in specific plant tissues or under target conditions through specific promoters. Nevertheless, elite allele pyramiding through transformation will likely be time consuming and/or expensive in the absence of major genes, although several genes can be transferred simultaneously. Over-expression of specific genes under stress in *Arabidopsis* has provided very promising results (Kasuga et al. 1999). However, in our view, this approach must be considered carefully. Indeed, when grain yield is the target trait, and a moisture deficit occurs prior to flowering, the gene expression required for optimal partitioning of plant resources at flowering and grain filling is not obvious. Moreover, if the over-expression of a single gene through a stress-induced promoter found in the plant is able to confer stress tolerance, why is the nature of plant response to an abiotic stress always so quantitative in a segregating population?

Once the characterization of genes involved in the drought response has been achieved, the screening of elite germplasm and promising genebank accessions should be conducted to evaluate the allelic identity at those loci based on field performance. Pyramiding and tracing of target alleles in new germplasm can be conducted through DNA markers using the same approach as that adopted for QTL (e.g., BC-MAS or SLS-MAS).

Under this scenario, MAS will likely become a dynamic area of investigation because optimal conventional and marker-based strategies will likely evolve together. Today, given the information available at the QTL and gene levels in relation to drought tolerance in maize, germplasm improvement can count on new molecular tools to complement conventional breeding. This complementarity should become more important in the near future and be information-driven, given the quantity and quality of the basic genomic research now being conducted at a global level.

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Genetic Analysis of Pre-Flowering and Post-Flowering Drought Tolerance in Sorghum

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Summary

Drought tolerance is an important agronomic trait in field crops, but the genetic and physiological mechanisms that condition its expression are not well understood. Sorghum (Sorghum bicolor (L.) Moench) is one of the more drought tolerant crops and is an excellent model for studying mechanisms of drought tolerance in higher plants. The purpose of the studies presented in this report was to use molecular markers to identify genetic loci associated with the expression of pre-flowering and post-flowering drought tolerance in sorghum. A recombinant inbred line population of 100 lines was developed from a cross between two parents with contrasting drought tolerance, TX7078 (pre-flowering tolerant, post-flowering susceptible) and B35 (post-flowering tolerant, pre-flowering susceptible). The RI lines were agronomically evaluated under conditions of pre-flowering drought, post-flowering drought, and under full irrigation. The population was also genotyped with 170 molecular markers using standard protocols. Analysis of genotypic and phenotypic data led to identification of regions of the genome associated with specific drought tolerance reactions. We identified quantitative trait loci (QTLs) for yield stability, yield under drought, stay-green, and other traits associated with drought tolerance. We developed and characterized near isogenic lines with contrasting alleles for markers linked to individual QTL and verified the marker-phenotype association observed in the RI lines. We conclude that our approach of narrowing on specific genomic regions associated with drought tolerance, coupled withphysiologic characterization, holds promise for developing a better understanding of this complex trait.

Introduction

Drought is perhaps the most important abiotic stress limiting crop productivity around the world. It is certainly of great significance in the semiarid tropics (SAT), where rainfall is generally low and its distribution is erratic. One of the most effective ways to alleviate problems of crop production associated with drought is the development of crops that withstand moisture stress. Identification and testing of drought resistant germplasm in a plant breeding program, however, is an arduous and slow process. Drought tolerance is a complex trait; the genetic and physiological mechanisms that condition its expression are poorly understood. Controlled by many genes and dependent on the timing and severity of moisture stress, it is one of the most difficult and seemingly intractable agronomic traits to characterize and study.

Sorghum (*Sorghum bicolor* [L.]) Moench is native to sub-Saharan Africa, a region generally characterized by unpredictable rainfall pattern. Adaptation of sorghum to the range of environmental conditions in semi-arid Africa has resulted in the evolution of extensive genetic variation for drought tolerance in this crop (Blum 1979; Doggett 1988). Consequently, sorghum is one of the most drought-tolerant grain crops and its rich and apparent genetic diversity for stress tolerance makes it an excellent crop model and choice for studying the genetic and physiological mechanisms of drought tolerance. Nonetheless, even in sorghum, direct selection for drought tolerance using conventional approaches has been slow and difficult. Although a number of physiological and biochemical traits

have been associated with the enhancement of drought tolerance, only a few of these mechanisms have been demonstrated to be causally related to the expression of tolerance under field conditions (Ludlow and Muchow 1990). We believe that use of molecular markers and quantitative trait loci (QTL) analysis based on carefully managed, replicated tests has the potential to alleviate the problems associated with the inconsistent and unpredictable onset of moisture stress or the confounding effect of other stresses such as high temperature. To this end, we undertook several studies where putative QTL involved in specific drought-tolerance reactions were identified and isogenic lines for some of these QTL were generated and characterized for relevant agronomic and physiological traits.

Materials and Methods

We developed a population of 100 recombinant inbred (RI) lines from a cross between two genotypes with contrasting patterns of drought tolerance—TX7078 (pre-flowering drought tolerant and post-flowering drought susceptible) and B35 (pre-flowering drought susceptible and post-flowering drought tolerant)—following a standard procedure for the Single Seed Descent method of plant breeding. RI populations allow replicated trials of genotypes for the collection of phenotypic data on drought tolerance and provide genetic material for mapping molecular markers. We characterized the RI lines for drought tolerance in field studies that we conducted on a farm near San Juan de Abajo, Nayarit, Mexico and at the University of Arizona Dry-land Experiment Station at Yuma, Arizona. At both sites, the trials were grown under three irrigation treatments: pre-flowering drought, post-flowering drought, and full-irrigation. Drought was imposed in each trial by appropriately withholding moisture at key stages of plant development (at the vegetative stage for pre-flowering stress and at the reproductive stage for post-flowering drought). The RI lines and their parents were genotyped with 150 RAPD and 20 RFLP markers using standard protocols (Williams et al. 1992; Saghai-Maroof et al. 1984). The field data and the 170 markers identified in the population were ordered into a linkage map to facilitate QTL analysis for drought tolerance. We also generated near isogenic lines (NILs) for major QTL linked to traits associated with drought tolerance and assessed the agronomic and physiological contributions of putative QTL involved in drought tolerance.

Results

Dissecting drought tolerance

Drought tolerance in sorghum is expressed in a developmentally specific pattern. Susceptibility to drought can occur when moisture stress is imposed at each of the following stages: early vegetative stage, period of panicle development prior to flowering, and from pollination to maturity, post-flowering (Rosenow and Clark 1981). Sorghum genotypes with good tolerance during one of the developmental stages are typically found to be susceptible to drought during the other growth stages. This developmental interaction further complicates the phenomenon of drought tolerance.

We have made slow but significant progress in the empirical breeding of sorghum for drought tolerance by breaking down drought tolerance into specific phenological stages. We developed drought-tolerant sorghum lines in diverse germplasm backgrounds. Sorghum germplasms that are uniquely pre-flowering or post-flowering drought tolerant and a few that combine tolerance at both stages have been generated. Our selection effort was based on reliable phenotypic markers associated with morphological and yield- related symptoms that occur at pre-flowering and post-flowering stages of crop development. Pre-flowering drought stress of a susceptible sorghum line produces leaf rolling, unusual leaf erectness, delayed flowering, floret abortion, reduced seed set and panicle size, and reduced plant height. Normal panicle development, good seed set and typical leaf morphology are indicative of a tolerance reaction to pre-flowering drought stress. Under post-flowering drought, susceptible lines exhibit premature leaf and stalk senescence and reduced seed weight. Tolerance to moisture stress at this stage is manifested by a stay-green phenotype and by normal grain filling.

Our approach has been to break down the complex trait of drought tolerance into simpler components by studying drought-stress expressions at specific stages of plant development. We have been particularly interested in midseason (pre-flowering) and late-season (post-flowering) drought expressions in sorghum germplasm. Our rationale is that if individual components associated with a

complex trait can be identified, we can measure the contribution of each of the factors or mechanisms independently, without the confounding effect of other factors.

Pre-flowering drought tolerance

A linkage map of the 150 RAPD and the 20 RFLP markers scored in the population was developed to facilitate QTL analysis for drought tolerance. Six QTL were shown to be specifically associated with pre-flowering drought tolerance. The allele derived from TX7078 was associated with increased drought tolerance at all but one of these QTL, which was consistent with the pre-flowering drought tolerance of this line through agronomic and physiological characterization (Premachandra et al. 1994). Although the severity of pre-flowering drought stress in each year was quite different, most of the QTL identified showed significant or near significant associations with drought tolerance in each year. Eight additional QTL were identified for traits generally associated with yield and yield components under fully irrigated conditions. Segregating markers accounted for between 43% and 14% of the phenotypic variation for traits associated with pre-flowering drought tolerance (Table 1). A large degree of the phenotypic variability associated with traits measured under fully-irrigated conditions was also accounted for by markers linked to those traits. The identification of QTL accounting for this substantial fraction of the phenotypic variability in drought tolerance is a significant first step towards a more detailed genetic characterization of this important trait.

Post-flowering drought tolerance

Significant differences in grain yield, seed weight, and stay-green were observed among genotypes in each testing environment. These differences indicate segregation in the mapping population for genetic factors contributing to these traits (Tuinstra et al. 1997). Differences among RI lines for both duration and rate for grain development were also significant.

Thirteen genomic regions were associated with post-flowering drought tolerance in our mapping populations (Table 2). Regions of linkage group F and I were associated with grain yield in the post-flowering drought trial. Together these loci account for about 12% of the variability in grain yield under post-flowering drought. These QTL were also associated with the staygreen trait. Four QTL were detected on linkage groups B, C, and E for yield stability and accounted for 36% of the variability in yield stability. Seven QTL on linkage groups A, E, G, and N were associated with duration of grain development and account for 51% of

Table 1. Effects of QTL identified using the combined and individual year means. The probability of the association between the marker and quantitative trait is shown (P > F).†

Linkage Group	Marker	Treatment	Trait	Combined year	1992	1993
A	bB13/35	Drt	Height stability	0.0006	0.0608	0.0001
		Irr	Yield per se	0.0074	0.0259	0.1047
	b257/167	Drt	Height stability	0.0001	0.0013	0.0015
		Irr	Height	0.0064	0.0053	0.0122
	247/116	Drt	Height stability	0.0001	0.0001	0.0059
		Irr	Height	0.0001	0.0001	0.0001
	t268/100	Irr	Yield per se	0.0092	0.0296	0.0232
B	ADH1	Irr	Maturity	0.0079	0.0049	0.0217
	tL19/62	Irr	Maturity	0.0011	0.0007	0.0040
C	t259/87	Irr	Height	0.0058	0.0112	0.0046
	b229/47	Irr	Maturity	0.0002	0.0007	0.0002
D	b465/140	Drt	Yield per se	0.0010	0.0407	0.0009
		Drt	Yield stability	0.0010	0.1233	0.0940
	UMC85	Drt	Yield per se	0.0094	0.0024	0.0893
	tK12/115	Drt	Yield per se	0.0019	0.2392	0.0021
		Irr	Yield per se	0.0059	0.0823	0.0064
	bD11/65	Drt	Yield per se	0.0008	0.0567	0.0016
		Irr	Yield per se	0.0002	0.0125	0.0002
E	b258/94	Drt	Seed set stability	0.0098	0.0101	0.5781
		Irr	Maturity	0.0001	0.0005	0.0001
	UMC109	Drt	Seed set stability	0.0088	0.0752	0.0226
		Irr	Maturity	0.0001	0.0001	0.0001
F	BNL5.62	Drt	Seed set stability	0.0035	0.0054	0.1211
	tM5/75	Drt	Yield stability	0.0031	0.0021	0.0383
	tC13/150	Drt	Yield stability	0.0003	0.0002	0.0615
	UMC84	Irr	Maturity	0.0001	0.0001	0.0001
G	tH19/17	Irr	Yield per se	0.0082	0.0236	0.0176
H	b374/75	Drt	Yield per se	0.0001	0.1262	0.0001
		Drt	Yield stability	0.0082	0.1775	0.1164
		Irr	Yield per se	0.0066	0.9593	0.0001
		Irr	Seed set	0.0028	0.6830	0.0007
	t380/67	Drt	Yield per se	0.0001	0.6905	0.0001
		Drt	Yield stability	0.0002	0.6768	0.4296
		Irr	Yield per se	0.0015	0.6742	0.0001
		Irr	Seed set	0.0025	0.5372	0.0009
M	bC18/820	Drt	Height stability	0.0012	0.0265	0.0011
N	tH19/50	Irr	Yield per se	0.0025	0.0184	0.0024

† Probability of a larger F-value by chance.
‡ Drt = pre-flowering drought; Irr = fully irrigated.

the phenotypic variability in duration of grain fill in the mapping population. QTL identified for rate of grain development accounted for 62% of the variability in this trait. Some of the QTL for rate of grain filling were also associated with higher seed weight. Several QTL were identified for grain yield and seed weight per se. QTL for seed weight on three linkage groups were associated with higher rate of grain development. One seed weight QTL was associated with short duration of grain development and reduced seed weight stability. These QTL together accounted for 44% of the variability in seed weight in the RI population, but under fully-irrigated conditions.

Markers associated with yield, yield stability, and seed weight were variable across testing environments. These results reaffirm the importance of multi-environment testing when evaluating drought tolerance and other traits subject to environmental interaction. Markers associated with stay-green and seed weight were more stable and consistent across environments. Markers strongly associated with stay-green or seed weight in one environment were usually also detected in other environments. Marker-assisted selection for these loci should be productive for enhancing the expression of these traits across environments.

Table 2. Percentage of the phenotypic variation explained by markers linked to QTL. Estimates were obtained by stepwise regression using markers significantly associated with each trait.

	R^2 (%)
Pre-flowering drought	
Yield per se	42.1
Yield stability	38.7
Seed set stability	14.2
Height stability	43.0
Post-flowering drought	
Yield	12.4
Yield stability	35.9
Seed weight stability	23.8
Staygreen	50.6
Duration of grain-fill	51.4
Rate of grain-fill	61.8

Evaluation of near-isogenic lines for drought tolerance QTL

Analysis of near isogenic lines (NILs) that differ at QTL can be an effective approach for the detailed mapping and characterization of individual loci. However, the use of NILs in analysis of important agronomic traits has been limited, perhaps because of the time and effort required to develop these lines. We developed a procedure (Tuinstra et al.1997) for drawing NILs for any region of the genome that can be analyzed with molecular or other genetic factors. The procedure utilizes molecular markers to identify heterogeneous inbred families that are isogenic at most loci in the genome from NILs that differ for markers linked to the QTL of interest. We used NILs drawn out in this manner to test the phenotypic effects of three different genomic regions associated with various measures of agronomic performance in drought and/or nondrought environments (Tuinstra et al. 1998). We reasoned that this process of identifying linkage between markers and traits in a mapping population and subsequently testing marker effects in NILs can provide strong evidence supporting QTL positions and effects. NILs also provide excellent material for fine-mapping and for studying the phenotypes of individual QTL.

In this study, we focused on the analysis of NILs contrasting at three loci and evaluated differences in the size of the genomic region differentiating each set of NILs by testing markers flanking each target QTL. Phenotypic evaluation of NILs indicated large differences in yield and seed weight associated with each QTL marker (Table 3). In most cases, NILs contrasting for a specific locus differed in phenotype, as predicted by QTL analysis. NILs contrasting at QTL marker tM5/75 indicated large differences in yield across a range of environments. Further analysis indicated that differences in agronomic performance may be associated with a drought tolerance mechanism that also influences heat tolerance. NILs contrasting at QTL marker tH19/50 also differed in yield under drought and nondrought conditions. The analysis of these NILs indicated that these differences may be influenced by a drought tolerance mechanism that conditions plant water status and the expression of stay-green. NILs contrasting at QTL marker t329/132 differed in yield and seed weight. These differences appear to be the result of two QTL that are closely linked in repulsion.

This study (Tuinstra et al. 1998) is one of the first in which the effect of specific QTL associated with grain yield and other agronomic traits under drought stress have been evaluated in near isogenic lines. The expression of drought tolerance QTL was strongly influenced by the environment and the genetic background in which they were evaluated. Nevertheless, we believe that the approach of focusing research efforts on specific genomic

regions associated with drought tolerance holds promise for developing a clear understanding of the physiology and biochemistry associated with this complex trait.

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Table 3. Analysis of yield, seed weight, stay-green, and leaf water potential in NILs tested in nondrought, post-flowering, or pre-flowering drought environments.

			Nondrought[2]		Post-flowering drought			Pre-flowering drought	
Marker	HIF	Genotype[1]	Yield kg ha^{-1}	Seed weight g(100 seeds)$^{-1}$	Yield kg ha^{-1}	Seed weight g(100 seeds)$^{-1}$	Staygreen 1 to 5	Yield kg ha^{-1}	XPP Mpa
tM5/75	1	0	4417 ***	2.83	3982 **	2.77	2.66 **	2826 **	1.37
		1	2851	2.71	3064	2.88	3.09	2186	1.30
	2	0	3649 **	2.58	3522 *	2.58	3.06	2367 *	1.56*
		1	2402	2.52	2549	2.68	3.03	1491	1.45
	3	0	5805 ***	2.78 *	4600	2.66 ***	2.75	3625	1.47
		1	4554	2.93	4287	3.30	2.69	3102	1.45
tH19/50	1	0	3830 ***	2.76 ***	4172 **	2.94 *	3.34 *	2814 **	1.55
		1	2533	3.15	2644	3.25	3.81	1360	1.55
	2	0	2938	2.42 *	2575	2.32 ***	2.25 **	1934	1.47**
		1	2664	2.54	2503	2.68	2.72	1828	1.59
	3	0	4392	2.56	4176	2.72	2.75	3133	1.54
		1	4152	2.54	3735	2.56	2.75	2567	1.59
t329/132	1	0	4351 ***	2.68 ***	3348 *	2.56	2.84	2781 **	1.63
		1	3409	2.42	2636	2.42	2.56	2073	1.53
	2	0	3842	2.62	3606	2.81 **	3.41	2696 *	1.55
		1	3612	2.44	3166	2.37	3.59	2386	1.55
	3	0	3192 **	2.64 ***	2940 **	2.71 ***	2.75 *	2052 *	
		1	3792	2.39	3737	2.41	3.03 2	456	1.57

*, **, *** Significant at 0.05, 0.01, and 0.001 level respectively

[1] 0 = B35; 1 = TX7078

[2] Nondrought = Irrigated Mexico and Arizona; Indiana.

Physiological Basis, QTL, and MAS of the Stay-Green Drought Resistance Trait in Grain Sorghum

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Summary

Stay-green is a drought-resistance trait in grain sorghum. When water is limited during the grain filling period, genotypes possessing this trait maintain more photosynthetically active leaves compared with genotypes not possessing this trait. An improved understanding of the physiological basis of stay-green was sought. Higher grain yield in stay-green compared with senescent genotypes was associated with increased green leaf area at maturity, leaf nitrogen status, and transpiration efficiency. Three genomic regions located on linkage groups B, G, and I were identified for QTL associated with stay-green, accounting for 14.4%, 10.2%, and 10.1% of the variation, respectively. One of the markers identified for stay-green on linkage group B is a PCR-based SSR marker that could be readily used in breeding programs.

Introduction

Production of sorghum in semiarid regions of the world is limited by drought. Developing plants that have an advantage under water-limited conditions is a major challenge for sorghum improvement programs. Results from breeding programs in the USA (Rosenow et al. 1983) and Australia (Henzell et al. 1992) suggest that advances in crop improvement under water-limited conditions are more likely if drought resistance traits are selected in addition to yield per se. Stay-green is one such trait, and genotypes possessing this trait maintain more photosynthetically active leaves than genotypes not possessing this trait (Rosenow et al. 1983).

The key issue is whether maintaining green leaf area under post-anthesis drought actually increases grain yield in stay-green compared with senescent genotypes. If this is the case, then pathways to increased green leaf area retention such as higher maximum green leaf area, delayed onset of leaf senescence, and reduced rate of leaf senescence should be sought to improve yield under drought. In addition to this empirical approach, the underlying physiological mechanisms of drought resistance should be investigated, providing a basis to modify the leaf senescence routines in a sorghum simulation model. The model could then be used to assess the value of stay-green in a wide range of target environments (Hammer and Muchow 1994).

Increased biomass accumulation under post-anthesis drought in stay-green compared with senescent genotypes can be achieved by increasing one or both of its components: transpiration and/or transpiration efficiency. Knowledge of the extent of genetic variation in these components is required to improve yield under drought. Since the longevity and photosynthetic capacity of leaves are related to their nitrogen status (Thomas and Rogers 1990), it is also important to determine the role of nitrogen in extending leaf greenness in stay-green genotypes.

Genome analysis provides a framework to link structural analysis of genes with the underlying physiological mechanisms, and

ultimately with phenotypic performance of crop plants in a range of target environments (McCouch and Xiao 1998). As stay-green is expressed only in those environments in which post-anthesis drought is sufficiently severe, neither efficiency nor reliability of selection is high when conventional breeding is used to select for this trait. Hence marker-assisted selection for stay-green should greatly enhance the efficiency of selection for the trait.

This paper reports on a series of field and associated laboratory studies undertaken in northeastern Australia between 1994 and 1999. The aims of these studies were twofold. Firstly, to determine the key physiological mechanisms responsible for enhanced yield in stay-green compared with senescent genotypes and, secondly, to identify quantitative trait loci for stay-green with consistent effects across a set of environments.

Materials and Methods

Physiological studies

Field experiments were conducted between 1994 and 1999 at Hermitage Research Station (altitude 480 m, latitude 28°10′S, longitude 152°02′E) in the sorghum cropping zone of southern Queensland. Most of the physiological data reported in this paper arose from an intensive experiment undertaken in 1995 (Borrell and Hammer 1999; Borrell et al. 1999a,b,c). Briefly, the experimental design was a split plot with three replicates. Three irrigation treatments were applied to main plots (No Deficit, ND; Post-Flowering Deficit, PFD; Terminal Deficit, TD) and nine genotypes varying in rate of leaf senescence were allocated to subplots. To examine the A35 and RQL12 sources of stay-green, nine genotypes were examined from crosses of three females varying in stay-green (AQL39, senescent; AQL41, intermediate; A35, stay-green) and three males similarly varying (R69264, senescent; RQL36, intermediate; RQL12, stay-green). All treatments were covered with black plastic prior to sowing to exclude rainfall and prevent evaporation losses.

Genome analysis

Molecular marker development for Australian sorghum breeding programs has been conducted in parallel to the construction of RFLP maps developed by several other groups overseas (McIntyre et al. 1997). One hundred and sixty random recombinant inbred lines (RILs) were developed from a cross between two elite lines BQL39 / BQL41; BQL39 is senescent and BQL41 is stay-green (Henzell et al. 1992). A genetic map has been established using the RI population and aligned with other major published sorghum maps (Tao et al. 1997). Field trials were conducted across five sites and three growing seasons using these RILs. Phenotypic data for stay-green was taken from five of the trials. Field trial design, statistical analyses and QTL mapping were the same as those previously reported (Tao et al. 1999).

Results and Discussion

Physiological basis of stay-green

Leaf growth and senescence:

Four classes of stay-green have been identified by Thomas and Smart (1993). Two classes are functionally stay-green and may occur after alteration of genes involved in the onset of senescence (Type A) and the regulation of its rate of progress (Type B). The remaining two classes are cosmetic, ie., the plants are green but lack photosynthetic capability. In a study of nine genotypes varying in rate of leaf senescence grown under terminal water deficit, Borrell et al. (1999a) found that genotypes possessing the A35 and RQL12 sources of stay-green retained more green leaf area at maturity compared with intermediate and senescent genotypes (Table 1), although the mechanism of leaf area maintenance varied with the source of stay-green. RQL12 genotypes displayed Types A and B stay-green, yet A35 genotypes displayed only Type A. Higher green leaf area at maturity in A35 genotypes was also due to increased total plant leaf area before anthesis

Table 1. Green leaf area at maturity (cm2 m-2) for six parents grown under three water regimes. Values were determined using a broken-linear model.

	Water regime		
Female parents	**No deficit**	**Post-flowering deficit**	**Terminal deficit**
AQL39	2650	1217	822
AQL41	2722	918	667
A35	2781	1390	1213
l.s.d. (*P*=0.05)	ns	187	187
Male parents			
R69264	2659	961	508
RQL36	2922	1389	888
RQL12	2573	1175	1305
l.s.d. (*P*=0.05)	187	187	187

which, in turn, was associated with increased leaf appearance rate as well as later maturity. That such differences exist in these mechanisms should not be surprising, since the A35 and RQL12 germplasm is derived from lines native to Ethiopia and Nigeria, respectively (Borrell et al. 1999a).

Dry matter production and grain yield:

In a study of nine genotypes varying in stay-green, Borrell et al. (1999b) found that grain yield was correlated positively with green leaf area at maturity ($r=0.75^{**}$) and negatively with rate of leaf senescence ($r=-0.74^{**}$), suggesting that sorghum genotypes possessing the stay-green trait have a significant yield advantage under post-anthesis drought compared with genotypes not possessing this trait (Table 2). They also reported that stay-green did not constrain yield when water was not limiting, since no differences in grain yield were observed among 8 of 9 genotypes under fully irrigated conditions. When water was limiting during grain growth, yield accumulation in stay-green genotypes was largely dependent on photo-assimilation in the remaining green leaves. Lower yields in the intermediate and senescent genotypes were associated with retention of less green leaves, although this reduction was offset to some extent in the intermediate hybrid by utilisation of stem reserves for grain filling. A positive correlation ($r=0.71^{*}$) was observed between rate of leaf senescence and the magnitude of stem reserves mobilised (Table 2), providing some evidence that stay-green genotypes are less reliant on nonstructural stem carbohydrates for grain filling compared with senescent genotypes, resulting in stronger stems and less lodging.

Nitrogen dynamics:

Longevity of a leaf is intimately related to its nitrogen (N) status (Thomas and Rogers 1990), and the attainment and subsequent loss of photosynthetic capability are linked to the rate of export of N (Thomas and Smart 1993). Nitrogen dynamics in stay-green and senescent sorghum genotypes were examined under varying levels of water supply by Borrell and Hammer (1999). They found that above-ground N content and green leaf area index were positively correlated at anthesis ($r=0.8^{***}$), mid-grain filling ($r=0.47^{*}$) and maturity ($r=0.69^{***}$) for sorghum plants grown under terminal water deficit. This raises an important question. Do the leaves in stay-green genotypes take up more N simply because they continue to grow, or do they stay green for longer because their leaves contain more N? There is some evidence to support the latter since specific leaf nitrogen (SLN) was higher in stay-green genotypes compared with intermediate and senescent genotypes at anthesis, mid-grain filling and maturity. This indicates that the leaves of stay-green types contained more N, even before the trait was expressed. In fact, differences in leaf N status between stay-green and senescent genotypes were observed as early as 27 days after emergence in the 1999 season (A.K. Borrell, unpublished). The strong association observed between leaf N concentration (LNC) at anthesis and grain yield under drought suggests that LNC at flowering should be considered as a selection index for drought-resistance in sorghum breeding programs (Borrell and Hammer, 1999).

Table 2. Correlation matrix for a range of yield determinants grown under terminal water deficit.

	Yield (g m-2)	AGDM (g m-2)	HI	Grain size (mg)	Grain number per m²	Grain growth rate (g m-2 d-1)	Duration of grain growth (d)	Rate of senescence (% LAI d-1)	GLAM (cm2 m-2)	Stem reserves (g m-2)
Yield	1.00									
AGDM	0.97***	1.00								
HI	0.39	0.15	1.00							
Grain size	0.46	0.39	0.46	1.00						
Grain number/m²	0.79**	0.80**	0.15	-0.18	1.00					
Grain growth rate	0.90***	0.94***	0.08	0.20	0.85***	1.00				
Duration of grain growth	0.70*	0.56	0.73*	0.70*	0.31	0.32	1.00			
Rate of senescence	-0.74**	-0.77**	-0.06	-0.33	-0.57†	-0.85***	-0.20	1.00		
GLAM	0.75**	0.82**	-0.09	0.34	0.57†	0.90***	0.15	-0.96***	1.00	
Stem reserves	-0.58=	-0.68*	0.26	0.18	-0.76**	-0.74**	-0.02	0.71*	-0.71*	1.00

Correlation significant at $P<0.1$ (†), $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***).
AGDM = Above-ground dry mass at matuirty. HI = Harvest index. GLAM = Green leaf area at maturity.

Transpiration efficiency:
Differences among nine sorghum genotypes in biomass production when grown under terminal water deficit were associated with variation in transpiration and transpiration efficiency (TE) for both the A35 and RQL12 sources of stay-green (Borrell et al. 1999c). These relationships, however, were highly dependent on the genetic background in which they were evaluated. Transpiration efficiency varied by 45% among the nine genotypes, ranging from 3.3 g kg^{-1} (AQL39/R69264) to 6.0 g kg^{-1} (A35/RQL36), although TE was similar for 7 of 9 genotypes (about 4.5 g kg^{-1}) (Table 3). After correcting for ambient vapour pressure deficit, the TE coefficient for A35/RQL36 was 12.5 Pa, or 39% higher than the standard of 9 Pa, suggesting that genotypes do exist with higher absolute levels of TE. Similarly high TE values were observed for A35/RQL36 in the 1999 season (A.K. Borrell, unpublished). These outcomes are further supported by a study of 17 sorghum genotypes grown under well-watered and water-limited conditions in which Mortlock and Hammer (1999) found that A35/RQL36 ranked second-highest in TE.

Genomic regions associated with stay-green

Three genomic regions located on linkage groups B, G, and I were identified for QTL associated with stay-green from overall means of five test environments (Tao et al. 1999). These regions accounted for 34% of total variance of stay-green, 14.4%, 10.2%, and 10.1% for each region, respectively (Figure 1). Pedigree analysis also strongly supports that these regions contain genes for stay-green. Phenotypic means of BQL39 were greater than those of BQL41 for the loci in regions of linkage groups B, G and I, suggesting that the stay-green regions were inherited from BQL41, a cross between BQL33 and B35. It is difficult at this time to align the regions identified with stay-green in this study with those identified by Tuinstra et al. (1997) because of insufficient common loci between the two maps.

Marker assisted selection for stay-green

QTL analysis enables the identification of genes responsible for superior performance across a wide range of environments. Since stay-green is a trait exhibiting significant genotype by environment interaction, at least in Australia, it is critical to collect phenotypic data in multi-environment tests which sample the target environment. The five trial sites used in this study were selected to represent the most common stress

Table 3. Above-ground dry mass (g m^{-2}), transpiration (mm), transpiration efficiency (g kg^{-1}) and transpiration coefficient (Pa) for nine sorghum genotypes during the period from panicle initiation to physiological maturity.

Hybrid	Above-ground dry mass	Transpiration	Transpiration efficiency	Transpiration coefficient†
AQL39/R69264	1193	362	3.27	6.9
AQL41/R69264	1414	313	4.52	9.5
A35/R69264	1307	297	4.43	9.3
AQL39/RQL36	1145	248	4.66	9.8
AQL41/RQL36	1254	268	4.67	9.8
A35/RQL36	1614	271	5.95	12.5
AQL39/RQL12	1295	289	4.52	9.5
AQL41/RQL12	1683	370	4.63	9.7
A35/RQL12	1560	373	4.28	9.0
l.s.d.	367 (*P*=0.06)	64 (*P*=0.05)	1.06 (*P*=0.05)	2.2 (*P*=0.05)

† Mean daytime vapour pressure deficit = 2.1 kPa.

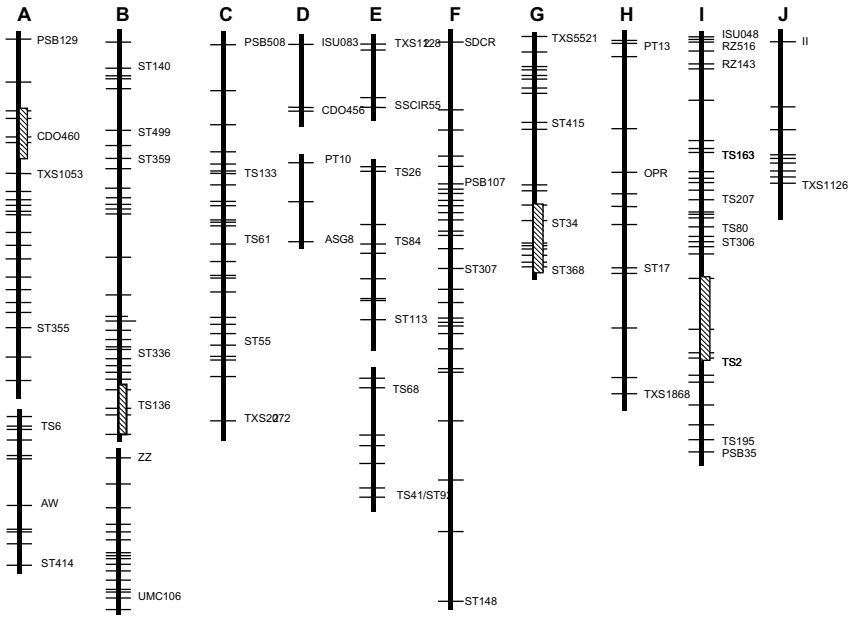

Fig. 1. Sorghum genetic map of the recombinant inbred population derived from a cross of BQL39 x BQL41. Regions associated with stay-green are indicated with blocked bars.

environments encountered by sorghum in Australia, namely post-anthesis drought. The association of regions on linkage groups B and I with the expression of stay-green across the majority of these environments increases confidence about the real value of marker assisted selection in the Australian sorghum industry. Significantly, one of the markers identified for stay-green on linkage group B is a PCR-based SSR marker. This type of marker is "breeder user friendly" and could therefore be readily implemented into breeding programs.

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Narrowing the Phenotype Gap: Genetic Maps and Gene Machines Connect Traits and Genes

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Summary

Advancements in technology and attitude have enabled the initial comprehensive molecular investigations of the biological repertoire of higher plants and other complex organisms. While the initial investigations are severely limited, they still provide unprecedented power and a foundation for understanding the biology of crop plants and their interactions with the environment. A key aspect of the foundation is the ability to establish valid associations between phenotypes and genes. Large-scale insertional mutagenesis with transposable elements ('gene machines') and integrated genetic maps are two elements that provide some of the necessary associations. In concert with other methods, these elements provide new options for short- and long-term approaches to understanding and manipulating complex traits such as drought tolerance.

Introduction

The advent of genome initiatives has created an abundance of limited information about many genes and a few genomes. Simultaneously, the initiatives have exposed the poverty in our ability to understand gene function and effects, the genotype, the interactions within genotypes, and between genotypes and the environment in the context of a complex system such as plants. The gap between the source molecule, DNA, and a trait (the 'phenotype gap') is a great mystery, a nonlinear path and one of the major challenges for basic and applied contemporary biology.

Closure of the phenotype gap is a major task. The complexity encountered in simple organisms (e.g., bacteriophage lambda and yeast) grown in controlled conditions has hindered efforts to relate genotype to phenotype. Certainly, the analysis and manipulation of complex traits (drought tolerance and yield) and organisms (plants) grown in stressful and dynamic environments will be more challenging. A recent summary of various perspectives and aspects of improving stress tolerance in maize provides some hints of the magnitude of the challenge (Edmeades et al. 1997). If one hopes to use some of the abundance provided by genome projects and genomics at the molecular level for crop improvement then one must reduce the gap and be able to integrate the new information within the context of the target organism growing in the target environment.

The phenotype gap will be narrowed in many ways in a series of steps that gradually reveal the function(s) of the genes and their connection(s) with phenotypes. Once the simple, primary aspects of identification have been achieved, more sophisticated schemes may be invoked to reveal the more complex nature of gene deployment and interactions that ultimately mediate phenotypes. This presentation will review two common approaches, genetic mapping and transposon tagging (a.k.a. 'gene machines'), that provide some primary information regarding the connection of genes and their phenotypes.

Genetic Maps

Maps based on estimates of genetic recombination have remained a central resource and starting point for many investigations. Loci detected by polymorphism at the DNA level have enabled construction of high density maps that allow one to collate and cross-reference information collected from numerous perspectives and methods. The maps permit genome-wide surveys with a power and sensitivity determined by the interaction between the plant and the environment (here, our perception and limits are not solely determined by models and machines). Despite the inherent uncertainty of estimating genetic map distances and other related problems (remember, recombination is just another phenotype), the information has been

a key component for alignment of physical maps and map-based gene cloning in plants with relatively small nuclear genomes such as *Arabidopsis*, rice, and tomato.

Technical advances will certainly influence the role and utility of genetic maps. Maps based on polymorphism in known genes might be more informative than maps based on mostly anonymous loci. As comprehensive physical maps and whole genome sequences become available, the role of genetic maps for gene hunting declines but does not disappear.

Comparative Mapping

The evidence from genetic maps of widespread conservation of gene content and order among groups of distantly related plants has been a major surprise (Gale and Devos 1998). Even though their last common ancestor may have existed tens of millions of years ago, groups of grass species (such as maize, sorghum, and rice) have retained a very similar repertoire of genes and maintained very similar arrangements of genes, at least for some components and regions of their genomes. Such circumstances provide the opportunity to evaluate groups of sexually isolated species as single genetics systems and compare allelic content, gene deployment and effect in diverse but related biological contexts (Bennetzen and Freeling 1997).

Such analyses will not only reveal important unique coding regions of genes and alleles contained in some species, but perhaps more importantly, they will reveal essential clues about differences in regulatory regions of the many (most) genes in common among all species of a group (the species will have the same coding regions but they will deploy them in different ways as influenced by the regulatory regions). For example, the degree of cuticular wax deposition in maize and sorghum are inverse with respect to the phase of leaf development (adult vs. juvenile). Perhaps the truly interesting sequences for comparative analyses should be those included in the regulatory regions.

A related area of research might be comparative physiology. After the repertoire of one species has been established, it might be routine to identify important differences in the gene content among species. For example, rice is not colonized by Puccinia species, but many other grasses are very 'good' hosts to several species of Puccinia —the results are the various and devastating 'rust' diseases. What does rice have, or lack, relative to the other stricken grasses? No doubt, there are many examples of slight differences in gene content that have important effects on the phenotype. Comparative physiology should elucidate some of the differences and suggest ways to exploit them for crop improvement.

The fact that species such as maize, sorghum, and rice have such similar gene content and order, despite the relatively large size of the maize genome and the millions of years since divergence, has created additional opportunities for gene hunting. By aligning related regions of the large and small genomes, one may conduct gene hunting more efficiently in the species with the smaller genome. The success of this strategy depends, of course, on the degree of conservation of gene content and order when one has arrived at the regions containing the few genes of interest. This strategy has met with mixed results (Bennetzen et al. 1998; Gale and Devos 1998; Leister et al. 1998) and the merit of this model for gene hunting may vary widely with the species and genes under consideration.

QTL Mapping and Marker-assisted Selection

Some of the motivating factors behind the interest in genetic maps have been the exploration of the genetic architecture of complex traits and identification of genetic factors underlying quantitative inheritance patterns (quantitative trait loci, [QTL]). The genetic maps based on loci defined by DNA polymorphism provide a convenient means for surveying the genome for associations between allelic variation at 'marker' loci (or real genes) and variation in traits of interest. The integrated genetic maps and the inclusion of marker loci defined by known genes (known by a DNA clone, sequence or phenotype—a 'mutant' and perhaps, by function) provide the basis for relating quantitative variation to some real genes (candidate loci) that might reveal something about the biological basis of the trait(s).

The strengths and weaknesses of QTL mapping have been well documented (e.g., Tanksley 1993; Beavis 1994; Lee 1995; Melchinger et al. 1998). With a foundation in recombination-based genetic maps, QTL detection is inherently ambiguous and destined to provide a mixture of true and false information. The problems trace to the detection methods, sampling of genetic and environmental reference populations, and the biological

complexity of the traits. Despite the problems, QTL mapping has provided some useful information for an array of inquiry ranging from gene cloning (Doebley et al. 1990) to marker-assisted selection (MAS) in some breeding programs (Lee 1995). Aspects of QTL mapping and the prospects of MAS have been addressed in several sections of this conference.

Gene Machines

More direct clues about the function of individual genes are derived from the large-scale insertional mutagenesis of genomes using well-defined transposons, the 'gene machines' (a.k.a. reverse genetics). The 'machine' consists of an active and well-characterized transposon (known DNA sequence), a large population of plants (approximately 40,000 individuals) segregating for the transposon, DNA samples and progeny of each individual in the population, and a partial DNA sequence of the genes of interest (the targets of the new insertions by the transposon). The population is created in such a way that the transposon inserts into many new sites in the genome. Some insertions into or near genes will modify an aspect of gene expression, possibly affecting gene function and resulting in an observable change in phenotype (a mutant). Since the transposon's DNA sequence is known, the new site of insertion is effectively identified or 'tagged'. Using the DNA sequences of the transposon and the partial sequences of the gene(s) of interest, the entire population is surveyed by PCR and/or hybridization for new insertions into DNA sequences related to the gene(s) of interest and for evidence of cosegregation of the new insertions and a mutant phenotype. DNA fragments that cosegregate with the new mutants may be readily cloned, sequenced, analyzed, and identified regarding their information content and relation to the change in phenotype. Many of the DNA fragments will be pieces of genes and will provide the first clues as to the role of that gene in the context of that organism. The details of various strategies for transposon tagging have been summarized (Sundaresan 1996; Maes et al. 1999) and the plans and background of a maize-specific transposon-tagging gene machine have been made available on a website (http://www.zmdb.iastate.edu/zmdb/nsf_grant_online.html).

Several gene machines have been established for maize (e.g., Pioneer, DuPont, DeKalb, Novartis/Cold Spring Harbor, Max Planck Institute, University of Bristol, and Stanford University). Most of the maize gene machines utilize the transposon "Mutator" because of its high forward mutation rate at many loci, the tendency to insert into hypomethylated DNA (genes), and known molecular and genetic features. The main design features of the Mutator machines are essentially constant, but there are a few notable exceptions. For example the Stanford machine utilities a genetically engineered version of a nonautonomous Mutator element (Mu1), RescueMu, that has been introduced into maize by particle bombardment. In that design, the construct containing the Mutator element also contains a copy of the *Lc* gene whose sequence has been interrupted by the element. When that element excises, the coding sequence of the *Lc* gene is restored and is under the influence of the 35S promoter. Complete restoration of the Lc gene is indicated by the appearance of purple spots of anthocyanin (*Lc* gene is a transcription regulator of structural genes involved in the synthesis of that pigment). In addition to the interrupted *Lc* gene, the design includes an addition of Rhizobium DNA sequence to the Mutator element (to distinguish the modified elements from endogenous Mutator elements and from each other) and sequences of pBluescript to assist cloning of the new insertions.

The maize populations were created by crossing pairs of parents (inbred lines or more variable genetic stocks) with one member of each pair containing active copies of the autonomous member of the Mutator family of transposons (MuDR). The other member of the pair does not contain an active MuDR but it does contain several copies of one or more nonautonomous members of the Mutator family (e.g., Mu2, Mu3). In the F1 generation (40,000 to 50,000 plants), and generations thereafter, the nonautonomous members will transpose in the presence of MuDR and insert into new regions of the genome. The F1 generation plants are self-pollinated to produce the F2 generation. Each F1 plant is identified by one or more samples of DNA isolated from one or more leaves (several leaves should be sampled to distinguish between somatic and germinal mutations because the somatic mutations are essentially false positives). The DNA samples are systematically pooled or combined so that the entire population may be surveyed for new insertions at specific sequences by PCR on relatively few samples.

The F2 generation of this and other Mu-laden populations become the primary 'hunting grounds' for mutations produced by the new insertions of the Mutator elements. So, one potential limitation is the ability to screen for mutations affecting your trait of interest. The goal is to identify DNA fragments (e.g., PCR products) that cosegregate with a new mutation (although this is not absolutely necessary). The DNA fragments are produced by using pairs of primers to amplify DNA from the pools representing the entire population. The DNA sequence of one primer is derived from the sequence of the terminal inverted repeat of Mutator, while the sequence of the other primer may come from various other sources, (e.g., 'the partial sequences of the gene(s) of interest', EST projects, conserved regions of gene families, or a cosegregating fragment from another Mutator-laden population). Information needed to produce the non-Mutator primer may be a limitation. The cosegregating DNA fragments (likely, a region of the mutated gene) are readily cloned, sequenced, and used to verify that the new mutation was indeed caused by the insertion of a Mutator transposon.

Analysis of the amplified DNA sequence might enable classification of the gene that has been interrupted and cloned (e.g., another serine kinase?). Analysis of the mutant and normal siblings reveals something about the role and function of that mutation of that gene in the context of that population in that environment. Additional functional and genetic evaluations (e.g., combining mutant alleles in one genotype and different genetic backgrounds) will be required but the subsequent activities benefit from the hints provided by the primary connection between the mutant and the molecule (the gene).

Perspective, Conclusions, and Questions

Understanding and meaningful manipulations of complex traits will require information from myriad sources. To do so, one must obtain unprecedented insight into the mediation of function and form in higher organisms. The maps and gene machines merely define the 'endpoints' of a nonlinear circuitry between DNA and the phenotype. The mysterious middle, all points between the ends, should prove to be a significant puzzle and an expensive challenge.

Given the likely magnitude and complexity of the challenge of molecular-based enhancements of drought tolerance in maize and wheat, one may wonder if the most effective strategies would involve some concessions and redirection. For example, the native systems for drought tolerance might be finely tuned to plant growth and development and external signals. Such highly integrated and sensitive systems might be recalcitrant to most simple manipulations. Also, one may wonder if such attempts to stabilize the production of such crops, as opposed to lower cost enhancements of other crop species actually reduces the stability of the regional crop production systems.

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Proteomic and Genetical Approach of Physiological and Molecular Responses to Drought in Maize

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Summary

The multiple responses of plants to water deprivation are polygenic traits, which can be analyzed with the modern tools of quantitative genetics. Molecular markers allow the easy mapping of QTLs, but their identification remains a tricky task. The genetic analysis of proteome variations induced by water stress provides new means to tackle this problem through the mapping of the PQL (Protein Quantity Loci).

Introduction

The proteome is an important concept for post-genomics studies, because it gives access to the translated part of the genome in any given developmental or environmental context. Not only can the proteins excised from two-dimensional gels can be characterized, but also their amount, which is commonly genetically variable, can be quantified using dedicated computer software. The loci controlling these variations (protein quantity loci, PQLs) may be mapped on the genome, which provides the geneticist with a tool for identifying Quantitative Trait Loci (QTLs). In the context of the candidate gene approach, a necessary (but not sufficient) condition for retaining a candidate is that a QTL for the quantity (PQL) and/or activity of its product is detected in the chromosomal region exhibiting the apparent colocation of the structural gene and the trait's QTL. Beyond, the PQL may help identify "candidate proteins," that is to say proteins whose PQL appear colocated with trait's QTL(s), regardless of the map location of the structural gene. Given the large confidence interval of PQL/QTL positions, colocations in a single region could just be due to fortuitous linkage. But the same associations observed on two (or more) different chromosomes would reveal possible physiological link between the protein and the trait. This strategy (de Vienne et al. 1999) was applied to agronomic and physiological traits responsive to mild water deprivation in maize.

Materials and Methods

Three-week-old plants of a maize line sensitive to drought ('Io', an American dent line from the Iodent group) and of a line tolerant to drought ('F2', a French flint line) were submitted to progressive water stress for 10 days. Changes induced in 6th-7th leaf proteins were studied by two-dimensional electrophoresis and quantitatively analyzed using image analysis. Induced proteins were excised from the gels for microsequencing of internal fragments.

One hundred individuals of the recombinant inbred line (RIL) population derived from the cross between these lines were characterized in the field for agronomic traits (yield, anthesis-silking interval [ASI], leaf senescence), in a greenhouse for physiological and morphological traits (photosynthesis, water status, carbon metabolism, abscisic acid [ABA] content, survival, growth during application of the stress), and for the amounts of individual proteins affected by water-stress. A genetic map of about 150 RFLP markers (Causse et al. 1996) was used for mapping PQLs and QTLs for agronomic or physiological traits.

Results and Conclusions

Seventy-eight proteins out of a total of 413 showed a significant quantitative variation (increase or decrease) under mild water stress, with 38 of them exhibiting a different expression in the two genotypes. Microsequences of 19 induced proteins allowed the identification, or tentative identification, of 16 of them (Riccardi et al. 1998), among which ASR1, an ABA/water stress/ripening related protein (Iusem et al. 1993), increased in 'Io' but was not detected in 'F2'.

One to 5 PQLs were found for 47 induced or repressed proteins, some of them displaying apparent mapping correlations with QTLs that could be physiologically relevant. The RFLP observed with the cDNA of ASR1 (the maize cDNA was isolated by M. Hoefer et al., manuscript in preparation) cosegregated with the presence-absence of the protein. This locus mapped on chromosome 10, in a region exhibiting a QTL for leaf senescence and a QTL for ASI (Fig. 1). The latter traits were highly correlated in the progeny ($r = 0.58$, $P < 0.0001$). The result suggests that the polymorphism of the structural gene of ASR1 would be responsible for the presence-absence variation of the protein, which in turn would pleiotropically affect the other responsive traits. Experiments are in progress to verify this hypothesis.

Another drought-induced protein (P71, pI 5.5 and Mr 39 kDa), the sequence of which was not determined, had three PQLs with apparent colocation with QTLs of growth under stress on chromosome 1, 4 and 8 (Fig. 2). The high-alleles of PQLs are associated with the low-alleles for growth, i.e., the more abundant the protein, the more the growth is reduced during the stress. Further experiments are needed to confirm the role of this protein in the trait variation and that of other induced proteins. In this connection, it is worth noting that the quantity of a protein is only one component of the protein efficiency. QTLs for enzyme activity, which integrates both quantity and specific activity, can also be used as tools for candidate gene/protein validation (Causse et al. 1995; Prioul et al. 1997).

Complementary experiments were performed to better characterize the proteome responses to drought:

- The rate of protein induction and ABA content have been followed over time in the parental lines This experiment revealed that some genotypic differences in induction were related to differences in ABA content (Fig. 3).
- Comparisons between roots and leaves revealed that some proteins are organ-specifically induced
- The expression of proteins were studied along growing leaves in order to detect possible differences due to the state of differentiation of the tissue. Many proteins were found to be regulated by differentiation, including those induced by water deficit. For example, the position of the peak of caffeate O-methyl transferase quantity followed the displacement of the elongating zone.

We are currently studying the protein response to drought in leaves and kernels of plants grown in the fields. In the near future, an important effort on proteome

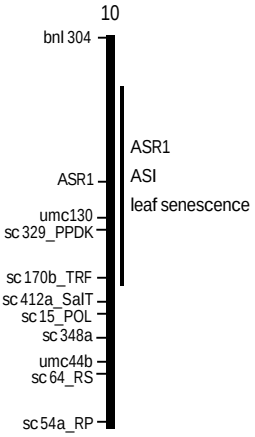

Figure 1. Apparent co-locations of ASR1 structural gene, ASR1 PQL, and QTLs for ASI and leaf senescence on chromosome 10.

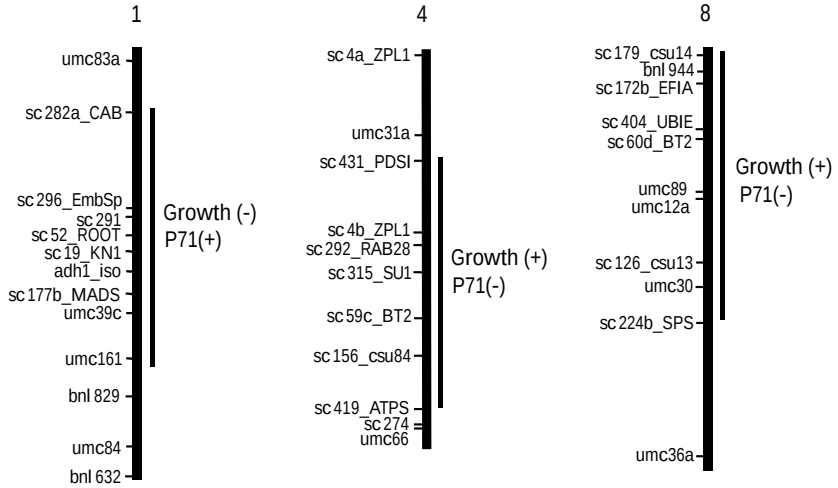

Figure 2. Apparent co-locations of PQLs for protein P71 and QTLs for growth upon drought. (+) and (-) refer to the high and low alleles from the parental line 'Io'.

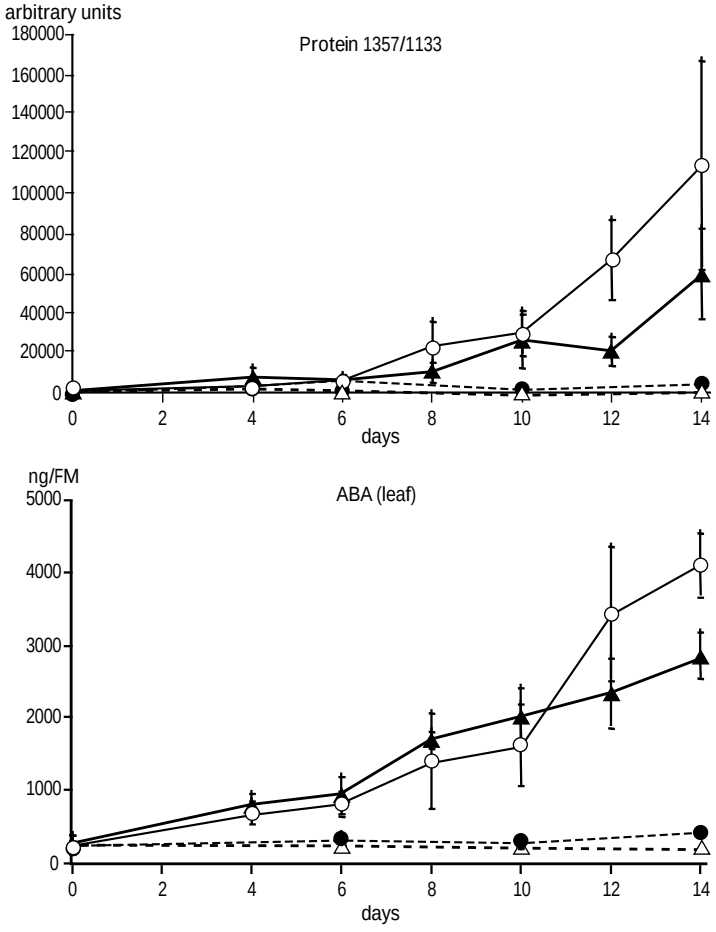

Figure 3. Comparison between the kinetics of leaf ABA content and the kinetics of protein 1357/1133 quantity in F2 (triangles) and Io (circles). Solid lines: droughted plants; dotted lines: controls. Proteins 1133 and 1357 are allelic forms of the protein. This unidentified protein has been shown to be induced by exogen ABA (data not shown).

characterization will allow a better understanding of the relations between protein expression and variation of traits of interest.

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Utilizing New Technologies to Investigate Drought Tolerance in Maize: A Perspective from Industry

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Drought induced yield losses can be substantial and researchers have been attempting to improve the tolerance of crops to limiting supplies of water for decades. Physiologists, breeders, biochemists, agronomists, and molecular biologists have all used specific tools from their disciplines to unravel the complexities of the drought response. Their efforts have resulted in improved knowledge of drought tolerance; however, predictable improvement remains elusive. We believe that one key to improving drought tolerance is the identification of critical genes whose expression controls the plant phenotype. This effort has been aided recently by the identification of chromosomal segments associated with drought tolerance via quantitative trait loci (QTL) analysis. Unfortunately, large numbers of genes fall within these chromosomal segments and the identification of key genes within QTL responsible for drought tolerance have not yet been identified. With the advent of genomic technologies, it is now possible to efficiently analyze thousands of genes simultaneously and, hopefully, identify drought responsive genes in maize.

Genomic technologies can be divided into structural and functional categories. Structural genomics entails some aspect of sequencing genomes and this activity has been ongoing for some time in many species, including maize. At Pioneer, we have been sequencing the maize genome since 1996, and we currently have over 200,000 ESTs in our database, which we estimate represents 60% of the genes in the genome. We are applying these sequenced genes to various functional genomic tools. Functional genomics involves using various technology platforms to determine transcript levels of sequenced genes. In our case, we are using these tools to better understand the molecular mechanisms of maize plants growing under water deficits. We are using both open and closed expression profiling technologies. By definition, an open system allows one to survey all transcripts and compare their levels between two different RNA pools, but the identity of the genes may not be known a priori. In contrast, with the closed system one can analyze only those genes that one has isolated a priori; however, once the analysis is complete the genes showing differential expression are immediately know.

The open system we are using is a gel-based technology that was developed by the CuraGen Corporation (http://www.curagen.com/index.html). We are using this technology to compare RNA pools between droughted and well-watered tissues. RNA is extracted from these two tissues and is converted to cDNA. The cDNA is fragmented and adapters are ligated to the DNA ends. A fluorescent dye is bonded to one of the adapters, which allows the quantification of gene fragment levels. The DNA then goes through several rounds of amplification, and the gene fragments are separated on a proprietary gel system, which can precisely differentiate the size of fragments. The gel is then scanned and the following information is acquired: (1) the size of the fragment, and (2) the amount of fluorescence emitted from each fragment. These fragment sizes are then compared against a virtual digestion of the Pioneer maize EST database and candidate genes are identified for individual fragments. The relative changes in transcript levels between

droughted and well-watered tissue are expressed as an N-fold increase or decrease, which is based on the amount of fluorescence detected in the image analysis step.

We are currently using two closed systems at Pioneer, the first of which was developed by Affymetrix Inc. (http://www.affx.com/index.html) and is a high-density array of oligonucleotides bound to a glass slide (chip). Pioneer is currently testing a prototype chip that contains 1,500 maize genes. These genes represent housekeeping, secondary metabolism, defense related, and several unknown ESTs. There are 20 paired oligonucleotides per gene and these consist of 20 perfectly matched oligonucleotides and 20 oligonucleotides containing a one base pair mismatch. cRNAs synthesized from the drought and well-watered samples are labeled with biotin and these are allowed to hybridized to the oligonucleotides on the chip. The chip is stained with a streptavidin/phycoerythin conjugate and differences in fluorescence from the perfect and mismatched oligonucleotides are then used to assess the fold change in steady state transcript levels between droughted and well-watered tissues.

The second closed system is from MD/Amersham (http://www.mdyn.com/); with their microarray one can analyze 1,500 genes (at 2 reps) per microscope slide. Unlike the Affymetrix system, actual maize DNA from our EST sequencing project is spotted on specially treated slides. Initially, we developed a slide that contains maize genes involved in carbohydrate metabolism. Similar to the Affymetrix system, RNA is extracted from two different tissues and converted into cDNA. The cDNA is labeled with either Cy3 or Cy5 dyes and is amplified via PCR. The labeled cDNA is then allowed to hybridize with the spotted DNA. The slides are placed in an image analysis scanner and the amount of fluorescence given off from each spot is quantified. This value is then used to calculate the message level of a particular gene in droughted versus well-watered tissues.

Upon identification of drought responsive genes in maize, they are mapped and compared with known drought QTL as well as map positions of known mutants. In addition, reverse genetic technologies such as the Trait Utility System for Corn can be used to assess the importance of candidate genes. It is our belief that the use of genomic technologies will enable us to identify drought responsive genes and that this information will in turn allow us to predictably improve drought tolerance in maize.

Cataloging Stress-Inducible Genes and Pathways Leading to Stress Tolerance

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Summary

We have chosen a molecular approach to investigate the reactions plants use to attenuate or ameliorate osmotic stress. We ask several questions of increasing complexity: (i) how many osmotic stress-responding genes might be contained in plant genomes? (ii) what types of genes constitute the stress response? and (iii) where and how strongly are these genes expressed. Because of recent technological advancements and breakthroughs in genome analyses meaningful answers to such questions have become possible (Bouchez and Höfte 1998). We have begun working on a project designed to detect the number and nature of all transcripts that respond to salinity and drought stress in higher plants. Main components of the project are mutant generation, the analysis of several non-plant models, and the large-scale determination of osmotic stress-regulated transcripts. DNA sequencing of approximately 10,000 ESTs each from two halotolerant (Mesembryanthemum, Dunaliella) and two salt-sensitive (Arabidopsis and Oryza) species is underway. The combination of transcript sequencing, microarray analysis, and bioinformatics will result in defining the set of genes for osmotic stress responses common to all plants.

Introduction

Although the rate of increase in world population declines, the mass of humanity and the desire to live well will require increased food production. This current cycle of Malthusian prophesy can either be viewed as yet another problem that needs to be solved or as an insurmountable problem that finally has exhausted our capacity to respond. Whatever the scenario, the competition between agriculture and the space and energy needed to equally feed, house, and wash all humans is obvious. In addition to population increases destroying tillable land and polluting our resources, there is increasing competition between humankind, feedstock, and crop plants for fresh water. This is not an insignificant problem because fresh water is a limited commodity that is unequally distributed across the planet.

One way of solving the problem would be to breed varieties of crop plants that can be grown on marginal soils with limited water supply or water with low osmotic potential (saline soils). Naturally drought- and salt-tolerant bacteria, fungi, algae, and higher plants already exist, indicating that the genes exist in organisms to cope with low osmotic potential environments. Drought tolerance breeding has met with some success, as contributions published in these proceedings indicate, but progress in salinity stress tolerance breeding has remained elusive (Flowers and Yeo 1995). We have argued before (Bohnert and Jensen 1996) for renewed attention to experiments that provide mechanistic understanding of the term tolerance. To be sure, for successful application it is not necessary that we understand the mechanisms and the functions of the genes that need to be expressed to make a plant stress tolerant. However, understanding these mechanisms would provide a new addition to the breeder's toolbox in the form of genes for pathways. Marker-assisted breeding could become more precise, QTL designations could be more precise, and transgenic lines could be designed for integration into breeding programs. In addition, genome-wide expression patterns can now be analyzed (Bouchez and Höfte 1998; Walbot 1999). For this reason, a

group of seven biologists from three universities (Arizona, Oklahoma State, and Purdue) designed a program on the "Functional Genomics of Plant Stress Tolerance", which has been funded by the National Science Foundation (Plant Genome Initiative) and is now in the middle of its first year. We will outline the program briefly and indicate the first conclusions from this work in the sub-project on EST-sequencing of stress-induced transcripts.

Transcript Induction

How many genes are induced and how many transcripts increase following stress? Is there a temporal order that may reflect physiological necessities? What is the extent of tissue- or organ-specific transcription? Is the induction of transcripts different in immature plants when compared to flowering plants? We are trying to answer these questions by sequencing clones from a variety of cDNA libraries from the halophytic and glycophytic models.

The rationale for much of this work comes from a comparison of randomly sequenced transcripts from stressed and unstressed *Mesembryanthemum crystallinum* (common ice plant) leaves. We detected a significant difference between the transcript populations with respect to sequences that were unknown. While less than 20% of the sequences obtained from unstressed plants belonged into this category, more than 35% of the sequences from stressed plants had not previously been found in other plants or organisms. These numbers decline as more and more sequences become available, but the difference remains between conditions. Based on numbers, assuming an average of 30,000 genes in higher plant genomes, the percentage of unknown sequences detected indicates that approximately 2,000 to 3,000 (6-10%) transcripts may be expressed only in plants that experience osmotic stress. Approximately the same percentage of yeast genes is induced by salinity stress (Yale et al. 1999).

For example, 12 cDNA libraries from Mesembryanthemum will ultimately provide approximately 15,000 sequences. All libraries have been constructed with RNA from plants that were stressed by the addition of NaCl (between 200 mM for seedlings and 500 mM for adult plants) for different time periods (1 hour to 5 days) and different tissues were harvested (including roots, leaves, stems, flowers, meristems, epidermal cells, and developing seeds). Procedurally, for some libraries we have sequenced between 500 and 1,000 clones at random and then will sequence another 500 to 1,000 clones after eliminating all those transcripts that were already obtained in the first random selection. We will eventually do this for all libraries. In this way, we can assure that transcripts of low abundance will not be missing.

The differences between the stressed and unstressed states are best documented by considering transcript abundance. Stressed plants execute a completely different transcription program during osmotic stress. Within the 15 most abundant mRNAs from unstressed plants, the list is dominated by transcripts for Rubisco subunit proteins, carbonic anhydrase, and transcripts for components of the photosystems of chloroplasts (8 of 15). There are two functionally unknown proteins in the top 15 from unstressed plants, both of which have been found in other plants before. In contrast, the transcript population after 30 h of salt stress is completely different. Only three transcripts appear that are also abundant in unstressed plants. Instead, there are now six unknown proteins among the top 15 and two of those are completely novel, i.e., they have never been encountered among bacterial, yeast, *C. elegans*, plant, or mammalian sequences.

A Case Study—Rice

Rice is a salt-sensitive crop species, and soil salinity in irrigated land is a primary factor in depressing yields of rice production. Plant geneticists have been able to compensate partially for this loss by breeding for increased salt tolerance in commercial varieties of rice, but the mechanism by which tolerance is conferred remains unclear. Until now, many rice cultivars have been investigated for salt-sensitivity. For example the Indica varieties Pokkali, Nona Bokra, and Bura Rata are low-yielding but tolerant to salinity stress whereas the IR29, M-1-48, and Taichung are sensitive, high-yielding cultivars. Genetic studies revealed that salt tolerance of these varieties is principally due to additive gene effects, however, the underlying molecular mechanism for their salt tolerance has barely been investigated. In recent years, some of the salt-induced proteins, which were produced by the salt tolerant species, were identified by two-dimensional electrophoresis (Claes et al. 1990; Moons et al. 1995, 1997; Ramani et al.

1997; Singla et al. 1998). More than 35 polypeptides have been detected in this category. Prominent rice proteins responsive to salinity stress are the SalT, Lea (GroupII, III), HSP100 family, and OSR40 proteins, active oxygen scavengers such as SOD and ascorbate peroxidase, betaine aldehyde dehydrogenase, and several other novel and functionally unknown proteins. Technical advances in 2-D protein analysis makes it easy to identify accumulated proteins at specific stages of salinity stress, but mechanisms of metabolic changes and genetic regulation under salinity conditions remain unclear.

Our goal is to understand the molecular and genetic control of gene expression under high salinity conditions. With cDNA libraries from several varieties, both Indica and Japonica varieties, and with a major focus on the moderately salt-tolerant Indica line Pokkali, we will generate 10,000 ESTs. DNA microarrays containing virtually every stress-regulated transcript of salinity-tolerant seedlings of Pokkali will be made for a comprehensive investigation of the temporal program of gene expression accompanying the metabolic shift from normal growth to salinity-stress. These arrays will also be useful for the analysis of stress responses between salt-tolerant and salt-sensitive varieties.

Corn—Transcripts and Microarrays

Corn cDNA libraries have been generated from stressed roots and shoots/leaves from immature two-week-old plants. The sequencing of a representative number of transcripts is underway, and sequences have been deposited on the corn database (http://www.zmdb.iastate.edu/) and in dbEST. A preliminary analysis of the first go-around of sequencing by the Stanford group (Dr. Virginia Walbot) provides some indications about osmotic stress responses in maize leaf and root tissues.

In shoots/ leaves from stressed maize plants, transcripts for photosynthesis-related functions and metabolism connected to carbon fixation and nitrogen metabolism are found, but they do not constitute a major fraction of the transcripts. Instead, the number of unknown sequences (18 out of 66; i.e., approximately 26%) was surprisingly high considering how much sequence information on corn and other grass species is already contained in the databases. In some instances this may be due to sequences only covering the 3' ends of transcripts. Another seven transcripts from this first population (i.e., approximately 10%) showed homologies with functionally unknown proteins from cyanobacteria, insects, or other plants. The surprising complexity and the surprisingly high amount of novel sequences in leaves in such a small sample population was also reflected in root sequences. Twenty (out of 71, i.e., approximately 28%) sequences included functionally unknown protein coding regions, five of which were novel while the others had been found before in other organisms. Although a more in-depth analysis will have to wait for a larger dataset, several features characterize the sequences that have come from this preliminary set. A number of sequences are present for functions that we think reveal stress responses. These sequences are for scavengers of radical oxygen species, for chaperones, protein turnover, for typical defense proteins such as osmotin, and for functions that may alter transcription, such as transcription factors. The value of these sequences will become fully appreciated, however, only when the next step in our project is included, microarray analysis of the cDNAs.

A Case Study—Changes in Meristematic Activity Following Stress

Two directionally cloned cDNA libraries from meristems and leaf primordia have been generated. One library was derived from RNA from five-week-old Mesembryanthemum grown without salt stress, and a second library was made from six-week-old plants grown for three days in the presence of 500 mM NaCl. As a progress report, more than 1,000 cDNA clones have been entered into the EST-sequencing pipeline. First results for the stressed library give insights into the expression pattern of meristem under salt stressed conditions by functionally classifying the transcripts. The main categories include functions in photosynthesis, general metabolism, and protein modification. Unknown sequences amount to approximately 18%. As a surprising finding, the most abundant sequence (5% of all ESTs) in stressed plants was a novel chlorophyll a/b-binding protein that is more divergent from all other ice plant CABs than those are from each other. From the analysis of these clones we expect clues about gene expression programs that enable Mesembryanthemum to continue growth of the meristems under severe osmotic stress condition.

How Many Up-regulated Genes?

The most advanced work is on the stress-induced transcript complement of *Mesembryanthemum*. This halophyte has become the best-studied higher plant model for tolerance-conferring mechanisms following salinity stress and drought (Adams et al. 1998). With the EST sequencing portion of the work well underway, the focus will soon shift to microarray analysis of sequences. Preliminary data (arrays for approximately 1,000 ESTs,) indicate that from among the randomly picked sequences present in cDNA libraries from stressed plants, approximately 15 to 20% are for up-regulated transcripts.

Based on preliminary analyses the categories of genes that are up-regulated indicate that photosynthesis-related functions in particular decrease, functions of photorespiration, protein turnover, chaperones, oxygen radicals scavenging, and defense-related proteins (PRP) increase, with drastic accumulations for some transcripts. We will present examples during the conference.

Models for Studying Osmotic Stress Tolerance——Yeast

Included in the project is the analysis of model species, *Saccharomyces cerevisiae*, *Aspergillus nidulans,* and *Synechocystis* PCC6803. Data are available from work with baker's yeast (Yale et al. 1999; Matsumoto et al. 1999). Yeast is well established as a model for plant-specific reactions for a number of reasons (Nelson et al. 1998), the most important one for our consideration being the availability of microarrayed coding regions of the entire genome. It is also an excellent model for studying and understanding the elements of cellular tolerance to osmotic stress.

We have used yeast microarrays to find gene expression modulations during salinity and high temperature stress (Yale et al. 1999). In addition, microarray experiments have been done comparing wild type to mutants in which two important signal transduction pathways are disrupted (Matsumoto et al. 1999). The HOG and calcineurin signal transduction pathways control aspects of the osmotic and ionic stress response in yeast. Mutants in either one of the two cannot induce all stress responses — unless crosstalk exists between different pathways. We can compare our results with those of others interested in responses to elevated levels of H_2O_2 (oxygen radical stress) (Godon et al. 1998). Among the approximately 6,000 coding regions that make up the yeast genome, about 10% are strongly affected by osmotic stress and respond by either up or down-regulation. We are still in the process of analyzing the large sets of data that result from such experiments, but a preliminary summary is possible. Most up-regulated transcripts are in energy production, cellular defense and transport facilitation. The latter category does not simply include sodium transport proteins, but a large number of other transporters, possibly indicating severely altered intracellular protein and metabolite trafficking. Another category is also over-represented (i.e., more than the average 10% of transcripts in a group). This group includes ORFs for proteins (or putative proteins) of unknown function. We found more than 100 transcripts in this category are up-regulated. A detailed account of these data is being prepared at the moment (Yale et al. 1999).

Information Management

Possibly the greatest challenge to making sense out of sequence and microarray data lies in setting up intelligent dissemination and bioinformatics tools. The dogma of large-scale DNA sequencing and gene discovery projects is to process EST sequencing data through "interpreters," "assemblers," and "comparers." Translating sequencing data, matching overlaps into "contigs," and finally comparing overlapping contigs as well as nonoverlapping "singlets" to a database of well-defined genes or transcripts using the BLAST algorithm are the main tasks. This process generates a wealth of data that may be further scrutinized using an ever-growing number of programs. These are built to detect similarities potentially missed by a first comparison, to find features known to be similar or conserved in DNA or deduced protein sequences as functional units or domains that have been defined by other studies. Using these programs in concert, personalized interfaces may be created to arrive at useful solutions for a particular lab.

Our group is developing an automated BLAST output-parsing tool that will prepare alignment output for import into 3rd party database software using macros created with Visual Basic for Applications™. Similar tools will be created to extract necessary information from all steps of the sequence analysis process. With this

database we will be able to service our requirements: evaluate contig quality and alignment significance, to score transcript abundance, to identify domains in unknown sequences, and to correlate the sequences with their behavior as described by microarray analysis experiments. Ultimately, this automated process should proceed until expression patterns in transcript behavior become discernible. At this stage, input by trained investigators becomes paramount.

Perspectives

Inclusion of genome and chromosome mapping data, application of marker assisted breeding, and tracing of QTL assignments are now trademark tools to help in the generation of crop plants for characters that are multigenic. Osmotic stress resistance of plants, including drought, low temperature, and high salinity, is certainly a multigenic trait, but we would like to add some considerations that qualify this statement.

Over the past ten years, views about the principles of osmotic stress resistance have changed. In the 1980s, multigenic characters were sought in a multitude of individual biochemical reactions that translated into physiological, phenotypical characters. A number of the characters/ mechanisms have been listed (Figure 1). Tolerant species, it was believed, had "better" enzymes, pathways, or water, nutrient, and ion transport processes. This translated into higher water-use-efficiency. There is now enough evidence indicating that metabolite synthesis and, to some degree, metabolite accumulation, scavenging of reactive oxygen species (in particular hydroxyl radicals), and control over sodium uptake, excretion, or storage, AND control over water uptake or loss are important mechanisms. There are others, represented by the synthesis of, for example, LEA proteins and there will be additional mechanisms (many of which will be family- or species-specific). Then, the detection of a multitude of signaling processes in animal systems and yeast brought about a reinterpretation of many plant-specific reactions in terms and in language of signal recognition and transduction. Stress tolerance, consequently, became a matter of signal transduction chains. Tolerance indicated how well the pathways from signal recognition to biochemical responses were developed in different plants, and how they were wired for crosstalk with those pathways that signal development, flowering, and/or senescence.

Expression data alone cannot provide the important information about essential or even ancillary genes. Changes in transcript amounts, however, represent the most parsimonious approach towards what might be important. In order to understand mechanisms, functional analysis must be done. With understanding, it might be possible to start designing completely new pathways and completely new crop plants, but such dreams are far in the future. At present and for the near future, the information from a stress genomics

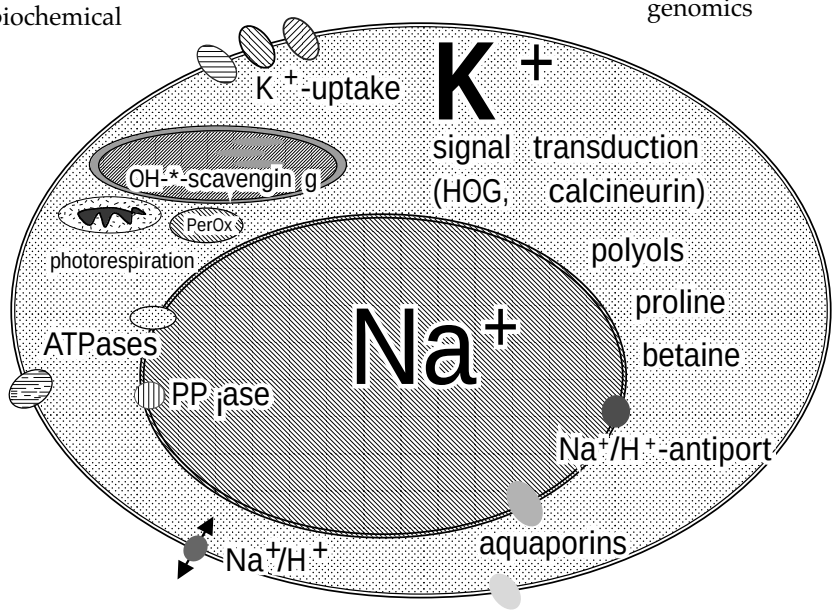

Figure 1: Essential aspects of cellular salinity stress tolerance.
The schematic drawing of a cell includes biochemical pathways that have been shown to contribute to enhanced salinity (and drought) tolerance. Important are different intracellular location for potassium and sodium, and preferential uptake of potassium over sodium — or export of sodium that enters the cells (Nelson et al. 1998; Zhu et al. 1998). The latter is a questionable strategy because sodium excreted by one cell will generate problems for a neighboring cell in land plants, but it is a successful strategy for algae and yeasts. Additional biochemical activities are control over aquaporin amount and location, lowering of the internal osmotic potential by increased metabolite amounts, and protection against radical oxygen species. Signal transduction and the processing of information leading to changes in the regulation of many genes are likely wired through pathways that are homologues of the yeast HOG and calcineurin signaling pathways (Li and Zhu 1998; Nelson et al. 1998). Additional materials will be made available during the conference.

project—integrated with marker assisted, mutant-based, OTL-integrated information, can be used for making predictions about which characters should be searched in breeding programs. Among the characters, which seem to result from the preliminary analyses, the most important pointers towards tolerance acquisition seem to be in several different pathways. These are radical oxygen scavenging, enhanced photorespiration (in C3 species) with a possibility to convert carbon into compatible solutes, control over water uptake, sodium/potassium discrimination (in salinity stress), and—possibly—efficient synthesis of chaperones.

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Computer Simulation Linked to Gene Information Databases as a Strategic Research Tool to Evaluate Molecular Approaches for Genetic Improvement of Crops

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Summary

The role of computer simulation tools linked to information management systems for data generated from analysis of genotype-environment systems is discussed as an enabling technology to evaluate alternative molecular-conventional breeding strategies. There is a growing need for such a strategic research approach as we improve our understanding of the relationships between gene and phenotype for important traits targeted in breeding programs. A proposed decision support system that can be tailored for individual breeding programs is described and discussed in relation to collaborative work between Australian and CIMMYT wheat breeding programs.

Introduction

Today, a wide range of molecular tools and powerful computers provides us with an unprecedented capacity to generate data that reveals the architecture of genomes, genes, traits, and how these contribute to the value of the germplasm units (populations, lines, varieties, hybrids, cultivars) used in breeding programs. Complementary work is characterising the key environmental variables that influence the expression and value of genes and improving our understanding of the composition and structure of the Target Populations of Environments (TPE) faced by breeding programs. Combining these two avenues of research provides a foundation for understanding gene function and expression and definition of the value of the alternative alleles of genes within the context of our reference breeding populations and target genotype-environment systems. The availability of these data, in appropriate information management systems, promises opportunities for accurate, rapid multi-gene manipulation strategies, with greater precision than previously achievable. The processes for generation of these gene-environment data are critical to the design and implementation of optimised molecular-conventional breeding strategies that build on the successes of our current suite of strategies. However, the data generation processes by themselves are insufficient to enable the implementation of the range of possible molecular breeding strategies. Mature breeding programs examining the potential of molecular tools to assist in achieving breeding objectives have proceeded beyond the phase of concentrating solely on data generation. There is recognition of the need to link the growing body of data on the value of alternative alleles with the germplasm units used within breeding programs. Without a link between the data generation processes and the units of manipulation in the breeding program the data are not accessible as real-time information to assist the breeder with cross and selection decisions. The large private sector breeding programs are well advanced compared to their public domain counterparts in this bioinformatics research and development area.

As our understanding of the genetic networks that control variation for traits advances, there is a growing realisation that we are often faced with the task of manipulating complex networks of genes that involve genotype-by-environment

(G×E) interactions and also epistatic interactions between the genes regulating variation for the traits. Based on our work on yield improvement of wheat in the rainfed system in northeast Australia, both G×E interactions and epistatic components of genetic variation are frequently encountered in experimental studies (e.g., Cooper et al., this volume). To achieve many of our stated breeding objectives, including the improvement of drought resistance, we need to be able to work with genetic networks ranging from simple to complex. Quantitative genetic theory provides one approach for making predictions about the likely outcomes from applying particular breeding strategies using knowledge of the inheritance of the target traits. However, it is widely recognised that this theoretical framework makes many simplifying assumptions to enable the construction of the prediction equations for breeding programs. Kempthorne (1988), in his review of the state of the quantitative genetics framework, made the case for the use of high-speed computers to investigate a wider range of genetic models. Clearly, to evaluate breeding strategies we need relevant genetic models and data on the relative value of alleles. Investigations linking data on genetic variation at the molecular and phenotypic levels for adaptation and performance traits provide an exploding information base that can be used to guide these investigations. We are using computer simulation to complement and extend the predictions from quantitative genetic theory in a way that deals with more complex genetic networks and genotype-environment systems than are often assumed in the theoretical models and their derivations. The simulation platform we have developed for this purpose is QU-GENE (QUantitative GENEtics; Podlich and Cooper 1998).

Decision Support Systems for Breeding Programs

In collaboration with a number of groups around the world we are investigating the opportunities that exist to develop decision support systems for plant breeding programs in major cereal crops. These programs have established breeding procedures that have, and are expected to continue to produce improved cultivars and hybrids for their target genotype-environment systems. However, for a range of reasons, these programs need to increase their efficiency and success rate. The aims of these investigations have elements that are both strategic and tactical, including (1) optimising resource allocation within existing breeding strategies; (2) evaluating the opportunities to use molecular marker assisted selection (MAS) to improve on the effectiveness of the current strategies; (3) identifying benchmarks for technology development that are required to realise an advantage from alternative breeding strategies (e.g., MAS); and (4) assisting real-time cross and selection decisions within the operational limitations of the breeding program. Some of the background to components of this research has been discussed elsewhere (e.g., Cooper and Hammer 1996; Podlich and Cooper 1998; Cooper and Podlich 1999). The objective of this paper is to give an overview of the components of such a decision support system, using current collaborations with the CIMMYT wheat breeding program as an example to demonstrate some of the processes being undertaken. In this example, the potential for benefits to the Australian and CIMMYT wheat breeding efforts are being examined. Comparable research programs, at different stages of development, are underway for rainfed lowland rice in Asia (Fukai et al. 1997; Fukai and Cooper 1999; Cooper et al. 1999) and grain sorghum in Australia (Cooper and Chapman 1996; Borrell et al., this volume).

Figure 1 is a schematic representation of a cyclical process we are considering as a decision support system for a breeding program. There are three major components: (1) the data generation processes used to characterise the genotype-environment system; (2) the information management system that links the data generation processes with the germplasm units of the operational breeding program; and (3) the QU-GENE simulation platform used to examine the operational breeding program and quantify the merit of alternative breeding strategies and refinements to the existing strategies.

Genotype-Environment System Characterisation

Processes contributing to the characterisation of a genotype-environment system include research programs conducted to (1) determine the types of environments within the TPE that influence expression of genetic variation and G×E interactions, and quantify their frequency of occurrence and spatial and temporal distributions; (2) develop Geographical Information

Systems (GIS) methodology that integrates the environmental characterisation data to generate a representation of the TPE; (3) determine the traits, genes or Quantitative Trait Loci (QTL) required for adaptation to the different types of target environments; (4) define the combinations of traits required for improved performance in the TPE; (5) characterise the architecture of the traits, their relative value and the function of alternative alleles of genes and detected QTLs; and (6) determine the distribution of the favourable alleles for the required traits among the germplasm units available to the breeding program. Versions of these activities are common to many of the inheritance and adaptation studies reported in the literature and also discussed at this workshop. A common representation of the outcomes from these studies is the

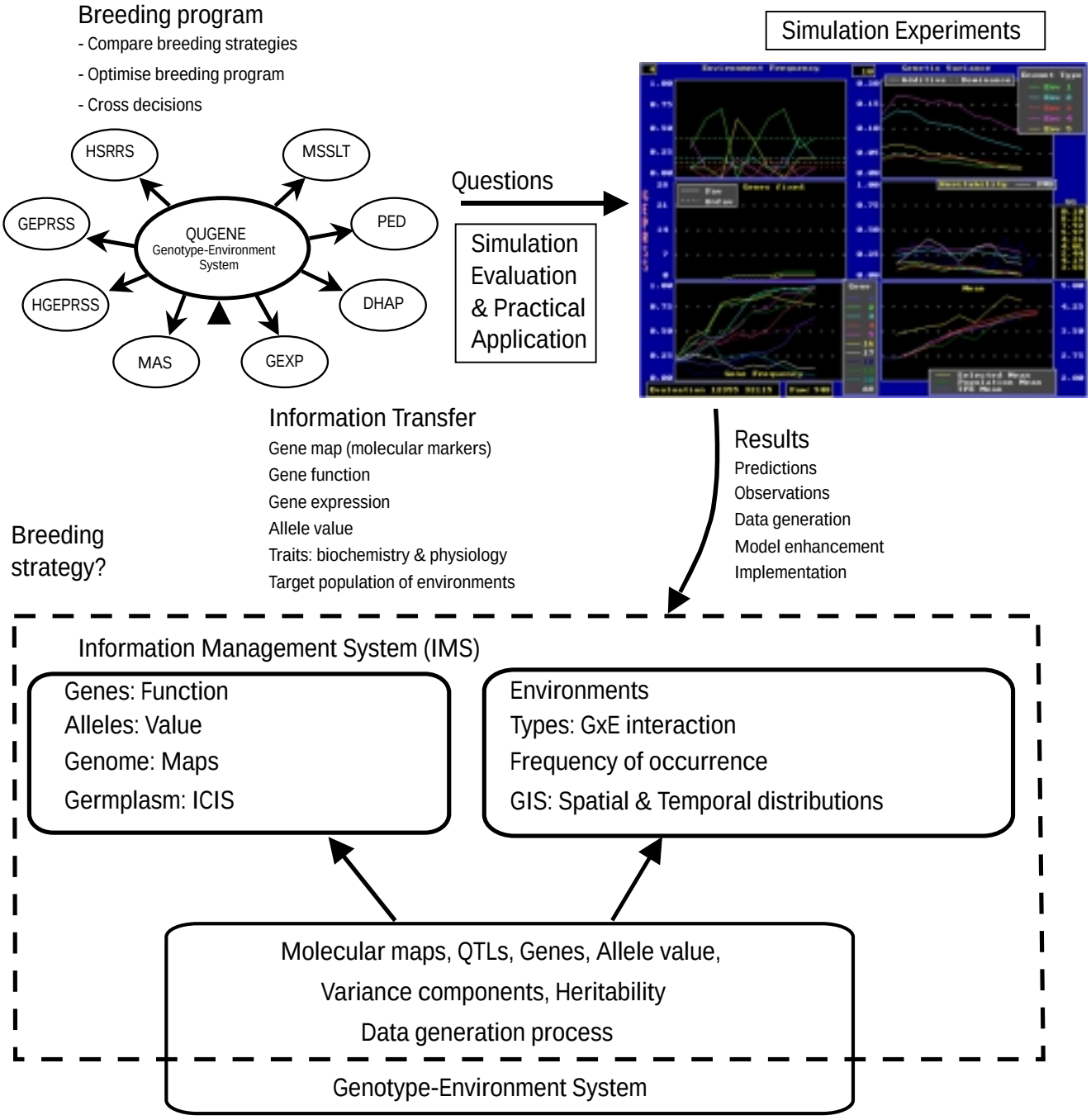

Figure 1. Schematic representation of the components of a decision support system for a plant breeding program. The central ellipse of the QU-GENE simulation platform represents the engine and the surrounding ellipses represent alternative application modules that simulate the breeding programs.

construction of a consensus molecular marker map and the localisation of QTLs for the traits on the map, together with a quantification of the relative value of allelic variants. Progression of this work to a stage where breeding programs can take advantage of the data on allele value for important genes and QTLs involves measures of the distribution of the favourable alleles among the germplasm units used by the breeding program.

Information Management System

The components of the information management system are database modules that link gene location, function, and allele value data with target environment characterisation data and the germplasm units used in the breeding program, together with the tools for querying the database. The importance and enormity of this task cannot be overstated. There are currently a range of database structures being used around the world to hold gene-based data for plants and animals. In Figure 1, the International Crop Information System (ICIS) is identified as the key component that can link the gene, gene value, and target environment data with the uniquely identified germplasm units used and manipulated in breeding programs. The unique identification of the germplasm units, documentation of their coancestry, and the association of gene information with these units are critical to practical implementation of any molecular-gene based breeding strategy. Given access to this database and capacity to query it, the aim of a decision support system for a breeding program is to identify efficient breeding strategies, based on the germplasm units, to generate new gene combinations (genotypes) that are closer to the target gene-trait combinations for a TPE. The information management system can be used to monitor the flow of genes through pedigree-based ancestral relationships and suggest likely pathways towards the desired new gene combinations. The ICIS initiative has already established protocols for unique identification of germplasm units and is working on procedures for linking molecular marker and gene maps with the allele variants for marker and gene loci possessed by the germplasm units.

QU-GENE Simulation Platform

While it may be possible to query the database through the information management system, the task of defining optimal, or at least efficient, breeding strategies is formidable. The complexity of deciding on preferred crosses to achieve the desired flow of genes towards a target genotype is not unlike that of the widely studied "Travelling Salesman Problem," with which the task is to find a minimal route to visit multiple cities (sales venues). Once a relatively moderate number of traits and genes are considered, a high level of combinatorial complexity emerges. In addition, practical implementation of the selection process in a breeding program deals with finite numbers of individuals (breeding units) and the stochastic nature of sampling variation in terms of both the genotypes generated and examined from crosses, and the environments in which the genotypes are evaluated. Quantitative genetic theory can provide some useful guidance on average expectations and general features of the merit of alternative strategies, but it has limited power to deal with many of the specific questions relevant to individual breeding programs. It is in this area that simulation tools, in combination with speed computing capacity, have a role in complementing theoretical predictions. QU-GENE was developed as a tool for such applications.

The architecture of QU-GENE is based on an engine that models the genotype-environment system and modules that represent the specific breeding strategies to be simulated (Figure 1). The engine integrates information on the genetic models proposed for multiple traits with the structure of the TPE to determine the relative values of alternative alleles and genotypes in relation to individual environment types and the TPE. This information is used to define a reference genotype-environment system that simulates the key features of the physical system. The core model within the engine can incorporate many of the features of the architecture of traits that are revealed by the genotype-environment system characterisation work described above. These include multiple traits and QTLs with different effects, genome positional information such as that provided by molecular maps, epistatic interactions within gene networks, differential gene expression and G×E interactions, and structure within the TPE. Molecular marker maps, based on recombination frequencies, QTL data, and gene data can be directly imported into the engine. Following definition of the key features of the genotype-environment system, the

engine generates a reference population of genotypes. The application modules, representing alternative breeding strategies, sample or select genotypes from the reference population to commence the simulation of the breeding strategies. The results of the simulations can be analysed to quantify the expected efficiency and power of the breeding strategy. A comparative approach can be adopted and breeding strategies can be compared for their relative efficiency in developing target genotypes. Cooper et al. (1999) discussed an example of this approach for comparisons between conventional phenotypic and marker-based selection strategies.

In collaboration with the ICIS group, we are investigating the potential to link the QU-GENE engine with ICIS as a basis for accessing genotype and gene specific information relevant to Australian and CIMMYT wheat breeding programs. Modules that simulate the breeding programs of the Northern Wheat Improvement Program are well advanced and are in the planning phase for the CIMMYT wheat breeding program. If this link can be achieved, given the multi-crop nature of ICIS and the modular structure of QU-GENE, the procedures developed for the current wheat-based work should be extendable to other crops.

Conclusions

While research programs generate data and promote our understanding of the traits and relevant alleles required to improve plant adaptation and crop performance in agricultural systems, there is a need to develop decision support tools that enable the implementation of breeding strategies that draw on the data generation process. We have described the components of a decision support system that we are working on. One of our highest priorities is the design of an efficient information management system that links gene and phenotype data in target environments with the germplasm units used in breeding programs. Computer simulation is being used as a tool to integrate the large body of data on QTLs, genes, and allele values available to breeding programs and answer questions posed by breeders. Here computer simulation is used to complement predictions from quantitative genetic theory in order to deal with more complex models and the specifics of individual breeding programs. Linking the QU-GENE simulation tool with ICIS is viewed as a critical step in the process of developing a decision support tool that will enable breeders to evaluate the merit of alternative cross and breeding strategies for their target environments and program specific germplasm pools.

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The Dehydrin Multigene Family in Triticeae and Maize

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Summary

One objective of our work has been to determine the map position of every member of the dehydrin (Dhn) gene family in barley and maize. The purpose is to provide a foundation for testing associations between alleles at specific Dhn loci and phenotypic variation related to drought and temperature responses. Dehydrins are a species of protein produced by plants during drought or low temperature stress. Eleven barley Dhn genes were identified, sequenced, and mapped (using gene-specific PCR) to chromosomes 3H, 4H, 5H, and 6H. Wheat Dhn genes were mapped to 4DS, 5BL, and 6AL using seed protein data from cytogenetic stocks. Six maize Dhn probes identified eight maize Dhn loci on chromosomes 1, 3, 4, 5, 6, and 9. Expression (determined by gene-specific RT-PCR) varied; some genes are induced by drought, others by low temperature. This variation, together with cross-hybridization between Dhn genes, highlights the necessity of gene-specific methods to study the Dhn multigene family.

Introduction

Dehydrins (DHNs, a.k.a. LEA D11) are one of the typical families of proteins that occur in plants as a consequence of dehydration, low temperature, osmotic stress, seed drying, and exposure to abscisic acid. Inheritance studies, including QTL analysis, in several crop plants have revealed apparent cosegregation of *Dhn* genes with phenotypes associated with dehydrative stress, such as drought and freezing. The first such observation was in barley (*Hordeum vulgare*), in which a cluster of *Dhn* loci (*Dhn*1/2 and *Dhn*4a) overlapped the major QTL for winterhardiness in a winter (Dicktoo) by spring (Morex) dihaploid mapping population (Pan et al. 1994) on chromosome 7(5H). Several additional instances have been summarized previously (Campbell and Close 1997).

DHNs are unified by the presence of one or more copies of a putative amphipathic a-helix-forming domain (the K-segment), which is highly conserved in higher and lower plants and has a 15 residue consensus sequence EKKGIMDKIKEKLPG. This and other distinct domains of DHNs, including a phosphorylatable (Vilardell et al. 1990) tract of Ser residues (the S-segment) and an N-terminal consensus sequence (the Y-segment), are pieced together in a consistent manner, interspersed by other lesser-conserved and usually repeated domains (the f-segments). The assembly of these domains into numerous, yet consistent, permutations has resulted in a range of DHN polypeptide lengths from 82 to 575 amino acid residues. The number of occurrences of the K-segment varies from one to 11 within a single polypeptide. The bulk of the DHN polypeptide in most cases contains regions or domains (f-segments) that are rich in Gly and polar amino acids (especially Thr) and are tandemly repeated between K-segments. But, there are contrary examples in which the f-segments located between the K-segments are rich in other amino acids or do not exist as tandem repeats. For example, the f-segments between K-segments in all SK_3 DHNs are not Gly-rich but in many cases are rich in Pro and Ala. Because of this distinction and the fact that the SK_3 and some other DHNs tend to contain high percentages of acidic residues, it has been proposed that the DHN family may contain a biochemically distinct acidic sub-group (Danyluk et al. 1994).

Immunolocalization and sub-cellular fractionation studies have established that DHNs can be present in the nucleus or cytoplasm (reviewed in Close 1997). Two studies have clarified the location of DHNs in the cytoplasm. The major embryo DHN of maize, which is a YSK_2 DHN, seems to be associated with a cytoplasmic endomembrane (Egerton-Warburton et al. 1997), while an acidic wheat DHN, which is an SK_3 type, is located in the vicinity of the plasma membrane (Danyluk et al. 1998). Most DHNs contain putative bipartite nuclear-targeting signal sequences (for example, Monroy et al. 1993; Godoy et al. 1994).

The predicted molecular weights of DHNs based on primary amino acid sequences are invariably less than their apparent molecular weight SDS-PAGE. This anomaly is also observed with DHNs translated in vitro (Close, unpublished); retarded migration thus seems to be due at least in part to secondary structure in 0.1% SDS. A ~35 kDa DHN of cowpea (*Vigna unguiculata*), which is associated with an increment of chilling tolerance during seedling emergence, has been shown to take on a-helical structure in the presence of SDS (Ismail et al. 1999). A simple interpretation of these observations is that DHNs are lipid-binding proteins. Possibly, DHNs and other LEA and COR proteins function in a lipoprotein environment at an interface between phospholipid bilayers and aqueous compartments. If so, then the effects of biochemical (representing allelic) variation could be manifested by a diversity of phenotypic consequences.

With this mechanistic intrigue as a background, we have made an effort to locate all of the *Dhn* genes in the Triticeae and maize genomes.

Materials and Methods

Initially, we produced lambda genomic libraries of Dicktoo and Morex barley and utilized a collection of Himalaya barley *Dhn* cDNA and genomic clones to isolate a collection of 56 positive *Dhn* clones. In 1998, a 6.3X Morex barley BAC library became available (http://www.genome.clemson.edu), and has also been utilized. We produced a genomic library of maize inbred Oh43 and from it identified a total of 15 *Dhn* clones by screening with a maize *Dhn1* cDNA. More recently, we have been using a maize BAC library (http://www.genome.clemson.edu) produced from inbred B73. Following the identification of *Dhn* positive clones, we employed techniques including restriction mapping, Southern blot hybridization, sub-cloning, and DNA sequencing to assemble the lambda clones into contigs and determine the number and identity of each *Dhn* gene. Gene-specific oligonucleotides have been developed for each sequenced *Dhn* gene. These oligonucleotides have been used for PCR-based genetic mapping using wheat-barley addition lines and a barley doubled haploid mapping population (Dicktoo x Morex). In maize we have employed a range of clones, oligonucleotides, immunological probes, and various mapping populations, particularly two RIL populations obtained from Benjamin Burr. When screening BAC libraries, we first identify *Dhn* positive clones by nucleic acid hybridization, then determine by gene-specific PCR which of the positive BAC clones carry previously identified *Dhn* genes. Finally, we sub-clone and sequence any new *Dhn* genes. A collection of 200 wheat (*Triticum aestivum* L. cv. Chinese Spring) cytogenetic stocks (nullisomic, tetrasomic, nulli-tetrasomic, ditelosomic and deletion lines, addition and substitution stocks from intra- and inter- specific crosses; from Adam Lukaszewski, Univeristy of California, Riverside) was utilized to determine the proteins encoded by some of the wheat and barley *Dhn* genes, using a seed protein Western blot procedure. Gene-specific RT-PCR have also been conducted in order to explore the expression of each barley or maize *Dhn* gene without noise from other *Dhn* genes.

Results

The *Dhn* clones present in Dicktoo barley lambda genomic libraries were sorted into 10 contigs containing 11 *Dhn* genes, all of which were sequenced in their entirety. The corresponding 11 Morex *Dhn* genes were also recovered and completely sequenced gene-specific oligonucleotides for *Dhn1* through *Dhn11* derived from their nucleotide sequences were used for genetic mapping by PCR and gene expression analysis by RT-PCR. The map locations (Figure 1) and other properties of barley *Dhn1* through *Dhn11* are summarized in Table I. In general, the *Dhn* genes are dispersed on four chromosomes, 3H, 4H, 5H, and 6H. Most of the *Dhn* genes are drought-induced, while two (*Dhn5* and *Dhn8*) are cold-induced. These results have been presented in more detail elsewhere (Choi et al 1999). In the 6.3 X Morex BAC library, additional *Dhn* genes have been discovered.

Proteins encoded by *Dhn* loci in chromosome arms 4DS, 5BL, and 6AL of Chinese Spring wheat were assigned. There was also evidence of a regulatory factor on 5B in the vicinity of the *Dhn* genes. These results have been presented in more detail elsewhere (Werner-Fraczek et al. 1998).

The approximate map locations of seven maize *Dhn* loci detected by five *Dhn* nucleic acid probes (Campbell and Close, unpublished data), *Dhn2* mapped using seed protein data (Campbell et al. 1998), and further information on the nature of each maize DHN protein, is given in Table 2. As with the barley *Dhn* genes, the majority are drought-induced while some are cold-induced (Campbell, unpublished data).

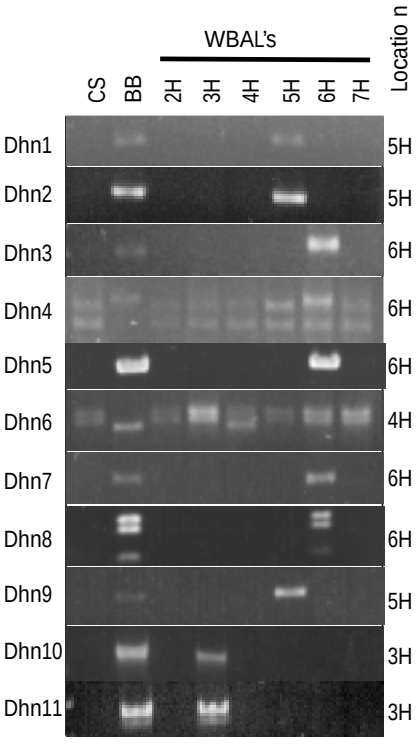

Figure 1. Chromosome assignment of 11 *Dhn* genes.
Genomic DNA from Chinese Spring wheat (CS), Betzes barley (BB) and six disomic barley chromosome addition lines (2H, 3H, 4H, 5H, 6H and 7H) were amplified with *Dhn* gene-specific primer sets as in Choi et al. 1999. Amplified DNAs were electrophoresed in a 1.2% agarose gel. The *Dhn8* gene-specific primer set amplified the same size DNA fragment from CS and BB genomes, but only the BB PCR product was digestible by *HinfI* (into three sub-fragments). Only the *HinfI*-digested *Dhn8* PCR products are shown.

Conclusions

Dhn genes are present as dispersed multigene families in the Triticeae and maize. In barley there are at least 12 *Dhn* genes, which implies that in hexaploid wheat there are at least three times this number. In maize there are at least 8 *Dhn* loci. At low hybridization stringency all of the *Dhn* genes cross-hybridize with each other. Given the presence of numerous *Dhn* cDNA and EST sequences in databases from other well-studied plant systems, such as rice and *Arabidopsis*, it seems likely that the dispersed multigene nature of *Dhn* genes is universal to higher plants. The expression patterns of *Dhn* genes is highly variable from one *Dhn* gene to another. In barley, in which *Dhn* gene expression studies have been more comprehensive than in any other system, the majority of cases are strongly induced by drought, while others are induced principally by low temperature. It can be surmised from the literature and public databases that variation in *Dhn* gene expression also is consistent across higher plants. Given the dispersed nature of this gene family and the variation in expression characteristics, the inherent cross-hybridization of *Dhn* genes means that all techniques that rely on nucleic acid hybridization, particularly Southern blot hybridization and cDNA arrays, will provide imprecise information in regards to both map position and expression characteristics of specific *Dhn* genes. The imprecision of Southern blot and cDNA array data can potentially obscure subtle allelic differences at specific loci within multigene families when comparing genotypes, and

Table 1. Summary of barley (Dicktoo) *Dhn* genes

Gene	Location	Type	Amino Acids	kDa	Expression
Dhn1	5H	YSK_2	139	14.2	drought
Dhn2	5H	YSK_2	143	14.4	drought
Dhn3	6H	YSK_2	155	15.7	drought
Dhn4	6H	YSK_2	247	24.7	drought
Dhn5	6H	K_9	575	58.5	cold
Dhn6	4H	Y_2SK_3	502	47.6	drought
Dhn7	6H	YSK_2	181	18.1	drought
Dhn8	6H	SK_3	255	27.7	cold
Dhn9	5H	YSK_2	146	15.1	drought
Dhn10	3H	YSK_3	295	29.2	drought
Dhn11	3H	Y_2SK_2	232	23.5	drought

Table 2. Summary of maize *Dhn* genes

Gene	Location	Bin	Type	Amino Acids
Dhn1	6	6.05	YSK_2	168
Dhn2	9	9.02-9.03	?	?
Dhn3	4,5	4.05-4.06, 5.05-5.06	SK_3	289
Dhn4	1,5	1.09, 5.02-5.03	KS	92
Dhn5	9	9.02-9.03	YSK_3	325
Dhn6	3	3.08	Y_2SK_2	232

potentially will preclude the discovery of important germplasm variation. This type of imprecision probably applies to all multigene families—not just *Dhn* genes. In contrast, quite precise mapping and expression data can be obtained with gene-specific methods, including PCR for mapping and RT-PCR for expression analysis. In conclusion, gene-specific tools are needed to most effectively achieve the genetic improvement of drought and low temperature tolerance in the Triticeae and maize, whenever the relevant genetic determinant is a member of a multigene family.

Acknowledgments

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Improving the Tolerance of Irrigated Rice to Water-Stressed Conditions

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Summary

Drought is one of the most important constraints of rice production in China. We are initiating a program with the long-term goal to improve the water-efficiency of rice production in China. The objectives of the program are to improve tolerance of lowland cultivars and hybrids to reduced water supply by incorporating germplasm from upland rice varieties, and to increase the yield potential of upland rice varieties. Molecular markers will be used to identify loci for drought tolerance. Molecular marker-based strategies will be formulated on the basis of genetic analyses, and practiced in the breeding program.

Introduction

Drought is one of the most important constraints of rice production in many rice producing areas of the world (Herdt 1991). A large-scale survey listed drought at seedling period, vegetative period, and the anthesis stage, individually, among the top 20 constraints of rice production in China (Lin and Shen 1993). In fact, drought can cause severe damage at any stage of rice growth and development, which would lead to yield loss.

With the global shortages of water, reducing water consumption in crop production has now been generally recognized as an essential strategy for sustainable agriculture. It has also been gradually recognized as an important strategy for rice production, even for areas where water supply is still abundant. In addition, reduced levels of irrigation will decrease the level of water contamination and also save energy consumption, thus having a fundamental and positive impact on environment.

We have recently initiated a program that combines conventional breeding and molecular technology with the objective of increasing the tolerance of rice varieties to reduced irrigation. The long-term goal of our program is to improve the water-efficiency of rice production in China. We will present the strategies that we are following in our research program.

Breeding for Varieties with Increased Tolerance to Reduced Irrigation

The main objective of our breeding program is to improve the water efficiency of the best performing cultivars and hybrids currently used widely in rice production in China, so that the same yield levels can be achieved with reduced irrigation and/or under water-stressed conditions. The strategy is to introduce genes from upland rice materials to lowland irrigated rice varieties by molecular marker-assisted selection. We have identified more than a dozen upland varieties as the donor parents for the drought tolerance traits. A large number of crosses have been made between the upland rice varieties and top lowland cultivars and hybrid parents. The progenies for several crosses have now been advanced to the F_3 generation, and will be selected in water-stressed conditions and also in fully irrigated conditions.

Another objective of the breeding program is to increase the yield level of the upland rice varieties by developing new cultivars and hybrids. Availability of superior upland rice varieties will not only increase the yield of upland rice, but will also allow conversion of the marginal rice fields currently encountering difficulties with

irrigation to rain-fed upland rice fields, thus eliminating the need for irrigation.

Molecular Marker-based Dissection of the Genetic Basis of Drought Tolerance

The objectives of molecular marker-based analyses are to dissect the genetic basis of drought tolerance and to determine the genetic control of yield traits. The results will lead to the formulation of strategies for improving the drought tolerance of lowland varieties and for increasing the yield potential of upland rice varieties.

We have now made a cross between an Indonesian upland variety and "Zhenshan 97," one of the parents for "Shanyou 63," the best performing hybrid in China. A doubled haploid (DH) population is now being developed for molecular marker analyses and also for testing the performance under both fully irrigated and water-stressed conditions. A molecular marker linkage map will be constructed based on the segregating data collected from DH lines of this population. The genetic bases of drought tolerance and yield traits as well as loci controlling these traits will be identified using QTL analysis.

More crosses will be made between high-yielding lowland cultivars (also hybrid parents) and upland varieties. Segregating populations, produced either via doubled haploid or recombinant inbred lines approaches, will be obtained from these crosses, which will be subjected to molecular marker analyses and field testing.

A major technological difference between our strategy and that used by many other studies is that drought tolerance in our study will be directly evaluated under field conditions rather than through indirect indicator traits such as root morphology (Champoux et al. 1995; Yadav et al. 1997), root penetration (Yu et al. 1995) and osmotic adjustment (Lilley and Ludlow 1996).

Formulating the Strategies for Varietal Improvement

The results from the above analyses will lead to the formulation of strategies and also provide tools for improving drought tolerance of lowland rice cultivars and hybrids and for increasing the yield potential of upland rice varieties. Molecular marker-based systems will be developed and practiced to achieve the breeding goals.

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