



Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice



International Crops Research Institute for the Semi-Arid Tropics

The Rockefeller Foundation

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Abstract

There are two options for the management of crops in water limiting environments: the agronomic and the genetic management. With the genetic management option drought-tolerant varieties, once developed, would be a low economic input technology that would be readily acceptable to resource-poor, rainfed, small land holding farmers. Development of this genetic management technology requires robust, reproducible, simple, and rapid field, pot, and laboratory screening methods for identification of traits of drought tolerance in germplasm, and incorporation of the same in high-yielding varieties using conventional and biotechnological tools. Scientists working in various national and international crop drought research programs use different methods of screening appropriate to the crops on which they work, and keeping in view their target drought environment. This workshop focused on the methods used in different cereal (including rice) and legume crops. The rationale for the use of various methods and their advantages and disadvantages (if any) were discussed. Papers contributed by the participants, either as full papers or only abstracts of their paper, are included in this book. An overview and synthesis of the workshop and crop drought research in general is presented in the final chapter of the book.

Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice

*Proceedings of an International Workshop on
Field Screening for Drought Tolerance in Rice*

**11-14 Dec 2000
ICRISAT, Patancheru, India**

Edited by

N P Saxena and John C O'Toole



ICRISAT

**International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh**



The Rockefeller Foundation

The Rockefeller Foundation, New York, New York 10018-2702, USA

2002

Workshop Sponsors



**International Crops Research Institute for the Semi-Arid Tropics
(ICRISAT)**



The Rockefeller Foundation (RF)

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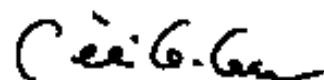
Foreword

Drought management is a major challenge to rainfed grain and fodder crop production. More than 80% of the global agricultural land area is rainfed. Drought is known to cause substantial reduction in the economic yield of crop plants. It is a major threat to food security, sustainability of production systems, and the well being of people living in drought-prone areas. It adversely affects the lives of 2.6 billion people (43% of the world population) that are engaged in agriculture. Most rainfed farmers, in general, are resource poor, with small land holdings and a limited capacity to adopt high-input technologies. For farmers trying to minimize the effects of drought on their crops, drought-tolerant varieties are an appropriate farmer-friendly, seed based technology that is easy to disseminate.

This book is a major step for ICRISAT because the information presented would have a significant impact on increasing agricultural productivity and sustainability of production systems, and assuring the well being of the poorest of the poor: small landholding, resource-poor, rainfed farmers. It is an example of *Science with a Human Face*, and portrays the vision and mission of ICRISAT.

This book on *Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice* is comprised of full-length papers and extended abstracts of papers presented at the *International Workshop on Field Screening for Drought Tolerance in Rice*, cosponsored by ICRISAT, India, and the Rockefeller Foundation, USA. Scientists working on management of drought in very diverse agroclimates and crops, both cereals and food legumes, have shared their wealth of experience. The document would be very useful in planning strategies on economizing the use of scarce global water resources, and promises that increasing agricultural productivity per unit (drop) of rainfall and per unit (mm) of supplemental irrigation is feasible. I am happy that ICRISAT's multidisciplinary team of scientists has very actively participated in achieving this important objective of the workshop, thereby contributing towards achieving the institute's mission.

The principles, practices, and knowledge presented will be of great practical value for drought researchers, not only in rice but also applicable to all programs on food crops. The book is focused and holistic as it encompasses both the strategic and applied aspects of genetic improvement of drought tolerance research, using methods of conventional breeding coupled with modern tools of biotechnology (marker-assisted breeding), and places it in a global perspective.



William D Dar
Director General
ICRISAT

Session 1: Overview

1.1 An Introduction to the ICRISAT-RF Rice Workshop

John C O'Toole¹

The Rockefeller Foundation's Food Security Theme is pleased to cosponsor, with ICRISAT, the International Workshop on Field Screening for Drought Tolerance in Rice. The Rockefeller Foundation (RF) thanks the members of the e-organizing committee (A Blum, FR Bidinger, NP Saxena, N Seetharama, R Lafitte, and R Ortiz) for the hard work they put into getting the workshop together.

This workshop, the first in a series, was organized to bring together those few scientists with significant experience in this field, with researchers, some of who are only beginning their screening and breeding projects. Thus the workshop was an excellent opportunity for all concerned to share experiences in field screening technologies and knowledge. Research on and breeding for drought tolerance in crop plants has shown limited success over the past 50 years. *This workshop and the national and international efforts it represented aimed to change that fact.* In addition to the scientific exchanges of information, the purpose of gathering so many experts together with researchers worldwide working on field screening of rice for drought tolerance, was to establish informal communications links and enable participants to develop collaborative partnerships and personal relationships with those pursuing this common goal.

The experts assembled at the workshop, many with over twenty years' experience in field screening for drought tolerance in crops, draw heavily on the sustained efforts and resources of the international agricultural research centers El Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), better known as the International Maize and Wheat Improvement Center, and ICRISAT. The RF appreciates their sharing the hard-earned experiences of their careers. Their valuable inputs, confirmed by examples, illustrate that sustained science-based efforts in field screening will pay enormous dividends, if only those involved are provided sustained support and opportunities for national and international collaboration.

1. Associate Director, The Rockefeller Foundation, Bangkok Regional Office, Thailand.

Genetic improvement of maize and rice is a primary component of a new initiative in The Rockefeller Foundation's program entitled *Resilient Crops for Less Favored Environments*. This program aims to apply science and technology to the most pressing problems of farm families in developing countries that were bypassed by the Green Revolution. The program focuses on genetic improvement of maize and rice as well as the delivery of resulting improved varieties to farm communities in drought-prone or poorly irrigated production environments. The term *drought tolerance* is most commonly associated with rainfed regions. However, given the demand for water in the 21st century, the cultivation of irrigated crops, especially irrigated rice, may soon see water become critical in formerly irrigated rice environments, as well as rainfed systems.

By supporting this workshop and the resulting network, The Rockefeller Foundation is supporting the process of building a community of scientists, capable of carrying out high quality science-based field screening and breeding for improved drought tolerance. The few successes of the past have made it clear that only by working in multidisciplinary teams, and networking with other research teams over a protracted period of years, can this effort succeed. Thus this workshop was the initial step in accomplishing three basic functions leading to the goals of the program:

1. Building specialized capacity in the national agricultural research systems, necessary for successful breeding and seed distribution of improved varieties.
2. Fostering multidisciplinary teams that network with others, nationally and internationally.
3. Assuring that this scientific resource is linked directly to poor farmers and seed delivery systems appropriate for those in less favored production environments.

Although this workshop focused on rice, the participants in the workshop attested to the broad geographic range and diversity of production conditions being addressed by the program. The national networks represented at the workshop came from China, Thailand, and India, and from WARDA in West Africa. In each of the institutions/national teams assembled here the RF have sought to sponsor a breeder, molecular breeder, and phenotyping (field screening) expert. Expert consultants have recommended this mixture of disciplinary expertise for each team, working together and linked to other

teams, in order to be successful. The basic premise is, this trait is quantitative in nature and will require teamwork and collaborative research across multiple environments. No individual or team is expected to be successful working alone.

In addition to the crop breeding teams focused on screening and breeding more drought-tolerant varieties, the Foundation's program is supporting other activities that will contribute to an integrated and unified approach in each country. Research is being supported by social scientists and economists to better understand the losses due to water-limitations and the full impacts of drought on farm families. Farmer-participatory breeding efforts that engage the farmer directly in varietal improvement are also being supported, and may involve social scientists and local nongovernmental organizations in collaboration with breeders. In addition, support is also available for experiments with new and innovative seed delivery systems, involving both the public and private sector seed industry. The program being initiated by this workshop represents a vertically integrated enterprise ranging from basic to applied research and further to extension of new germplasm to farm communities who have not had previous access to improved genetic technology specifically developed for "water-limited" rice environments.

Finally, some of the lessons learned in previous Rockefeller Foundation programs should be emphasized here. Collaboration is a key element of any national/international effort of this nature. Collaboration, as two Indian sociologists recently pointed out, requires "cognitive empathy"....or, "thinking alike", regardless of discipline, gender age, seniority, or national origin. Collaboration by thinking alike does not require one to be in the same place/institution nor to be in frequent communication (especially with internet/e-mail opportunities for discussion and data exchange). However, collaboration and cognitive empathy do require honest and equal interactions between those in a team or between teams. They noted that true collaboration required:

- a shared view of the problem;
- a shared research planning; and
- shared recognition and credit in professional communications.

Many changes are taking place in the world of agricultural research today. Multidisciplinary research teams linked nationally and internationally, are the *agents of change* with regard to the function and participation in a team or collaborative research effort. Today's multidisciplinary research teams

illustrate the benefits of moving away from the old idea of a single scientist working alone being able to make a significant contribution. Proving to be equally useless is the perceived superiority among disciplines. The real engine of effective research teams and their successes is "true collaboration" among team members who exhibit and gain from mutual respect. The Rockefeller Foundation's experience in the International Program on Rice Biotechnology (1984-2000) has amply demonstrated the power of true collaboration among equals. It is hoped that the participants of the workshop would jointly be able to demonstrate substantial progress in the resolution of the problems that confront drought research and researchers as outlined in Chapter 7 (Overview and Synthesis of Crop Drought Research).

References

Haribabu, E. and Laxmi, T. 2000. Rice Biotechnology Research: A Study of the Community of Rice Researchers in India. Project Report of the University of Hyderabad to the Rockefeller Foundation. 149 p

1.2 An Overview of ICRISAT and the ICRISAT-RF Rice Phenotyping Special Sub-Project

R Ortiz¹ and N P Saxena²

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, apolitical, international organization for science-based agricultural development. Established in 1972, it is a Future Harvest Center of the Consultative Group for International Agricultural Research (CGIAR). It is one of 16 CGIAR centers, and is supported by more than 50 governments, foundations, and development banks. ICRISAT has approximately 1200 staff, and an annual budget of about US\$ 24 million.

Future Harvest is an organization that builds awareness and support for food and environmental research for a world with less poverty, a healthier human family, well-nourished children, and a better environment. Future Harvest supports research, promotes partnerships, and sponsors projects that bring the results of research to rural communities, farmers, and families in Africa, Latin America, and Asia. ICRISAT is a Future Harvest Center.

Research and technology development ensuing from genetic improvement is organized at ICRISAT according to major topical thrusts, rather than crop mandates, to create more flexibility. The Genetic Resources and Enhancement Program (GREP) was established at ICRISAT in 1997 to help developing countries to:

- Rescue and preserve endangered crop biodiversity;
- Introduce and apply new biotechnological tools to the needs of the semi-arid tropics;
- Identify valuable new traits for resistance to biological and environmental stresses; and
- Improve breeding populations as a vehicle for sharing new traits with national agricultural research systems (NARS).

ICRISAT agreed to implement the ICRISAT- Rockefeller Foundation (RF) special project on *Field Phenotyping of Rice under Defined Drought Conditions* because of its long standing experience in field research on genetic

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2. Consultant Scientist (Crop Physiology), PI ICRISAT-RF-Rice Phenotyping sub-Project, and Workshop Coordinator, ICRISAT, Patancheru 502 324, AP, India.

improvement of its mandate crops for drought tolerance. The Institute's accumulated experience cuts across cereals and legume crops, built upon since its' establishment in the early 1970s. During this period it has developed and refined protocols of field screening for drought tolerance in order to identify and verify sources of drought tolerance in germplasm and breeding material. It has also identified traits of drought tolerance that have been incorporated in high-yielding genetic backgrounds (chickpea and groundnut) through conventional breeding methods. The current thrust is on identification of Quantitative Trait Loci (QTL) of drought tolerance for root characters, and to incorporate these root traits with other drought-tolerant traits, thereby enhancing the genetic level of drought tolerance.

The other comparative advantage that ICRISAT has is its team of multidisciplinary scientists seeking holistic solutions to the problem of drought by incorporating drought-tolerant traits together with other essential biotic and abiotic stress tolerant traits, thereby realizing the objective of multiple stress tolerance and high and stable yield.

Also, necessary physical facilities for drought tolerance research were available at ICRISAT that could be spared for this special project on rice which, with some modification, could meet the requirements of the project. Furthermore, a senior Crop Physiologist was identified to guide the implementation of the project by a group of well-trained staff.

Genetic Resources and Enhancement

The director of the program is based at ICRISAT's facility in India, where the Center's gene bank and biotechnology laboratories are located. Problems that confront agricultural production in different regions of the semi-arid tropics (SAT) vary, and therefore solving these will require specific strategies. For example, the thrust in Asia is on diagnostic, strategic, and applied research involving national partners. There is a different thrust in research in sub-Saharan Africa, where the development of breeding populations will emphasize vigorous NARS partnerships with greater direct ICRISAT involvement. Genetic resources conservation and delivery of improved germplasm are the focus of GREP activities in Africa. Likewise, GREP scientists are conserving and enhancing crop genetic resources by applying conventional and new tools at ICRISAT's headquarters in India. Other scientists are also identifying useful characteristics to improve crop adaptation in the SAT.

GREP scientists are expected to continue building partnerships and submitting targeted proposals to apply molecular and bioinformatic tools for the genetic improvement of most important crops of the SAT, which have been included in the research agenda. Likewise, ICRISAT scientists continue applying conventional and innovative cross breeding methods for the genetic enhancement of ICRISAT mandate crops. In this way, they are working along the lines suggested by the last CGIAR Systemwide Review and ICRISAT External Program and Management Review, which advocated an integrated gene management approach. Furthermore, the partnership-based refinement and exchange of useful breeding materials balances the GREP strategy.

The four research areas advocated in the new Medium Term Plan (MTP) by GREP are a logical progression from an appropriate conservation, management, and utilization of plant genetic resources and the genes available in the different crop gene pools. GREP scientists expect that their work will culminate by sharing of products with local research and technology transfer partners and making impact together in the fields of farmer clients.

The four research areas coordinated by GREP are:

- Rescue, analysis, and conservation of biodiversity to sustain crop productivity;
- New tools: adaptation and application of new science methods to SAT crop improvement;
- New traits: the biology and improvement of disease and pest resistance, stress tolerance, and quality; and
- Partnerships to share breeding materials in farmer-ready forms.

GREP scientists are also supporting research of graduate students from developing and developed countries whose investigations focus on crop improvement for the SAT

ICRISAT is implementing a number of special research projects in partnership with NARS, agricultural research institutions (ARIs), sister CGIAR institutes, and research foundations. These projects are of common research interest and strengthen ICRISAT's efforts to achieve goals that match those of its partners.

The two special projects that ICRISAT is implementing jointly with the Rockefeller Foundation, USA, are:

1. Field phenotyping of rice mapping populations and exploitation of synteny between rice and sorghum, for improving field response to drought.

The rice-sorghum synteny project has two components:

- The synteny component, in which ICRISAT's participation is to benefit from the intensive research done and progress made in gene mapping for rice, for the improvement of its mandate crops, beginning with sorghum.
- Field phenotyping of rice under defined drought conditions.

2. Workshop on *Field Screening for Drought Tolerance in Crop Plants*.

This paper focuses on the phenotyping component of the first project under defined and regulated drought conditions.

Research and technology development, ensuing from genetic improvement, is organized at ICRISAT according to major thematic thrust areas rather than crop mandates, in order to create more flexibility.

Although rice is not one of the mandate crops of ICRISAT, rainfed cropping and the semi-arid tropics of the world cover the agroecological mandate of this Institute. In the cropping or production system context, ICRISAT has worked on agronomic management of other crops, including rice.

Genesis of the Project

The Indian Rice Mini-Network on Abiotic Stress Research (IRMN) approached ICRISAT for collaboration in the field phenotyping of rice mapping populations for drought response, and in exploiting the synteny among cereals to identify QTL governing crop response to drought that are common to rice and sorghum.

The field phenotyping part of the proposal involved the development and management of a set of controlled field environments for the evaluation of both whole crop response to drought, and the statement and importance of specific traits related to drought tolerance. These environments will be used to evaluate existing rice mapping populations developed by the International Rice Research Institute (IRRI) and other partners of the IRMN. Data generated by these experiments will provide the basis for the identification and evaluation of potential molecular markers for improving drought tolerance in the crop. The environments will be designed to emphasize different types of drought encountered by the crop and therefore to identify markers for different types of drought, and to generate data to assess environment and genotype x environment effects on both drought-related traits and on the markers themselves.

Objectives and Expected Outputs of the Field Phenotyping Sub-project

The objectives are to:

- Develop and manage three field phenotyping environments, each of which will provide a different drought (and non-drought control) environment, to measure the response of the test materials to different types of drought, and emphasize putative drought resistance or tolerance traits or mechanisms.
- Conduct one phenotyping experiment per year in each of the three environments, to generate data on whole crop performance (shoot mass, grain yield, and yield components) and on specific yield component or statement of traits (effective tiller percent, spikelet fertility, and grain filling) under drought.
- Provide supporting soil, atmospheric, and crop data to fully describe the drought environments used in each experiment, and to quantify/model actual drought levels experienced by test materials.

The expected outputs are:

- Identification of morphological and functional traits;
- Data sets to identify DNA markers for both field performance and for the specific statement of putative drought tolerance traits under simulated drought;
- Data sets to evaluate the effects of environment and genotype x environment interaction effects on both field performance and marker statement or importance;
- Identification of specific rice lines with superior adaptation to specific drought environments, for direct use in Mini Network collaborators' breeding programs; and
- Valuable experience in the field phenotyping of rice mapping populations.

About the Current Workshop

In South and Southeast Asia, 18 million ha (21% of total) out of 88 million ha of total land area are drought-prone, compared to 7.1 million ha (8% of total) that are favorable for rice cultivation. Losses in yield due to drought in India, for example, range from 30-70%. Since the drought-prone area is large, a

modest increase in yield under drought conditions would make a large and significant impact on increase in rice production. The first step in achieving this objective is to generate the capacity to reliably screen for drought tolerance in field experiments. Results of screening need to be relevant and applicable to on-farm occurrence of drought. Simple indices or traits associated with drought-tolerant germplasm need to be identified for use in trait-based (ideotypic) conventional breeding programs and for marker-aided selection (MAS) methods for improvement of drought tolerance in rice.

Research on the drought tolerance of rice has attracted considerable attention since the 1st International symposium on this subject held at IRRI in the 1970s; yet field methods of screening have still not been precisely defined. This is essential to initiate a successful and systematic program on genetic improvement of drought tolerance. Identification of traits and mechanisms of drought tolerance in field experiments have become even more relevant in the present context of use of DNA marker technology as an option to manage the complex constraint of drought. Various groups of researchers in South and Southeast Asia have considerable information on and experience in the subject of rice research with respect to drought.

ICRISAT is one of the partners in the IRMN, constituted jointly by the Indian National Rice Biotechnology Network and the RF. The objective of this ongoing project is to identify molecular markers for morphological and physiological traits that would confer advantages under drought, and to use these in marker-assisted breeding programs to reduce yield losses due to drought, for which accurate and consistent phenotyping is crucial. ICRISAT will also be conducting field phenotyping of rice, mapping populations for whole plant response to drought, and the statement and importance of specific drought-related traits. The data will also be used to assess environment- and genotype x environment effects, on both drought-related traits and on the markers themselves.

ICRISAT planned to host a workshop during the initial phase implementation of its special project activities as a member of the IRMN program, in order to adopt field protocols that are developed on a sound basis. This was done to benefit from the accumulated information and experience of other drought research workers in developing a protocol for the project. This objective was achieved by inviting to this workshop 40 participants actively involved in drought tolerance research, from seven countries and international programs. The spillover benefit of this project was expected to be equally large

because the methodology discussed and evolved would be available to other national and international crop research programs on drought.

Objectives of the Workshop

To:

- Share experiences in field screening for drought tolerance in crop plants under simulated and defined drought conditions.
- Identify approaches and most appropriate methods (experimental design, layout, etc.) for conducting field trials on screening for drought tolerance in rice.
- Design methods for creating defined and reproducible drought environments.
- Verify or identify simple to observe morphological, phenological, and functional (mechanism- or process-based) traits associated with superior and stable yield under drought-prone conditions.
- Establish causal relationships between traits and functional response, and use these traits in ideotype breeding program on genetic improvement of rice for drought-prone areas.
- Consolidate a common data set on soil, climate and crop to interpret rice response to drought across test environments.
- Identify common research interests, establish informal communication links, and foster a close partnership and personal relationship in pursuing common goals.
- Generate greater awareness about the Dos and Don'ts in drought tolerance research.
- Arrive at a robust and common research methodology on "Field and pot methods of screening for drought tolerance in crop plants".

Expected End Outputs of the Workshop

- The first purpose of the project, that of bringing together researchers engaged actively in drought research for cross-program interactions to evolve a common and a robust protocol of field screening for drought tolerance in rice, is accomplished with the opening of this workshop.
- Proceedings published would document methods of screening for drought tolerance used by researchers in different programs.

- Rice drought research network activities would be strengthened and communication channels established.
- There would be a strong emphasis on uniform and reliable data collection, and on integration and synthesis of results obtained across locations and groups of researchers in the ongoing drought management research in rice, thereby accelerating progress in genetic improvement of rice for adaptation to water limiting conditions.

1.3 Genetic Improvement of Rice for Water Limited Environments: Identification of DNA Markers and QTL and Marker-Assisted Selection - A Network Project

Arjula R Reddy¹

The National Rice Biotechnology Network/Rockefeller Foundation International Program on Rice Biotechnology set up different mini-networks in June 1997, each one focusing on a specific aspect of rice improvement. The major objective was the establishment of collaborative research teams among national scientists for the application of modern tools of biotechnology for the improvement of rice. One such mini-network was on abiotic stress resistance in rice with the specific objective of the development of screening techniques using molecular markers for QTL relating to osmotic adjustment. Subsequently, a meeting of the participating scientists at Malacca in September 1997 led to the broadening of the objectives of the mini-network. Accordingly, a working group of the participating scientists from State Agricultural Universities, universities, CGIAR system, and ICAR met at the University of Hyderabad in April 1998 and deliberated upon the specifics of the project. Nine institutions participated in this meeting. A broad consensus was arrived at on two different but complementary approaches to the genetic improvement of rice for abiotic stress tolerance, namely, marker-assisted selection (MAS) and molecular biology of tolerance to drought and salinity. After a series of meetings and discussions, five institutions were finally identified as suitable partners. These include ICRISAT, Hyderabad; TNAU, Coimbatore; UAS, Bangalore, IGAU, Raipur, and the University of Hyderabad (coordinating center), Hyderabad. After several field visits ICRISAT was identified as a suitable field site for phenotyping. The project proposal was submitted to the Rockefeller Foundation in August 1999 with the following objectives: a) Development of central facilities for evaluation of crop genotypes for drought tolerance and its components; b) Assembly and evaluation of appropriate germplasm, mapping populations, and other genetic

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resources, and initiation of practical applications; c) Development of genetic linkage map and identification of markers or QTL; d) Development of a central facility for marker analysis and related DNA technologies for crop improvement; and e) Application development, technology exchange, international collaboration, and manpower development. A final preparatory meeting of participating scientists was held during March 2000 at Hyderabad to detail the institution-wise tasks and time schedule. The project grant received the administrative approval of the Rockefeller Foundation in April/ May 2000. Currently experiments are being carried out in parallel at different centers dealing with phenotyping, synteny, physiology, mapping, and development of Expressed Sequence Tags (ESTs).

1.4 Drought Tolerance - is it a Complex Trait?

A Blum¹

Abstract

Drought tolerance is a very complex trait. This is a declaration and complaint often expressed by scientists entering into this area of research. Consequently, suggestions are offered on the need for identifying all of the many putative drought-adaptive genes followed by their pyramiding in order to consolidate an improvement in tolerance. However, both conceptually and functionally, drought tolerance is not a complex trait if two major arguments are considered:

1. Most of the crucial plant traits that control plant water status and plant production under drought are constitutive and not stress adaptive.
2. Plant water status, more than plant function, controls crop performance under drought.

Once these arguments are considered, the concept and design of a drought-resistant crop ideotype for a plant-breeding program becomes uncomplicated.

Introduction

Drought tolerance is a very complex trait. This is a statement frequently made by scientists entering into this area of research. The assumed complexity of drought tolerance as repeatedly stated in research reports, reviews, and especially in research proposals, is becoming almost axiomatic. The advertisement of drought tolerance as being very complex is also becoming a liability to the development of a realistic research approach to the problem. It is often expected that solutions to a complex problem such as drought tolerance are naturally complicated and costly. This is not necessarily always the case. Often, and depending on the drought scenario, drought tolerance may be controlled by simple plant traits that can lead to simple and cheap breeding solutions.

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This presentation challenges the notion that drought tolerance is necessarily complex. Drought tolerance will become much less complex conceptually and strategically if one considers the following axioms:

1. Most of the crucial plant traits that control plant water use, plant water status, and plant production under drought are constitutive and not stress adaptive.
2. Plant water status, more than plant function, controls crop performance under drought.

Constitutive Plant Traits Controlling Plant Water Status and Productivity under Drought

Phenology

Phenology has a major effect on plant performance under drought, in two respects:

- (a) A short growth duration enables evasion of severe end-of-season (terminal) stress; and
- (b) Plants of short growth duration generally tend to use less water because of their shorter growing period and smaller leaf area.

The expression of phenology in terms of time to flowering or time to maturity evidently does not require drought responsive genes. Genes that control flowering by their determination of photoperiod, temperature, or vernalization response are relatively independent of plant water status. Drought can modify phenology, such as advancing (wheat) or delaying (rice) flowering, but this has little to do with the inherent constitutive control of flowering time.

Root Traits

Root traits are key components of plant adaptation to drought environments. Root depth and extension into deep soil is crucial for crop performance under limited water supply if there is moisture available at deep soil level. The expression of deep rooting capacity does not require stress conditions. Roots do not grow into deeper soil *in pursuit* of the receding wetting front. The

potential capacity for deep roots is expressed constitutively and can be phenotyped under non-stress conditions. Root development can be modified by soil conditions such as soil moisture status and soil strength. Topsoil moisture conditions and strength can modify root distribution in the soil. However, the basic difference between shallow and deep-rooted genotypes will be expressed irrespective of stress conditions. The capacity of roots to penetrate hard soil is not necessarily linked to deep rooting capacity.

Plant and Organ Size

Plant and organ size exercises a major control over plant and crop water use. Small plants of small leaf area and leaf area index (LAI) use relatively less water and are expected to enter a state of plant water deficit later than large plants of greater LAI. Indeed, smaller plants generally offer a lower yield potential than larger plants. The trade-off between water economy and reduced yield potential is an important consideration in designing a drought-resistant crop ideotype. However, it still remains an undisputable fact that plant size and leaf area are expressed constitutively and the potential for developing large or small leaf area is expressed independently of stress. Again, stress may reduce plant size and leaf area through stress responsive systems that are not necessarily within the domain of the basic genetic control of plant size.

Leaf Surface Properties

Leaf surface properties affect the radiation load on the leaf canopy and, subsequently, leaf temperature and transpiration. Leaf surface properties are derived from various leaf surface traits such as the form, shape, and composition of cuticular and epicuticular wax (EW); leaf pubescence; and leaf color. All these traits are constitutively well expressed. There are numerous crop plant mutants for leaf surface properties such as *EW*, pubescence, and color. Again, leaf water deficit for a prolonged period of time will increase EW load and leaf reflectance within a given range, but the main control still remains constitutive. Epicuticular wax load will increase with leaf water deficit but the extent as compared to the effect of one gene, such as the *Bm* gene in sorghum, is relatively small. Protection by a constitutive statement of high EW load rather than by depending on the stress-imposed modification is therefore the desirable approach in formulating a resistant genotype.

Non-senescence

Non-senescence or "stay-green" is the delayed or reduced rate of normal plant senescence as it approaches maturity. Senescence is mainly expressed in the breakdown of leaf chlorophyll, reduced photosynthesis, and the general reduction in cellular capacity for various life functions. The normal progress of senescence is accelerated when drought occurs during the late developmental stage. There are several known major genes and quantitative trait loci (QTL) that delay the onset of senescence or reduce its rate. These generally do not require drought for their expression. However, when normal senescence is amplified by drought, the statement of these genes is more pronounced phenotypically. Thus, constitutive non-senescence becomes more effective towards plant production when stress occurs as compared with non-stress conditions, but these genes are expressed irrespective of stress.

Stem Reserve

Stem reserve is a major resource providing carbohydrates and nitrogen for grain filling when the transient photosynthetic source is inhibited by stress. The support of grain filling by stem reserves is effective under any stress that inhibits transient photosynthesis such as drought, heat, or even leaf diseases that develop during grain filling. This mechanism depends on the accumulation of reserves before flowering and the transport of the reserves during grain filling. The two processes are independent. The accumulation of reserves before grain filling is not stress responsive in the sense that it is totally independent of stress during grain filling. The signal for reserve (e.g. fructan in cereals) conversion into soluble fractions that can be transported from stems to grain can be stress responsive. However, evidence from wheat shows that certain genotypes use reserves extensively for grain filling even under non-stress conditions. In this sense stem reserves are constitutive as a backup source for grain filling under stress.

Stress Adaptive Plant Traits Controlling Plant Production under Drought

Stress adaptive traits are those controlled by genes that are expressed only in response to stress, often a specific stress and sometimes a non-specific stress.

The most widely common statement of adaptive genes involves the accumulation of *cellular compatible solutes*, which can be products of photosynthesis (e.g. sugars), metabolism (e.g. proline), or ions taken up from the soil (e.g. potassium). Solute accumulation responds linearly or exponentially to plant water status. Time is a crucial component of solute accumulation, whereas very rapid desiccation does not allow sufficient time for accumulation even though tissue water status may be reduced appreciably.

A major role for solute accumulation is osmotic adjustment, which in terms of plant-water relations serves to sustain turgor and cellular hydration and delay wilting. Turgor and cellular hydration dependent functions are better conserved under drought if osmotic adjustment occurs.

Some of the accumulated solutes are implied to have a role in protecting cellular organelles or cellular functions. A protective role has been argued for the following plant, algal and bacterial metabolites: proline, glycinebetaine, mannitol, trehalose, fructan, sorbitol, and inositol/ononitol.

An increase in certain *antioxidant* agents has been observed in response to stress. The increased antioxidant activity is generally believed to be associated with drought tolerance. Even though the increase in antioxidant activity in response to stress can be appreciable, the effect on what is being defined as 'drought tolerance' is rarely quantified. Consequently it is not always clear to what extent the increase in antioxidant activity under stress is really important for stress tolerance in terms of plant production or survival.

Heat shock proteins and *molecular chaperone proteins* have generally been observed to accumulate or be synthesized de novo in response to drought. Molecular chaperones are widely implicated in human, animal, and plant tolerance to stress. The exact role in quantitative terms of expressed molecular chaperones under drought is not fully resolved.

Taken as a whole, our present state of knowledge indicates that the role of constitutive traits towards drought tolerance may be regarded as greater, quantitatively, than that of stress responsive/adaptive traits.

Plant Water Status - a Major Control of Plant Function under Stress

A reduction in vital plant functions is of course often observed under drought stress. The capacity to sustain plant function under stress is generally taken as a statement of tolerance in that function to stress. For example, photosystem

activity can be reduced in plants subjected to drought. If a certain cultivar is found to sustain relatively better photosystem activity under stress it would often be assumed to possess some form of tolerance in photosystem function under stress. In most cases, however, the reason is its relatively better capacity to sustain higher leaf water potential, relative water content (RWC), or turgor under stress. Sustained water status or turgor is the primary reason for sustained function under drought. Cases for sustained function at low water status as a major reason for drought tolerance are comparatively rare.

Furthermore, differences among genotypes in the statement of stress responsive genes might first be driven by respective differences in plant water status among genotypes. Proof of differences among genotypes in functional stress responses must be normalized for plant water status before a conclusion is made on a relative advantage in stress adaptive traits. This is not always the case in reported research that claims a genetic difference in stress responsive traits.

Since plant water status has a major impact on plant function and adaptive processes in plants under stress, the factors that affect plant water status under stress are the most crucial for drought tolerance. The major control of plant water status in crop plants subjected to drought is exerted mainly by constitutive traits, such as leaf area, root extension, plant phenology, and leaf surface properties.

In conclusion, it is proposed that crop plant drought tolerance is not complex if one considers that the major impact on tolerance is exerted by constitutively expressed major plant developmental traits. These traits control plant water status, which triggers various stress responsive genes, some of which are considered to be stress adaptive. A role for stress adaptive genes in the context of the whole plant must be established with the background of the dominating impact of developmental traits expressed constitutively on plant water status.

Session 2: Field Screening and Genetic Improvement of Drought Tolerance in Rice

2.1 The International Rice Research Institute's Experience in Field Screening for Drought Tolerance and Implications for Breeding

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Abstract

There has been considerable investment in trying to understand the physiological basis of genetic variation in tolerance to water deficit in rice. The practical goal of this work has been to develop ways to reliably reveal useful genetic variation in the context of a breeding program. Rice cultivars differ in many traits, such as root depth and morphology, leaf senescence and stomatal conductance under stress, and osmotic adjustment, which are expected to result in different yields under stress. The demonstration of variation among genotypes has been successful, but the link to performance is less clear. Anticipated correlations between traits and performance are confounded by differences in plant type, phenology, and disease and insect resistance, and also by the difficulty of reproducing stress levels across seasons. Managed environments such as dry season nurseries with controlled irrigation can be used to produce fairly repeatable screening experiments. If the objective of the project is to improve grain yield under drought, the stress must be applied at a growth stage that influences grain yield. For rice, this generally means that stress should be applied near flowering. Earlier stress is simpler to apply, but it has not been possible to relate the results of seedling screening to grain yield under stress. The authors have observed that many genotypes perform poorly during the dry season even when the soil water potential is maintained above -20 kPa, indicating that the plants are experiencing mild continuous stress. Yields in these control plots in the dry season tend to be well correlated with yields in stress plots, where water is withheld for 14-20 days around flowering. If yield under these conditions is relevant to performance in farmers' fields in a given target environment, dry season screening can be included in a cultivar improvement program. Results from multilocation testing with climate data are required to make this decision.

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Comparisons between cultivars can give misleading indications of causal relationships between individual traits and yield, because different varietal types tend to have different preferential association of alleles covering a wide range of traits. Thus, contrasting groups of traits tend to be found in upland vs. lowland cultivars, improved vs. traditional cultivars, etc. An evaluation of the interaction of yield with managed stress environments suggests that root depth, leaf fraction fresh weight (an indication of cell size and supporting tissue), and anthesis date are important in determining how a variety responds to stress. It has been assumed that the effect of anthesis date on drought response was associated with escape from drought, but the effect is observed even when drought is imposed at the same growth stage across cultivars. Mapping populations offer the advantage of disassociating many characteristics that normally occur together in cultivars, and can thus allow a clearer assessment of the value of individual loci. Most current mapping populations still comprise a wide range of plant types and flowering dates, and these complicate the interpretation of results. The presence of major genes with pleiotropic effects or closely linked gene clusters also limits our ability to identify other important loci. In the IR64/Azucena DHL population, the presence of the *sd-1* gene affects yield and many other traits, and also influences the correlations between yield and secondary traits. Populations of near-isogenic lines offer an alternative to mapping populations, but with this type of material it is difficult to accurately estimate the effects of alleles, because donor-parent alleles are present in only a few lines in a backcross-derived population. In addition, the differences among lines are often smaller than in mapping populations, and opportunities to detect epistatic interactions are reduced. Results from controlled, replicated experiments would need to be combined with different types of germplasm in order to make progress in identifying the physiological basis of genetic variation in drought tolerance.

Introduction

Plant breeders and physiologists have the common goal of identifying traits that confer an advantage under drought. Traditionally, their approaches to this problem have differed. Breeders have worked to identify lines with superior performance in the target environments through multilocation testing, in order to gradually accumulate favorable alleles in improved cultivars. Physiologists have used the current knowledge about how plants grow to

hypothesize which traits might be advantageous, and have then looked for genetic variation in those traits that can be correlated with yield in the target environments. Simply stated, breeders tend to work from yield to alleles to traits (ideotype), while physiologists tend to work from traits to yield, and then seek the link to genes at the molecular level. Only limited synergy has been achieved between these approaches, and progress has been particularly insufficient in the area of improving tolerance to water deficit. Advances in molecular and statistical tools have enabled researchers to reach a position where a much more productive partnership between physiology and breeding activities can be forged.

Managed Environments - Necessity and Advantages

Drought is unpredictable, and it does not occur in all years in the target environment. This means that screening in the target environments will only result in stress in perhaps one-third of the years. In order to make greater selection progress, managed environments, such as a dry season nursery with controlled irrigation, can be used to increase the heritability of yield and other traits. These environments are only useful, however, if they either: 1) result in similar yield differences among genotypes as stress in the target environment, or 2) consistently reveal variation in secondary traits that are known to confer an advantage in the target environment.

The International Rice Research Institute (IRRI) has conducted extensive dry season field screening for tolerance to water deficit at the seedling stage (Mackill et al. 1996). It has, however, been difficult to demonstrate a consistent relationship between seedling drought scores and yield performance in target environments. In addition to the fact that different genes are expected to influence maintenance of green leaf area in seedlings under severe stress and grain formation under moderate stress, the seedling screen is confounded by differences in leaf area at the beginning of the stress period (Mitchell et al. 1996). It has also been difficult to demonstrate that seedling stress tolerance is a secondary trait relevant to the target environment. For those environments where plant loss due to early drought is common, there is probably some justification for seedling screening, but the authors are not aware of documented cases where this approach has resulted in the production of improved varieties.

Screening at the reproductive stage during the dry season resulted in repeatable yield differences among rice varieties (Garrity and O'Toole 1994).

That screen employed staggered planting dates under sprinkler irrigation, and trial management was quite complex. Similarly, late-season screening of advanced breeding lines with staggered planting dates has been employed in Brazil, to characterize new varieties as tolerant or intolerant of drought. Data are still being collected to assess how well dry season screening with reproductive stage stress mimics performance in the target environment. Unfortunately, in the case of rice, specific secondary traits analogous to the anthesis-to-silking interval in maize have not yet been identified (see Banziger, this volume) that can be measured in a managed dry season environment and are known to be relevant to yield in the target environment. Much of IRRI's current work on drought tolerance in aerobic rice is directed toward identifying such traits.

Over the past four years, the authors have used sprinkler, furrow, and drip irrigation systems for dry season screening. The advantages and disadvantages of each are indicated in Table 2.1.1. A set of 46 cultivars was

Table 2.1.1. Advantages and disadvantages of irrigation methods used in managed drought environments at IRRI.

Method	Advantages	Disadvantages	Frequency
Sprinkler	<ul style="list-style-type: none"> • Robust components • Efficient water use • Easy to measure application 	<ul style="list-style-type: none"> • Uneven distribution pattern • All entries receive same timing of stress • Large border areas needed between water levels • Leaks, blockages and wind increase variability 	3x/week
Furrow	<ul style="list-style-type: none"> • Minimal equipment required • Uniform application • Strips of the field can be irrigated differently if bordering is adequate 	<ul style="list-style-type: none"> • Inefficient water use • Requires land leveling • Hard to measure amount of water applied • Requires raised beds so row spacing is wide for rice 	2x/week
Drip	<ul style="list-style-type: none"> • Individual plot irrigation is possible • Efficient water use • Uniform application 	<ul style="list-style-type: none"> • Expense of components (tape and filter) • Limits weed control options • Labor required to check quality of irrigation 	3x/week

evaluated over several years using different irrigation systems to produce 9 managed environments. The environments were conducted on the IRR1 experiment station over three seasons, with water provided by rainfall or by sprinkler, furrow, or drip irrigation. Irrigation frequency was altered to apply stress at different developmental stages or for different periods. The 46 varieties were mostly of upland adaptation, and spanned a range of average flowering dates from 53 to 85 days after sowing. When water exclusion was for a specific period near flowering, it was imposed relative to the growth stage of the individual cultivars by using independent drip irrigation lines for each plot. Pattern analysis was conducted using IRR1STAT with mean polishing to remove the main effects of cultivars and locations. The interaction biplot of first and second principal components showed that the drip irrigated control, and drip irrigated treatment with stress at the vegetative stage interacted differently with variety than the furrow irrigated control, sprinkler with vegetative stage stress, and wet season experiments. These differences resulted in separation on the first principal component axis, which explained 46% of the observed variation. The differences between these water treatments can be explained by three observations: 1) Some of the cultivars that performed well in the dry season were affected by disease and lodging in the wet season. 2) The furrow-irrigated system used at IRR1 requires wide spacing between beds, which earlier varieties with limited leaf area index exploit less successfully than late varieties. 3) Early varieties were disadvantaged in the sprinkler-irrigated experiment with early stress because stress was applied to all lines on the same date, which was nearer to flowering in the early entries.

Both the method of stress application and the subset of cultivars selected have a major influence on the conclusions that can be drawn from these experiments. In studies of mapping populations (described below), which compare fewer alleles than the set of cultivars, significant line-by-water level-by irrigation method interactions for yield were not observed.

Another question that arises in designing managed drought environments for rice is in defining what constitutes stress. The authors' experience suggests that most varieties, even upland varieties, yield more grain in flooded plots than in aerobic plots, as long as lodging is prevented. For example, the lowland cultivar IR72 produced from 20 to 35% less grain in an upland field with high N and water supply compared to the lowland control (Table 2.1.2). With water stress or moderate N, the reduction was much greater. Other workers have concluded that aerobic growing conditions at IRR1 in the dry season

Table 2.1.2. Grain yield (t ha⁻¹) and harvest index of rice lowland cultivar IR72 with different levels of water supply in experiments conducted at IRRI, Philippines, over 2 years.

Growing conditions	Yield	Harvest index
Lowland, 97WS, 60 kg N applied	3.7	0.4
Upland, sprinkler irrigation as needed, 97WS, 60 kg N applied	2.4	0.3
Upland, rainfed with water stress causing leaf rolling at flowering, 97WS, 60 kg N applied	0.5	0.1
Lowland, 99DS, 110 kg N applied (30 kg basal + 6 splits)	5.0	0.4
Upland, 99DS, drip irrigation 3x/week to apply 1.6 x pan evap., 110 kg N applied (30 kg basal + 6 splits)	2.4	0.3
Upland, 99DS, drip irrigation 3x/week to apply 1.6 x pan evap., except for period-8 days to flowering, 110 kg N applied (30 kg basal + 6 splits)	2.4	0.3
Upland, 99DS, drip irrigation 3x/week to apply 1.6 x pan evap., 170 kg N applied (40 kg basal + 6 splits)	3.9	0.4
Upland, 99DS, drip irrigation 3x/week to apply 1.6 x pan evap.+ basin irrigation 2x/week, 170 kg N applied (40 kg basal + 6 splits)	3.8	0.4

DS = dry season; WS = wet season

impose a continuous mild water stress for rice (Dingkuhn et al. 1989). The performance of genotypes in irrigated aerobic fields during the dry season can, in itself, provide useful information on aerobic adaptation, which is a type of tolerance to water deficit. This conclusion is supported by the substantial correlations frequently observed between yields in irrigated (aerobic) control plots and stress plots for both variety trials and experiments with mapping populations (Table 2.1.3).

Withholding water for about 14 days from about 10 days before flowering until 4 days after flowering can reduce yield by 20-40% relative to the fully irrigated aerobic control treatment (Table 2.1.3). This period was targeted because a relatively mild stress at this stage has a large impact on grain yield. Across seasons, the length of the stress period should be adjusted to compensate for either very high evaporative demand or rain during the drought period. If water supply to the plot is removed when the flag leaf ligule reaches the ligule of the penultimate leaf on the first few tillers in a plot, this generally coincides with a water exclusion period that begins about 9 days

Table 2.1.3. Phenotypic correlation between grain yields in control plots and within plots with water excluded near flowering.

Season	Irrigation method	Material evaluated	Grain yield, control	Grain yield, stress	Correlation coefficient
98DS	Drip, stress with water withheld for 20 d beginning at panicle initiation	46 cultivars	1.52	1.21	0.64 n = 38
98DS	Furrow irrigation 2x/week, stress had water for two 14d periods beginning 10d before flowering of earliest line	82 DHLs of IR64 x Azucena	0.78	0.29	0.51 n = 41
99DS	Drip irrigation 3x/week, stress had water withheld for 14d beginning 10d before 50% anthesis for each line	78 DHLs of IR64 x Azucena	1.27	0.79	0.80 n = 68
99DS	Furrow irrigation 2x/week, stress had irrigation frequency of 1x/week beginning 6d before flowering of earliest line, 14d before average flowering date	98 RILs of Bala x Azucena	2.58	1.18	0.40 n = 95
OODS	Drip irrigation 3x/week, stress had water withheld for 14d beginning at appearance of first panicle for each line	92 RILs of Bala x Azucena	2.84	2.36	0.36 n = 90
OODS	Drip irrigation 3x/week, stress had water withheld for 20d beginning 10d before 50% anthesis for each line, rainfall of 42mm during stress.	56 IR64 NILs introgressed with Azucena alleles	3.34	1.80	0.44 n = 58

DS = dry season.

before 50% flowering in the control plots. If an earlier onset of stress is required, it is generally based on the date of panicle initiation. Unfortunately, the determination of panicle initiation is time-consuming and destructive, and is not feasible for a large number of lines.

Mechanisms of Drought Tolerance based on Varietal Comparisons

Traditional physiology experiments designed to assess the value of specific traits have relied on comparisons among cultivars. Cultivars represent unique combinations of alleles that interact with each other and with the environment to produce grain yield, the integrated indicator of performance. A wide range of cultivars and traits has been evaluated at IRRI over the past decades (examples in Table 2.1.4), and many other rice researchers worldwide have conducted similar work. For upland rice, variation in tolerance to stress at the vegetative stage measured as maintenance of green leaf area is apparently linked to root depth and, less convincingly, to root thickness. Cultivar differences in yield when the crop is under stress at the reproductive stage

Table 2.1.4. Examples of putative drought-adaptive traits that show genetic variation among rice cultivars.

Trait	Range		Number of cultivars	Reference
Root metaxylem vessel radius	20-32	µm	6	Yambao et al. 1992
Cuticular resistance	30-68 s		cm ⁻¹	Yoshida and Reyes, 1976
Osmotic adjustment	0.4-1.5 MPa			Lilley and Ludlow, 1996
Peduncle osmotic potential	0.8-3.8 MPa		46	Lafitte, unpublished
Maximum root length (40 DAS)	75-114 cm		108	Courtois et al. 1996
Nodal root thickness	0.9-1.3 mm		108	Courtois et al.
Specific leaf area (~60 DAS)	17-28	m ² kg ⁻¹	46	Lafitte, unpublished
Leaf % fresh weight	64-76%		45	Lafitte, unpublished
Root pressure (sap exudation per tiller overnight)	0-460 mg DS* 600-2880 WS		33	Lafitte, unpublished
[ABA] in xylem sap	7-92 nM			Bano et al. 1993

*DS = dry season; WS = wet season; DAS = days after sowing.

have been related to differences in leaf drying, canopy temperature under stress, and to predawn panicle water potential (see Jongdee et al., this volume). These relationships seldom account for more than 15-20% of the observed variation in yield. Many other traits have been found to differ among cultivars, but they have not been related to yield differences under stress.

Across studies at IRRI and elsewhere, the yield component found to be most sensitive to stress near flowering is the fraction of spikelets that develop into grains (spikelet fertility). Many processes are involved in fertilization and the initiation of grain filling, including the production of viable pollen, panicle exertion, pollen shed and germination, and embryo development. All of these processes are reportedly affected by water stress, particularly when the onset of stress is sudden and severe, as in many pot experiments. There is limited evidence on which process dominates in realistic field environments. For some cultivars, poor panicle exertion is frequently observed with stress in the field, and it is clear that spikelets on the unexserted portion of the panicle remain unfilled. The stage of pollen meiosis has been suggested as a very drought-sensitive stage. This stage occurs about 10 days before anthesis, and stress at that time is expected to reduce the number of normal pollen grains. In field experiments with drip irrigation, a severe 20 day stress treatment beginning at panicle initiation did not result in greater spikelet sterility than in the control (yields in Table 2.1.3). A treatment in the same experiment with water withheld beginning 14 days after panicle initiation had a large increase in spikelet sterility, but this treatment did not result in a high proportion of sterile pollen in the majority of the materials tested. In contrast, in pot experiments with water stress beginning 7 days before flowering, anther dehiscence declined sharply at mid-day leaf water potentials of less than -1.5 MPa, and pollen viability also decreased (Ekanayake et al. 1990). The stress developed extremely rapidly in those experiments, and more information is needed on the importance of pollen development, pollen shed, and pollen germination on spikelet sterility in plants experiencing more realistic stress.

In most studies comparing yield response to water deficit, cultivars differed consistently in yield potential. Researchers have generally dealt with this problem by either reporting yield loss as a percentage of the irrigated control, or by calculating a stress index that removes the effect of yield potential statistically. Both of these approaches are open to criticism. Where yield reduction is expressed as percentage of the control yield, low-yielding unresponsive cultivars may be ranked higher than varieties with high yield

potential that, even when stressed, have a greater yield than the unresponsive entries. The stress index similarly removes the effect of yield potential, which in itself frequently results in better yields under stress.

An alternative analysis to evaluate the importance of secondary traits independent of yield potential is to assess the interaction of the variety with a set of drought-affected and unstressed environments, rather than concentrating on the main effect of the variety. It can then be established whether cultivars that respond to stress in a similar way do, in fact, differ in some secondary traits that are hypothesized to be important for yield under drought. In the experiments mentioned previously, pattern analysis identified nine groups of cultivars based on their interactions with managed drought environments. The second principal component axis separated the well-watered environments from those that experienced stress, and that axis accounted for 24% of the observed variation. Mean values of secondary traits were calculated for each cultivar group. This approach should allow an assessment of the value of a trait in influencing cultivar response to water deficit independent of yield potential and, because there are several cultivars in each group, across several genetic backgrounds. Cultivars that interacted positively with stress environments were generally early maturing, with minimal anthesis delay under stress, long roots, high root pressure, limited leaf rolling, and high canopy temperature with stress. The cultivars that interacted negatively with stress environments tended to have large delay in flowering with stress, rapid leaf rolling, low canopy temperature with stress, a large decline in fractional leaf fresh weight between the wet season and the dry season, and minimal root pressure. These data provide information on sets of traits that differ between cultivars that differ in their responses to water deficit. Traditional upland cultivars had minimum interaction with environment; these were of late maturity and had low yield potential. These can be considered stable and unresponsive.

Lessons from Mapping Populations

Mapping populations have been developed in order to identify quantitative trait loci (QTL) for traits that are controlled by many genes. They have also been successfully used to identify major genes whose expression is strongly modified by the environment, such as a gene conferring tolerance to submergence (Mackill et al. 1996). From a physiological perspective, however,

these populations offer the additional advantage of breaking up conventional allelic associations for relevant traits. Because of independent domestication events for *indica* and *japonica* subspecies, followed by centuries of farmer selection, certain groups of alleles have been brought together in what are known to be traditional varieties. For example, tropical *japonica* rice varieties can be characterized as having thick, dark green leaves, negative phenol reaction, thick roots, and limited tillering. These are only some obvious traits - there are undoubtedly many more allelic associations at the genetic and biochemical levels. A physiological relationship among these traits is not necessary, but they have, either through founder effects or gradual coselection or both, come together in these varieties. Such baskets of traits suggest causal relationships where none may exist, and also confound estimates of the value of each trait independent of the others. Mapping populations, which are developed through crosses of divergent parents with minimal selection in segregating generations, break up allele associations and thus allow us to look at loci more independently (though not completely independently, because some recombination events are less likely than others). Because of this advantage, mapping experiments allow not only identification of QTL, but also an assessment of the reliability of trait estimates, and the genetic correlation between traits and yield. It should be noted, however, that such estimates derived from mapping populations created from parents of very different adaptation might still be overoptimistic relative to what might be found in a population of breeding lines. Mapping populations often contain many low-yielding lines that may inflate correlations, just as the inclusion of unadapted varieties overestimate correlations between traits and yield in a cultivar comparison. It is rare in a mapping population to find many lines with yield superior to the better-adapted parent. As better markers become available, it will be possible to use polymorphism that exists between parents with much more similar adaptation than was possible when mapping was based on restriction fragment length polymorphism (RFLP) markers. Therefore, the next generation of mapping populations may be much less affected by this problem.

While mapping populations allow the independent evaluation of different traits, lines will still have associations among traits that are controlled by either a single gene or by closely linked genes. An example of this effect is found in the IR64/Azucena mapping population, because IR64 contains a semi-dwarf gene (*sd-1*) that apparently affects several yield components. In a

Table 2.1.5 Phenotypic correlations between grain yield and other traits for 70 lines of the IR64/Azucena population of doubled haploid lines grown in the 1999 dry season with drip irrigation, and correlations for subsets of lines with either the IR64 allele or the Azucena allele at the RZ730 location on chromosome 1. All correlations shown are statistically significant at $P < 0.05$.

Trait	AH lines		IR64 at RZ730		Azucena allele at RZ730	
	Control n=70	Stress n=70	Control n=34	Stress n=34	Control n=35	Stress n=25
Anthesis date	-0.47	-0.47	ns	-0.38	-0.70	-0.56
Panicles m ²	0.62	0.58	0.45	0.57	0.48	0.56
% sterile panicles	-0.68	-0.77	-0.49	-0.56	-0.71	-0.81
TGW*	0.23	0.32	ns	0.37	ns	ns
% sterility	-0.75	-0.78	-0.74	-0.85	-0.69	-0.70
Stemborer score	-0.47	-0.36	-0.36	-0.42	-0.42	ns
% FW ctl**	ns	-0.29	ns	-0.46	ns	ns
Yield, control	-	0.80	-	0.78	-	0.65

* T G W = weight of 1000 grains

**% FW, ctl = percent fresh weight of fully turgid leaf sampled from the control plots

set of 70 lines, the correlations between grain yield and yield components depended on whether or not the semi-dwarf allele was present (Table 2.1.5). In the presence of the Azucena allele, grain yield was strongly negatively associated with duration, but that association was weaker or not significant with the *sd-1* allele. This is indicative of epistatic interaction between the allele at the *sd-1* locus and alleles at other loci. The reduction in grain yield with stress was less dependent on reduced harvest index and increased sterility in the tall types. Programs for QTL analysis are now available that take this type of background genetic variation into consideration.

Another question that arises when searching for traits that confer genetic variation in drought tolerance is whether overall yield or individual yield components provide a more reliable estimate of tolerance. Because yield components represent a lower conceptual level of complexity, and because the stress can be scheduled to affect only some yield components, it may be assumed that measurements of components may be more repeatable than yield. In a lowland experiment with the IR64/Azucena DHL population, yield had a lower broad-sense heritability (H , within-experiment repeatability) than individual yield components (Table 2.1.6). In an upland experiment with

Table 2.1.6 Broad-sense heritability (H) of yield and yield components for 80 DHLs and 56 NILs of IR64/Azucena, measured in lowland or aerobic conditions with (stress) or without (control) a period of water deficit near flowering. The number of QTLs identified with LOD>2 is indicated for each trait.

Trait	DHLs		DHLs		DHLs		DHLs		NILs	
	Lowland 94DS	Control 98DS	Stress 98DS	Control 99DS	Stress 99DS	Control 98 and 99	Stress 98 and 99	Control 00DS	Stress 00DS	
Pan m ²	0.64 2 QTL	0.35 1 QTL	0.26 2 QTL	0.76 1 QTL	0.72 2 QTL	0.69	0.75	0.52	0.17	
SPP	0.79 3 QTL	0.70 3 QTL	0.60 0 QTL	0.63 2 QTL	0.14 0 QTL	0.61	0.24	0.26	0.00	
% sterility	0.68 2 QTL	0.79 1 QTL	0.64 1 QTL	0.57 1 QTL	0.20 0 QTL	0.72	0.55	0.83	0.45	
TGW	0.88 2 QTL	0.53 1 QTL	0.67 3 QTL	0.67 1 QTL	0.51 1 QTL	0.72	0.50	0.54	0.31	
Yield	0.52 2 QTL	0.49 3 QTL	0.20 0 QTL	0.61 3 QTL	0.71 2 QTL	0.69	0.64	0.67	0.56	

furrow irrigation, grain yield had low heritability, especially under stress. In contrast, in a drip-irrigated experiment with milder stress and more precise timing of stress, yield had a greater heritability under stress than the yield components that were expected to change with drought, spikelets per panicle, and sterility. Because of the sampling errors that accumulate in the calculation of yield components, these traits may not necessarily be better candidates for QTL analysis than yield. The fraction of fertile spikelets is particularly vulnerable to sampling errors because it relies on many data points: panicle number, total weight of filled and unfilled spikelets, and counts of individual filled and unfilled spikelets. The "panicle harvest index" (weight of filled grain/weight of panicle) is closely correlated with percent spikelet fertility in upland rice experiments, and involves much less labor and fewer sampling errors. In addition to these concerns, yield component compensation means that the variation in the total response (yield) may be considerably less than the variation in the parts (the yield components).

Near-isogenic lines (NILs) with random introgressions from a donor genotype offer an alternative to recombinant inbred or doubled haploid lines for assessing both the contribution of individual QTL to a given trait and, after

pyramiding the detected QTL, the contribution of individual traits to drought tolerance. At IRRI, a number of BC2 to BC4 lowland lines introgressed with genes from upland varieties or from wild *Oryza* species have been evaluated. It is rare to find lines that yield significantly more in the managed stress environment than the recurrent parent. This is a reminder that integrated production strategies, not individual traits or genes are the selection unit. In introgressed lines, linkage drag is also a problem. Nonetheless, interesting results are emerging from studies of NILs. In a set of NILs developed through marker-aided selection for chromosome segments that contain QTL for root depth (Shen et al. 2001), several lines differed significantly from the recurrent parent (IR64) in grain yield under stress or control conditions. These lines were of similar height and duration as IR64, but generally produced more panicles per square meter and had higher levels of spikelet fertility. These lines will now be included in more detailed physiological and genomic studies to identify how they differ from the recurrent parent in component traits. They did not differ consistently from the parent in the usual traits measured in the field, such as canopy temperature, sap production, leaf water loss rate, or relative water content, and the stress was not severe enough to expose differences in leaf drying.

The Implications for Breeding Programs

Despite the extensive work conducted at IRRI and elsewhere on the physiological basis of drought tolerance, IRRI's approach to cultivar improvement for drought tolerance has depended primarily on including natural drought-prone locations in its multi-location testing system. Two new initiatives will now explicitly incorporate managed environments into the process of cultivar development.

Research at IRRI has shown that genotype means under constant, moderate levels of moisture stress tend to be highly correlated with means when stress is imposed only during the reproductive period. However, constant stress is more easily applied to large nurseries exhibiting a range of flowering dates. Therefore, in IRRI's upland cultivar development program, early-generation lines will be subjected to selection for grain yield under drought stress imposed by restricted irrigation during the dry season, assuming that the relative performance of cultivars under this form of managed stress is predictive of their performance under naturally-occurring reproductive-stage

drought. This hypothesis must be tested by evaluating populations selected in this screening system under conditions of natural drought in the target environment.

Another approach that will incorporate screening for aerobic adaptation or drought tolerance is based on an advanced-backcross QTL approach (Li et al. 1999). In this project, donor alleles will be backcrossed into a recurrent parent, and selection with water stress will take place in the BC2F2 and BC3F2 generations.

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2.2 Perspectives of Drought and Rice in China with a Focus on the Yangtze Basin

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Abstract

Rice is the most important and most widely cultivated food crop in China. Most rice fields in China are concentrated in areas such as the Yangtze basin where water is relatively abundant and irrigation facilities are generally available. Traditionally, flood irrigation has been the only way to counteract drought, but this consumes huge amounts of water. However, with the rapid economic development and urbanization in recent years, China is now facing an increasingly serious crisis of water shortage. The National plan of transferring water from South (Yangtze River) to Northern China has been started to balance the distribution of water, and the project will be completed in the near future. The traditional practice of rice cultivation may not be able to continue for long in many areas unless new approaches to drought tolerance of rice are found. Major strategies under investigation include: 1) Water-saving irrigation and cultivation technologies, such as alternate dry-wet irrigation technology, dry nursery beds for rice seedling, plastic film covered dry land cultivation of rice, and upland rice cultivation. 2) Genetic improvement of the drought tolerance of rice cultivars, including the screening and use of drought-tolerant germplasm using molecular biology techniques.

Rice in China: Regional Distribution and Climate

The mainland of China consists of three geographic regions: the eastern monsoon region, the Xinjiang-Inner Mongolia arid region, and the Qinghai-Tibetan plateau region. Almost all the rice production takes place in the eastern monsoon region, which makes up only 45% of the total land area but is inhabited by 95% of the total population.

The eastern monsoon region can be further divided into four subregions: 1) Temperate humid and semi-humid Northeast China, that includes the three provinces of Liaolin, Jilin, and Heilongjiang. The temperature condition of

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this region generally allows one crop of *japonica* rice, but rice cultivation is limited in small areas where rainfall is relatively higher and/or irrigation is available.

- 2) Warm temperate humid and semi-humid Northern China, including provinces such as Hebei, Henan, Shandong, and Shanxi. Both temperature and rainfall conditions of the region are slightly better for rice as compared with that of Northeast China. Still, water shortage is the major limitation for rice production in this region. As a result, rice-growing area of the two regions together makes up only about 10% of the total. The rice-growing area in these regions has showed a tendency to decrease due to increasing shortage of water resources in recent years.
- 3) Subtropical humid Central and South China (Yangtze River region), including Hubei, Hunan, Jiangxi, Anhui, Jiangsu, Zhejiang, Fujian, and Sichuan provinces.
- 4) Tropical humid South China, including some parts of Guangdong, Guangxi, Yunnan, and Hainan province. The climate resources of both 3) and 4) are favorable for rice production. As a result, 90% of the rice-growing areas in China are distributed in these regions. And 70% of the total rice-growing area is concentrated in the Yangtze basin, which covers the most densely populated areas of Central and South China. The warm and wet climate of this vast area is well suited to rice production. The average yield of rice in the Yangtze basin is also relatively higher. (Table 2.2.1).

Table 2.2.1. Rainfall and temperature conditions of subregions of the eastern monsoon region.

Region	Consecutive days above 10°C (d)	Annual rainfall (mm)	Crops of rice per year	Rice area (%)
Temperate humid/semi humid Northeast China	125-150	400-1000	1	2
Warm temperate humid/semi-humid North China	150-225	500-1000	1	8
Subtropical humid Central and South China	200-250	1000-1800	1-2	70
Tropical humid South China	250-365	1400-2000	2	20

Drought in China: Water and Its Distribution

China's annual total of fresh water resources is 2812.4 billion m³, which ranks 6th in the world. But when divided by 1.3 billion people, the volume per person works out to only about 2,160 m³, close to just 1/4 of the world average. China's vast farming land is also generally in shortage of water - the volume per hectare farming land is nearly 21,900 m³, which is about 1/2 of the world average. Consequently, drought prevails and drought alone accounts for 60% of all the natural disasters in China. It is estimated that the annual water shortage in agriculture is about 30 billion m³. Annual drought-affected area of farming land is about 20 million ha and average loss of grain yield by drought amounts to almost 10 million tons. With the rapid economic development and urbanization taking place in China, the country is now facing an increasingly serious crisis of water shortage. It is estimated that the annual loss of national economy from water shortage amounts to over \$25 billion in China.

A prominent characteristic of drought in China is the uneven distribution of water resources between different regions. The eastern monsoon region has greater water resources compared with those of the Xinjiang-Inner Mongolia arid region. Within the eastern monsoon region, the Yangtze basin and South China are relatively richer in water resources, while the Yellow River basin and North China are poorer in water supply (Table 2.2.2).

The National plan of transferring water from South (Yangtze river) to Northern China was started in order to relieve the increasingly severe drought in Northern China. This ambitious project includes three water transportation lines. Water will be transported from the upper, middle, and lower reaches of the Yangtze and its branches to the Yellow basin and the big cities such as Beijing and Tianjin in North China. The construction of the middle line will start soon and the first stage project will be completed in the near future. After it is completed, an annual total of 14.5 billion m³ of water will be transferred

Table 2.2.2. Comparison of water resources of two major rivers and their associated regions in China

River	Annual runoff (%)	Basin		Associated region	Water resource (%)	Farmland (%)
		Area(%)	Population (%)			
Yangtze	37.7	18.8	35	Central/Southern	81	36
Yellow	2.2	7.8	8	Northern China	19	64

from River Han, a branch of the Yangtze, to Northern China, where water is badly needed. In the meantime, the runoff from the lower reaches of River Han will be cut off by one-third to half. Rice production in the Jiangnan basin, which is a major rice zone in Hubei province, could be affected because River Han is its main source of irrigation water. It was estimated that the annual agricultural loss in these areas would be about \$ 100 million.

Drought in China: Seasonal Variation

Most of the rainfall in the eastern monsoon region is brought by the summer monsoon. The monsoon cannot reach the Xinjiang-Inner Mongolia arid region because the Qinghai-Tibetan plateau blocks its way. The unstable activities of the monsoon and other climatic phenomena frequently cause the occurrence of seasonal drought in different regions (Table 2.2.3.).

Table 2.2.3. Frequency of occurrence of drought (%) in different regions of China.

Region	Spring	Summer	Autumn	Winter
Northwestern	70-90	>90	50-70	10
Northeastern	30-50	50-70	30-50	10
inner Mongolia	50-60	70	80-90	10
Yellow basin	50-70	30-50	50-70	10-20
Yangtze basin	30	50	30-50	20
South China	30	30-50	50-70	30-50
South-western	50-70	30-40	30-50	30-90

Although the wet monsoon normally brings ample rainfall to the Yangtze basin in most years, its arrival and duration is erratic. As a result, the rainfall is erratic and frequently causes seasonal drought or flooding in this area. Drought prevails in summer and drought stress is the single most important constraint in rice production in the Yangtze basin as well as in the other regions of China. Traditionally, irrigation facilities are available for most rice fields in the Yangtze basin. However, when prolonged droughts occur and the water is in short supply, the rice production suffers severe losses in yield.

Drought and Rice: Cause and Effect

While drought is a major factor that affects rice production, extensive rice production in turn may also be a cause of intensified drought in China.

National statistics show that agriculture consumed 70.4% of the total water used in China (1997), of which more than 70% was used for rice irrigation. That means rice alone consumed about half of the water resources used in China. Thus, developing water-saving rice production methods is not only necessary for the drought tolerance of this important food crop, but essential for the sustainable development of all agriculture in China.

Approaches to developing drought tolerance of the crop and its water-saving cultivation technology have achieved substantial progress in recent years. The water-saving cultivation technologies under development include:

- 1) Water-saving irrigation technologies, such as dry-wet alternative irrigation technology.
- 2) Technology of dry nursery bed for rice seedling.
- 3) Plastic film-covered dryland cultivation of rice.
- 4) Upland rice cultivation technologies.

The value of water conservation, the yield potential, and the suitable areas for extension of different technologies have also been under investigation.

Another major strategy enjoying high priority aims at the genetic improvement of the drought tolerance of rice cultivars, including the screening and the utilization of drought-tolerant germplasms using molecular biology techniques such as QTL mapping and marker-aided selection of traits associated with drought tolerance in rice. Breeding of upland and lowland varieties or hybrids with the combination of drought tolerance and high yield potentials is also being carried out. New research centers for this research have been set up at the Huazhong (Central China) Agricultural University and the National Rice Institute of China, with the support of the Chinese government and the Rockefeller Foundation.

Both water-saving cultivation and water tolerance breeding strategies require a more thorough understanding of the physiology of plant water status under drought stress. Physiological research of crop responses to drought stress, such as the root system and water uptake, plant water potential *via* soil water potential, leaf water potential and water use efficiency, and physiological responses to water deficit in rice also needs more attention.

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2.3 Classification of Drought Injury and Enhancement of Rice Drought Tolerance in Central and Southern China

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Drought injury is one of the major constraints to rice production, with high frequency of occurrence, clearly seasonal characteristics, and large area of occurrence in central and southern China. This paper classifies the major drought types during the rice-growing season as spring, summer, and autumn drought, describes their effects on rice production, and defines that the damage to the late stage of rice crop from hot summer or autumn drought is the largest limitation to the yield. In addition, the spring and early summer drought also bring severe damage to the crop establishment of early and middle season rice in drought years. Developing drought-tolerant cultivars and water saving cultivation methods are the fundamental solutions to the drought problem of this area.

Rice is mainly distributed in the humid and semihumid regions where the mean annual precipitation is above 700 mm, covering the middle and lower reaches of the Yangtze River and some parts of the Yellow River valley, and involving the southern China two-crop rice zone and the central China single- or two-crop rice zone. This area is particularly suitable for rice production, with the climatic characteristics of ample rainfall and warm weather in most parts of the year. It is thus the largest rice-producing area, occupying above 80% of the total rice-producing area of China, and holds a critical position in China's grain production (Wang et al. 1998). However, drought stress is still one of the major constraints to rice production in this area, mostly due to spatially and seasonally uneven distribution of rainfall, frequently causing regional and seasonal water shortage or even severe shortage. Due to lack of adequate drought control measures, the extent of severity of drought in these areas sometimes surpasses that in certain parts of northern China, such as Beijing and Xingjiang. Investigating cultivation methods for enhancing drought tolerance and developing drought-tolerant cultivars is strategically important for stabilizing the grain production in China.

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Some Characteristics of Drought Injury in Central and Southern China

Regionally High Frequency of Occurrence

According to the official statistics from 1470-1990 (NGHOFDC and NIHRR, 1997), there were 7 extremely severe droughts, 67 severe droughts, 318 slight droughts, and 12 two-year consecutive severe droughts that affected the middle reaches of the Yangtze River in the past 541 years. The situation in the lower reaches of the Yangtze and in southern China was not much better, with even six consecutive years of severe drought recorded (1963-1968 in the former, and 1898-1903 in the latter). From 1900 on, the frequency of occurrence and severity of drought have been showing a tendency to increase with the global climatic changes, in that severe drought occurs once or more every five years. In Hubei province, there were 14 severe droughts in the last 50 years, affecting over two million ha of crops (Hong, personal communication).

Large Drought-Affected Areas

In general, in the southern parts of the Yangtze River, the ratio of drought-affected arable area to the total arable area was around 10-30%, and the ratio of disaster-affected arable area to the drought-affected arable area about 30-50%. The recorded largest drought-stricken area reached 1.32 million km² and the largest severe drought-stricken area covered 808,000 km², which comprised 80.7% and 49.4% of the total area, respectively (NGOHFDC and NIHRR 1997).

Clearly Seasonal Characteristics

Every season from spring to winter may suffer drought injury in this area, but each different region has its own seasonal characteristics. In the middle and lower reaches of the Yangtze River, the major types are summer and autumn droughts, with the worst damage to the crop production from the hot summer drought; while in most parts of southern China and the Zhujiang River valley, the winter and spring drought prevail, with the worst damage caused by spring drought. In several other parts, the hot summer and/or autumn drought are most severe.

Types Of Drought Injury in Rice in the Drought-Prone Area of Central and Southern China and Their Effects on Rice Production

Types of Drought Injury

Spring Drought

A dry and low rainfall weather type that occurs from March to May, mainly attacking the coastal parts of Guangdong, Leizhou Peninsula, southwestern Hainan, most (especially western parts) of Guangxi, and several parts of Hunan and Hubei, with a frequency of occurrence of about once a year in some places.

Summer Drought

It can be divided into early summer drought and hot summer drought.

Early Summer Drought

A drought type that occurs mainly in the Yangtze River valley, from the last ten days of May to the 20th of June, when the rains are delayed or sometimes do not appear.

Hot Summer Drought

A dry and high temperature weather type that frequently occurs from the last ten days of June to the 10th-20th of August after the rainy season, mainly affecting Hubei, Hunan, Jiangxi, western Zhejiang and most parts of Guangdong and Guangxi, with a frequency of occurrence of about four times every five years.

Autumn Drought

A long dry weather type that occurs from September to November when the dry monsoon arrives, affecting wide parts of central and southern China, especially Hubei, Hunan, Fujian, Jiangxi, and Anhui, occurring about thrice every five years.

Seasonal Successive Drought

Several types of seasonal droughts may occur successively sometimes, which cause severe damage over a long period, such as hot-summer-autumn successive drought in the Yangtze River area; winter-spring successive drought in southern China; and spring-autumn and four-season successive drought in some other places in central and southern China.

The Effects of Different Drought Types on Rice Production

The early spring drought corresponds with field preparation, sowing, and/or seedling transplanting of early or middle season rice, which can hinder seeding, transplanting, and/or crop establishment. The late spring and early summer drought usually correspond with the tillering stage of early or middle season rice, which can cause reduction in the tillering and rooting capacities, root function, leaf senescence or even death, and result ultimately in decrease of effective heads and yield loss (Mackill et al. 1996).

Hot summer drought generally occurs at the critical water stages of grain filling and seed-setting of early season rice, and the reproductive stage of middle season rice, which usually causes severe damage to the rice production due to long duration of drought and high atmospheric temperatures causing strong evapotranspiration. Its major effects on early season rice include disturbing the assimilation and the assimilate translocation, leading to reduction of grain weight and yield loss. On middle season rice, damage to panicle initiation and the meiosis of pollen mother cells occurs, desiccating spikelets and anthers, disturbing anther dehiscence and pollen shedding, inhibiting panicle exertion, and causing severe reduction of seed setting (Mackill et al. 1996).

Autumn drought generally corresponds with the flowering and grain filling stages of late season rice, which can cause reduction of seed setting, grain weight, and grain yield. Drought for successive seasons can cause damage to rice crop at any stage and even result in crop failure.

Several Considerations on Enhancing Rice Drought Tolerance in the Area

Studies on the Mechanism and Traits of Rice Drought Tolerance

At present, there is still a lack of systematical knowledge of plant drought tolerance, although many studies have been carried out on the drought tolerance mechanism and traits. Further understanding of rice drought tolerance on morphological, physiological, and molecular levels will play a very important role in breeding drought-tolerant cultivars and developing new cultivation methods.

Drought damage at the late stage is the largest constraint to every rice crop. The mechanisms of maintaining continuous rooting ability, assimilation, and panicle water potential; improving assimilate translocation under stress at the late stage; and the correlation of morphological and physiological traits with yield thus urgently need investigation. Meanwhile, investigating the critical point of soil water content for root elongation and function maintenance defining the functional relationship between transpiration rate and plant physiological function expression under stress; correlations between xylem hydrological conductance with plant drought injury, and of osmotic adjustment with plant water status; and the mechanism of restorative and compensatory growth after stress at vegetative stage are also important for understanding the varietal difference of tolerance potential at different stages.

Selection and Utilization of Drought Resistant Cultivars

Screening the Available Germplasm and Developing Cultivars with Relatively High Drought Adaptation and Yield

Very limited systematic research has been done on rice drought tolerance and in breeding tolerant cultivars. It is, therefore, necessary to screen available cultivars adapted to drought-prone regions for their performance in experiments on drought. This would be a basis for establishing field screening methods suitable to the drought stress pattern prevalent in this area. The development of these drought-tolerant cultivars will bring direct impact on rice production.

Genetic Improvement of Drought Tolerance

Breeding tolerant cultivars is a fundamental solution to the drought constraints in rice production, based on studies on the physiological and genetic mechanisms of rice drought tolerance in this area. During those studies, incorporating the drought avoidance, known as active mechanism, with drought tolerance, as a positive mechanism to increase the overall drought tolerance of rice is a strategically long-term goal.

Root traits, shoot morphological characters, stomatal action, and water-use efficiency conferring drought avoidance; and osmotic adjustment and dehydration tolerance conferring drought tolerance, have received wide attention, and other traits or characters may be developed in the future.

New Cultivation Methods for Full Use of the Potential of Drought-Tolerant Cultivars

Improving cultivation systems has long been recognized as a practical approach to enhance crop drought tolerance. Raising seedlings on dry beds can stimulate rooting, producing healthy seedlings and improved transplanting survival, helping seedlings evade drought. Drying fields moderately, known as hardening, at tillering stage can stimulate nodal rooting from early tiller and forming deep rooting (Kondo et al. 1999) and improve water-use efficiency (Cabuslay et al. 1999). The plastic-sheet-covered cultivation developed recently in some parts of China has also proved practical for enhancing rice drought tolerance and water conservation.

Some plant growth regulators have been shown to be involved in the expression of plant drought tolerance. Examples are regulation of stomatal closure, induction of gene expression for drought adaptation (Mugo et al, 1999), and increase in drought tolerance and restoration after stress (Blum, 1999) by abscissic acid (ABA); and paclobutrazol promoting tillering and rooting. Mineral nutrients are also involved in expression of plant drought tolerance - phosphorus can increase rooting number and depth, while potassium can improve metabolism under stress.

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2.4 Yield Responses of Rice (*Oryza Sativa* L.) Genotypes to Water Deficit in Rainfed Lowlands

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and J C O'Toole⁵**

Different types of drought stress frequently reduce grain yield of rainfed lowland rice in northeast Thailand. This study aims to: 1) examine genotypic variation for grain yield and to assess the magnitude of responses of genotypes for grain yield under various types of drought stress conditions in target drought-prone areas in Northeast Thailand, 2) investigate genotypic expression of putative drought-tolerant traits and their contributions to grain yield under drought stress conditions, and 3) to evaluate the use of the dry season screening for drought tolerance to estimate grain yield under drought stress in the wet season. A further goal of the present study is to integrate the information acquired into the modified breeding strategy that has been recently proposed by a project funded by the Australian Centre for International Agricultural Research (ACIAR, Fukai and Cooper 1999), so that selection efficiency for drought-tolerant genotypes can be improved.

Four sets of field experiments were conducted under lowland conditions in the upper and lower parts of northeast Thailand. Each of the first three sets consisted of non-stress and drought experiments, and there were eight wet season experiments. The first three sets of experiments conducted in the wet season were used to investigate genotypic variation for grain yield and putative drought-tolerant traits in the wet season. To ensure application of drought stress, experiments were seeded later than the normal seeding time practiced in this region. Different types of drought stress were imposed in each set of experiments, i.e. mild drought stress during the grain filling period in experiment 3, severe drought stress just before flowering in experiment 5, prolonged severe drought stress during the reproductive to the grain filling

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stages in experiment 7, and prolonged mild drought stress during the vegetative and the grain filling stages in experiment 8. Grain yield of each genotype under the drought stress conditions in these four experiments was adjusted using potential grain yield and flowering time under well-watered conditions to determine drought response index (DRI, Bidinger et al. 1987). The fourth set of two experiments (experiments 9 and 10) were conducted over two dry seasons to examine genotypic variation for drought score (leaf death score) and investigate the reliability of using the drought score measured in the dry season to estimate grain yield measured under drought stress conditions in the wet season. Randomly sampled sets of 50 to 128 recombinant inbred lines derived from 4 biparental crosses (Immark et al. 1997) were used for all experiments.

A large genotypic variation existed for grain yield under both irrigated and drought stress conditions. Depending on timing, duration, and severity of plant water deficit and seeding time, grain yield of genotypes under drought stress, in relation to that under irrigated conditions, was reduced by 18% in experiment 3, 55% in experiment 5, 81% in experiment 7, and 52% in experiment 8. In each drought stress condition, the DRI described the magnitude of the response of genotypes for grain yield and identified drought-tolerant genotypes. The DRI of the genotypes was inconsistent across the four drought stress environments in experiments 3, 5, 7, and 8, indicating that genotypes respond differently with the change in characteristics of the drought stress conditions.

Genotypes that were able to maintain high panicle water potential (PWP) during the drought stress period that developed just before flowering time (experiment 5) produced higher DRI, grain yield, harvest index, filled grain percentage, and fertile panicle percentage (Fig. 2.4.1). Drought-tolerant genotypes maintained significantly higher PWP than susceptible genotypes (-1.83 ± 0.016 vs. -1.97 ± 0.043 ; $P < 0.01$), and produced more fertile panicles ($74.3 \pm 3.7\%$ vs. $34.3 \pm 8.7\%$; $P < 0.01$), filled grains ($58.7 \pm 2.5\%$ vs. $31.8 \pm 6.3\%$; $P < 0.01$) and grain yield (2.40 ± 0.10 vs. 1.02 ± 0.08 t ha⁻¹; $P < 0.01$). Genotypes with a larger amount of the above ground total dry matter at anthesis (TDMa), tall plants, and larger root systems depleted soil water more rapidly than genotypes with smaller TDMa, shorter plants, and smaller root systems, and resulted in the larger decrease in PWP.

Drought stress delayed the flowering time of genotypes on average 3 ± 0.22 days (experiment 5) and 10 ± 6.3 days (experiment 7). In both

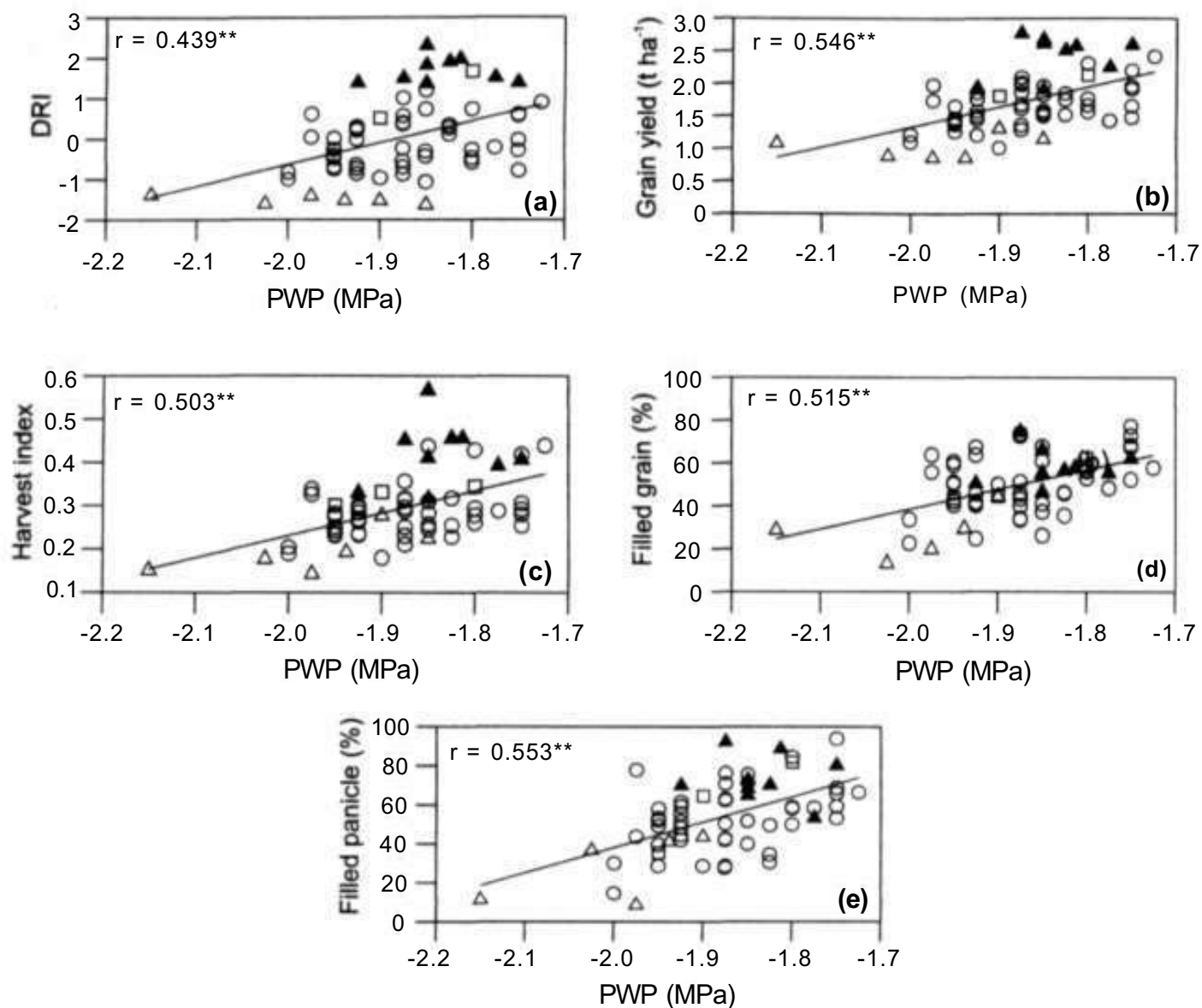


Figure 2.4.1. Relationship between predawn panicle water potential (PWP) and DRI (a), grain yield (b), harvest index (c), filled grain percentage (d), and fertile panicle percentage (e) of group 2 genotypes in experiment 5 and the check cultivar KDML105. % refers to drought tolerant, intermediate, + susceptible genotypes. (Pantuwan, 2000).

experiments 5 and 7, a larger delay in flowering time was associated with greater reduction in grain yield, harvest index, and filled grain percentage (Fig. 2.4.2 a-h). In experiment 5, delay in flowering time was strongly associated with low PWP ($r = -0.387^{**}$); hence genotypes with larger delay in flowering time suffered more drought stress since they flowered when available soil water was lower.

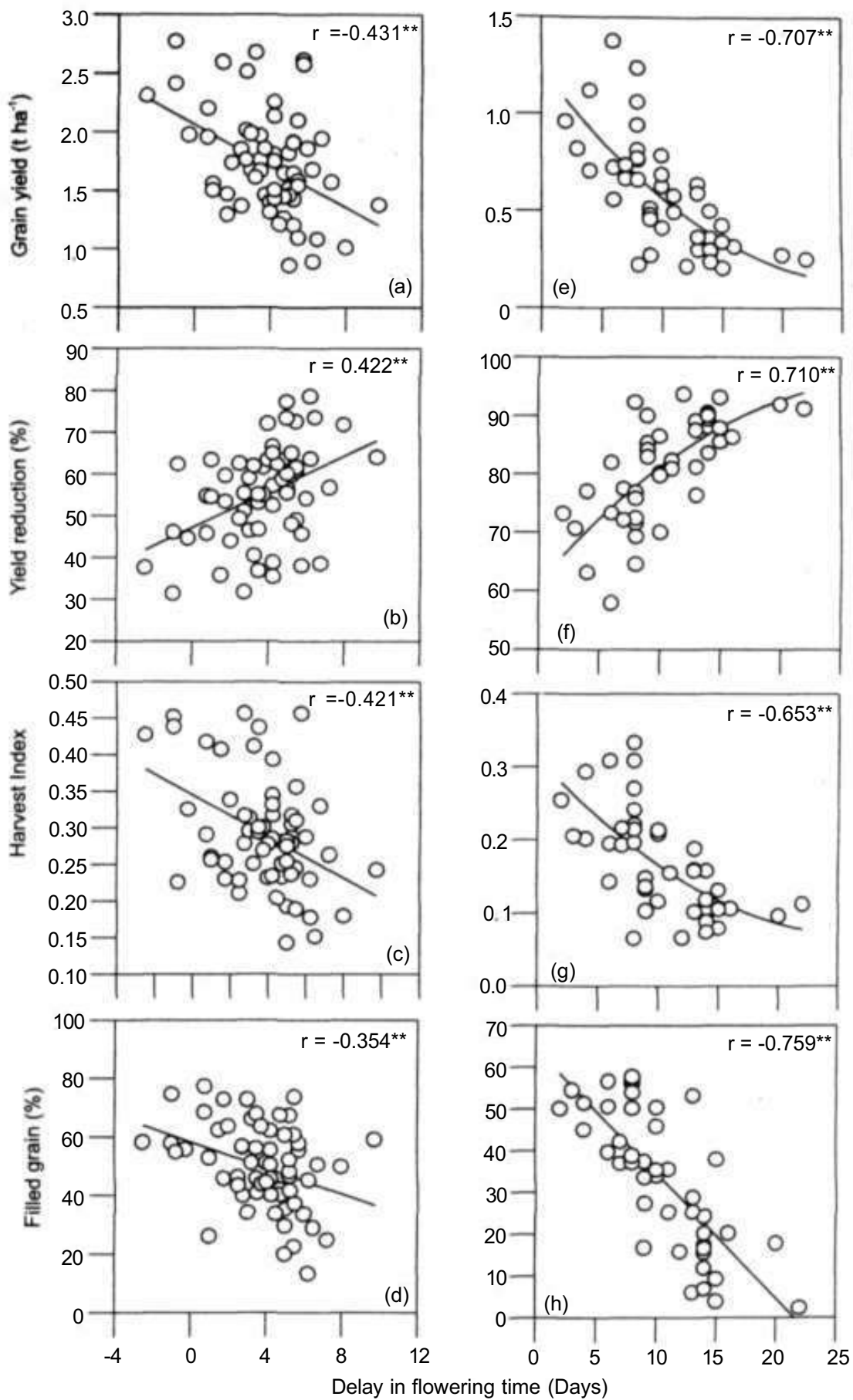


Figure 2.4.2. Relationship between delay in flowering time and grain yield, yield reduction, harvest index and filled grain percentage for genotypes grown under drought stress conditions in experiment 5 (a, b, c, and d, respectively) and 7 (e, f, g, and h, respectively). (source: Pantuwan 2000).

Larger drought score determined in the dry season was associated with lower grain yield under drought stress conditions in the wet season experiments; but the association was significant only in particular conditions, i.e. when patterns and severity of drought stress in both environments were similar. The dry season screening conditions for drought score screen should be managed to correspond to relevant types of drought conditions in the wet season in the target areas. Analysis of a data set of experiments for drought stress grain yield and drought score indicates that if 50% of lines had been discarded on the basis of high drought score (susceptible to drought), the mean performance of the remaining lines was improved by 272 kg ha⁻¹ (grain yield of high vs. low drought score genotypes was 1.57 vs. 1.84 t ha⁻¹, P = 0.000), or 17.3%.

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2.5 Morpho-Physiological Research on Drought Tolerance in Rice at WARDA

A Audebert¹, F Asch², and M Dingkuhn³

Highly variable rainfall in the forest and savanna zones of West Africa can introduce water stress to rice at any stage of crop development. The varying degree and duration of drought during the crop cycle can severely reduce rice grain yield in rainfed lowland, hydromorphic, and upland environments. Water stress has been identified as one of the most important production constraints in the West African rice environments. The diversity of affected ecosystems, variability of drought in terms of timing and severity, and the multiple traits involved, require strategic research to set priorities and develop environment-specific breeding approaches for drought resistance. Crosses of Asian rice, *Oryza sativa*, with African rice, *Oryza glaberrima*, aim at reducing the trade-offs between yield potential, weed competitiveness, and drought resistance. Research at the West African Rice Development Association (WARDA) shows that the morpho-physiological characteristics of these two rice groups are quite different. Drought tolerance is a complex trait involving several interacting physiological, phenological, and morphological mechanisms for escape, avoidance, resistance, and recovery.

The African rice, *O. glaberrima*, is weed competitive, characterized by high vegetative growth rate (shoot and root) resulting from low phyllochron, a large specific leaf area, a high light extinction coefficient, and increased partitioning of assimilates to leaf blades. This high specific leaf area is associated with a low stomatal density and low chlorophyll content. The rapid vegetative growth rate with the high light extinction coefficient maximizes the evapo-transpirative surface. In addition, carbon-isotope discrimination (CID) studies show high values for *O. glaberrima* like the lowland *O. sativa* subsp. *indica* in contrast to upland rice (*O. sativa* subsp. *japonica*).

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Rice reacted to drought stress with reductions in height, leaf area and biomass production, tiller abortion, changes in rooting patterns, and a delay in development. The effect on the plant depended on the severity of the stress. Assimilate partitioning between root and shoot was not affected by drought when the plants were gradually stressed and the drought stress was not lethal. Under constant drought, soil penetration resistance presumably interfered with root growth and partitioning. Roots grew deeper under drought stress, presumably following the soil moisture gradient. This was particularly the case in the upland adapted *Oryza sativa japonica*. Root/shoot partitioning provided no conclusive explanation for the superior performance of *O. glaberrima* under drought conditions. Therefore, it seems like *O. glaberrima* uses a large quantity of water.

Although high vegetative growth (shoot and root), large specific leaf area, and poor water use efficiency (CID) are not good characteristics for drought adaptation, *O. glaberrima* does show very good adaptation to the drought environment (low stomatal density, fast leaf rolling, and good maintenance of plant water status by the control of stomatal conductance).

These differences imply that there are different physiological mechanisms for drought avoidance in *O. sativa* and *O. glaberrima*. It is important for the development of interspecific varieties with enhanced drought tolerance that we know the physiological drought mechanisms used by *O. glaberrima*.

2.6 Field Screening for Adaptability in Drought-Prone Rainfed Lowland Rice: ACIAR Experience in Thailand and Laos

S Fukai¹ and M Cooper¹

The authors have explored genotype requirements for high yield of rainfed lowland rice for the past 10 years in Thailand and Laos through their activities in the Australian Centre for International Agricultural Research (ACIAR) and associated projects. The two main foci of the activities have been: 1) the determination of the consistency of genotypic performance for different environments by conducting multi-environment trials using a range of genotypes (35 to over 1000) evaluated over locations and years, and 2) the determination of physiological or morphological traits that confer drought tolerance and thus higher yield under drought stress.

For grain yield of rainfed lowland rice, a large genotype x environment (G x E) interaction component of variance has been consistently found. This is partly associated with the irregular pattern of drought development that causes differential effects on the genotype yield with different flowering times. Drought may develop early in the wet season causing delay or failure in transplanting, or it may develop later at around flowering time. The large G x E interaction for yield implies that it is necessary to have well-defined selection methods for efficient development of cultivars adapted to wide areas of rainfed lowlands. Selection of genotypes should be conducted in environments that are matched with those of the farmers' fields. However, when drought is mild and yield reduction is moderate, the genotype's potential yield under well-watered conditions largely determines the yield. It is therefore important to use parents with high yield potential or generally adapted to the region for crossing, to increase the chances of developing progenies that can perform well under a wide range of mostly favorable water environments.

Crop phenology is a major determinant of grain yield. Phenology can be manipulated to reduce the incidence of drought during the critical flowering period. In addition to phenology a key factor determining drought tolerance is

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the plant's ability to maintain favorable internal water conditions. Thus, the genotypes that can maintain high leaf water potential during drought are able to produce and fill a larger number of grains when drought develops just prior to flowering. This ability to maintain a high internal water status is not related to a larger root system, but to the smaller shoot. These results indicate that growing rainfed lowland rice is a conservative strategy for drought avoidance. The rainfed lowland rice genotypes adapted to drought-prone areas should have the capacity to avoid development of severe internal water stress during the period of drought, and also have high yield potential.

2.7 Breeding for Drought Tolerance in West African Upland and Hydromorphic Rice Germplasm

M P Jones¹, A Audebert¹, and M N Ndjiondjop¹

Rice grown in upland and hydromorphic conditions in West Africa is prone to varying degrees and duration of drought stress during the crop cycle, causing yield reduction and sometimes total crop failure. Breeding for drought tolerance in upland and hydromorphic rice germplasm is, therefore, a major activity at the West African Rice Development Association (WARDA).

Studies in drought tolerance at WARDA were initiated in 1991 and the breeding strategy emphasizes the need to understand the mechanisms of drought tolerance. A methodology for mass evaluation of rice germplasm for tolerance to drought at the vegetative or reproductive stages of growth was tested, found suitable, and used to screen available *Oryza sativa* and *Oryza glaberrima* accessions. Considerable variation for various traits was observed and several promising varieties have been identified that are currently being used in the breeding program. This provides a broad genetic base for drought tolerance studies. Recurrent selection to upgrade the levels of drought- and blast tolerance in upland and hydromorphic rice varieties has been implemented and several promising selections have already been made from the interbreeding populations. Considerable success has been made in the wide crossing program between *O. sativa* and *O. glaberrima* where backcrossing and embryo rescue has reduced spikelet sterility in the subsequent progenies. Anther culture of F₁ and F₂ progenies from the wide crossing is also showing success in reducing sterility and in attaining rapid homozygous lines. These progenies are showing drought tolerance at both vegetative and reproductive stages of growth. For example, there were significant correlations between tolerance scores after 21 days of moisture stress at the reproductive stage, and seedling vigor ($r = 0.22$, $P < 0.001$) and leaf-rolling ability ($r = -0.391$, $P < 0.001$) at 30 days of moisture stress in the vegetative stage. Leaf rolling, therefore, indicated that the plants suffered from stress, and did not effectively prevent moisture loss. Molecular

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approaches to the study of leaf rolling and root architecture will be used to better understand the genetic and physiological basis of drought tolerance found in rice. The strategies to develop the mapping interspecific population and to utilize the identified quantitative trait loci in improving drought tolerance in water-limited environments will be discussed.

2.8 Towards Better Understanding and Further Improvement of Drought Tolerance in Rice

Lijun Luo¹

Rice is the staple food for most Chinese people and rice production is seriously affected by drought. Due to the continued increase in population and decrease in water supply, developing high yield and drought-tolerant rice cultivars is the most important and the greatest challenge faced by rice scientists. In the past years, a total of 50 thousand rice germplasm resources maintained at the China National Rice Research Institute (CNRRI) have been screened and primarily about 400 rice accessions with different degrees of drought tolerance were identified. Some of them have been used successfully in breeding programs, and promising lines performed very well in water stress conditions. However, as drought tolerance in rice has been a very difficult problem to work with, new strategies on better understanding and further improvement of drought tolerance in rice were a particular focus. This included setting up reliable and reproducible field screening facilities and the National molecular breeding program network. The screening facilities should be based on a scientific level, should have the capacity to manage environments, water control (groundwater), schedule irrigation (time and quantity), as well as study existing soilborne biotic stress factors. The molecular breeding approaches were used to identify, characterize, and transfer the genes/QTL associated with drought tolerance of rice, to understand the physiological aspect of drought tolerance in rice, and develop high yield and drought tolerant rice cultivars.

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2.9 Improvement of Drought Tolerance of Rice In Thailand

Suwat Jearakongman¹

The Northeast is the largest region of Thailand, covering over 170,000 km². The region has a population of about 17 million, constituting over one-third of the population of the country. Rice is grown in the Northeast mainly for subsistence with any surplus being sold. Per capita income is the lowest in the country, primarily due to poor agricultural production. The major environmental constraints limiting crop yields are the poor soil quality and erratic rainfall. Drought of sufficient severity and duration to seriously affect growth and yield of rice is common in the region. Many agricultural projects have sought to overcome this constraint by finding ways to increase the water supply to crops. However, as it would be practically impossible to provide sufficient water throughout the huge rainfed areas, rice breeders are challenged to improve rice varieties for tolerance to drought. Although considerable research into drought tolerance is under way, progress in breeding to improve drought tolerance has been slow, mostly due to the lack of understanding of the genetics and physiology of adaptive traits and whether large genotype x environment (G x E) interactions are possible on the basis of grain yield. The main objectives of this research are basic knowledge and successful techniques for rainfed lowland rice grain yield improvement, in particular on screening methods used for drought tolerance in rainfed lowland rice in Northeast Thailand.

Previous experiments conducted by the author on *Growth and grain yield of contrasting rice cultivars grown under different conditions of water availability* in 1995, were carried out to elucidate how some rice cultivars achieve higher yield than others under water-limiting conditions (S Jearakongman et al. 1995). During 1997 to 1998, the study on *Effect of plot size on competition and yield of rice cultivars with different heights* was carried out to determine the influence of plot size on the yield of rice lines differing in height, to investigate how plot size influences the yield, and consequently to assess whether small plots could be used to accommodate large numbers of lines in multi-environmental trials (METs). These screening procedures will

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provide great benefit in reducing the cost and time involved in traditional screening on the basis of grain yield.

The Rockefeller Foundation provided graduate research funds to the author since 1998 for the application of molecular marker technology in rice improvement. The program is intended to strengthen the research partnership between Kasetsart University, Thailand, and rice researchers in one of the most exciting areas in rice genome research: quantitative trait loci (QTL) for late-season drought in rainfed lowland rice. The research project to identify QTL for complex traits associated with drought tolerance will enable us to reevaluate germplasm for positive alleles, and pyramid these alleles. Breeding crops for drought tolerance offers an excellent opportunity for international collaboration. The materials and the data development during such collaboration will also help better understand physiological and developmental phenomena in rainfed lowland rice. When a list of stable QTL is available, specific traits that show real prospects for increasing yield under moisture deficit, level of polymorphism, breeding objectives, and facilities available to each breeder will determine the most appropriate balance between marker-assisted selection (MAS) and conventional breeding. The expected benefits from this project will be modification of the existing plant breeding programs for rainfed lowland rice in drought-prone areas of Thailand by incorporating findings on selection strategies for the region. The breeding program, some years after completion of the project, could release new cultivars that will produce higher and more stable yields than are possible with existing cultivars. Increased production should then lead to improved welfare, mostly of subsistence farmers in the target regions.

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Session 3: Marker-Assisted Breeding in Rice

3.1 Marker-Assisted Breeding versus Direct Selection for Drought Tolerance in Rice

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Abstract

Traits related to drought tolerance are the focus of much research to identify quantitative trait loci (QTL). The objective of this research is to improve grain yield under drought stress by incorporating favorable alleles for these loci into useful cultivars. For this strategy to be successful, the traits in question must be highly correlated with yield under stress, at least as highly repeatable, and conditioned by alleles with large effects. Little evidence exists to support these assumptions for drought-related traits in rice. The repeatability or broad-sense heritability (H) of a trait and the number of progeny (N) evaluated determine the power of experiments designed to detect linkage between markers and QTL. Simulations indicate that, although mapping populations with as few as 100 recombinant inbred lines can be used to detect QTL with very large effects, populations of 300-400 lines must be phenotyped with $H = 0.6$ or greater to achieve useful power in detecting QTL of moderate effects (with 10% of genetic variance). Populations of this size have not been used in rice QTL mapping experiments. Phenotyping resources must be carefully allocated in designing molecular dissection experiments for drought, taking into account both within-experiment and across-experiment variances. Only variance component estimates derived from two or more experiments permit unbiased estimation of H . Estimates derived from a single field trial or controlled environment are invariably biased upward. H was estimated for several drought-related traits in mapping populations screened over more than one experiment. Except for leaf rolling and leaf drying, these traits were not more repeatably measurable than grain yield under stress. In all of the experiments, the genotype \times experiment variance contributed significantly to phenotypic variance, and within-experiment variances were high. Most H estimates were well below the level required to give adequate power to reliably detect QTL. The low H estimates indicate that it is unlikely

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that QTL with effects large enough to use in marker-assisted selection exist for most of these traits. Grain yield in managed stress environments has moderately high heritability, and is a more promising target for molecular dissection and breeding efforts aimed at improving drought tolerance in rice.

Introduction

Little progress has been made in improving the drought tolerance of rice, despite considerable effort devoted to physiological studies and cultivar screening. Much of this effort has been devoted to secondary traits considered to be determinants or indicators of drought tolerance, rather than to the direct study of grain yield under moisture stress (Fukai et al. 1999). These secondary parameters include anatomical traits (e.g. root system architecture), physiological parameters (e.g. osmotic adjustment), visible indications of plant stress (e.g. leaf rolling, leaf drying) or measures of plant water status (e.g. leaf water potential), and have recently become the focus of much research aimed at identifying quantitative trait loci (QTL). The objective of this effort is to improve grain yield under drought stress by:

- Locating genes controlling the component traits contributing to drought tolerance, and identifying favorable alleles at these loci via QTL analysis.
- Introgressing these favorable alleles into useful cultivars via marker-assisted selection (MAS).

For this molecular breeding strategy to be effective, the following criteria should be fulfilled:

- A gene or genes with large effects should control the secondary trait.
- The secondary trait should be highly correlated with yield under stress.

It is the purpose of this discussion to briefly set out the reasons for applying these criteria to the planning of molecular breeding programs. We will also examine the extent to which they are met for drought tolerance breeding in rice.

Reasons for Detecting Genes with Large Effects

Agricultural researchers often assume that QTL mapping can be reliably used to develop workable MAS systems for low heritability (H) quantitative traits affected by many genes. This is not usually the case. This is because H must be

high in mapping experiments and gene effects must be large in order for QTL locations and effect sizes to be reliably estimated, for reasons that will be discussed below. As a result, most successful applications of MAS for tagged stress-tolerance QTL have involved traits that are of high heritability and that are controlled by relatively few genes. The principal feature of traits of this kind is that phenotypic differences between susceptible and tolerant genotypes are large and highly repeatable across screening experiments. When QTL mapping experiments for these traits are conducted with small populations (N=100 to 200), QTL with very high LOD scores (>10) are often detected and account for a large proportion of the genetic variation for the trait. One example of such a trait in rice is submergence tolerance, which is greatly influenced by the *Sub 1* gene on chromosome 9. Xu and Mackill (1996) originally detected this gene in a population of 169 F₂-derived F₃ lines as a QTL with a LOD score of over 30, and with an R² value of nearly 70%. Another example is a gene for phosphorus uptake under P-deficient conditions, which was detected by Wissuwa et al. (1998) as a QTL with a LOD score of 10 in a population of 98 recombinant inbred lines. Both genes were later fine-mapped and successfully transferred to elite varieties via marker-assisted backcrossing, with the resulting introgression lines exhibiting high levels of tolerance (Wissuwa and Ae 2000, Mackill et al. 1999).

In a contrasting example, Yadav et al. (1997) conducted a QTL analysis on root parameters in a population of 105 doubled-haploid (DH) lines from the cross IR64 x Azucena. Four large Azucena chromosome segments putatively carrying alleles enhancing root length were then introgressed in IR64 via marker-assisted backcrossing. Of the 29 resulting BC₃ -derived introgression lines that were phenotyped, only 11 had root characteristics that differed significantly from those of the recurrent parent (Shen et al. 2001).

Why are the QTL detected and mapped in some studies reliably transferable and those in others not? How can efforts be focused on those traits that are likely to be amenable to MAS? To answer these questions requires a consideration of the factors affecting the power of experiments designed to detect linkage between markers and QTL. In QTL mapping studies power is determined by the repeatability or broad-sense heritability (H) of a trait, the proportion of the genetic variance accounted for by the QTL, and the number of progeny (N). If a single QTL explains a large proportion of the genetic variance for a trait and H is high (say, >0.6), the power of QTL mapping experiments is high, even when relatively small

populations are used. On the other hand, power studies have conclusively shown that QTL with small effects cannot be reliably detected or their sizes accurately estimated in small populations. In one such study, Beavis (1998) used genetic simulation to evaluate the effect of N and H on the power of QTL mapping experiments to detect QTL accounting for 10% of the genetic variance in populations of 100 or 500 F₂ individuals. Heritability levels of 0.3, 0.63, or 0.95 were assumed. The expected value of the R² statistic, generated by many QTL mapping programs, is the product of H and the proportion of the genetic variance explained by the QTL; expected R² values for these QTL were therefore 3, 6.3, and 9.5% for the three H levels, respectively. In populations of 100 F₂ individuals, the probability of detecting a QTL explaining 10% of the genetic variance was very low, never exceeding 40% even for the highest level of H. In populations of 500, power was highly sensitive to H, ranging from 57% for H=0.30 to 94% at H=0.95. Acceptable power was achieved at a heritability level of 0.63.

In addition to having low power to detect small-effect QTL, mapping experiments are unreliable in estimating both their effects and locations. When N= 100, Beavis (1998) reported that QTL effect sizes were consistently overestimated. In a simulated population of 150 doubled-haploid lines evaluated at H=0.5, Hyne et al. (1995) reported that the 95% confidence interval for QTL accounting for as much as 20% of the genetic variance (corresponding to an expected R² of 10%) was 35 cM. Thus, in summary, attempts to improve drought tolerance by MAS involving small-effect QTL identified by mapping at low H levels in small populations are likely to fail because:

- The QTL cannot be reliably detected;
- Of those that are detected, effect estimates are often biased upwards; and
- Detected QTL are too imprecisely mapped to be reliably transferred via MAS.

The Importance of Maximizing Heritability for QTL Detection

As noted above, the prospects for detecting large-effect QTL for drought-related traits in mapping populations of 100 to 200 lines are much more favorable than for genes with small effects. This is confirmed both by power studies (Zeng, 1994) and the empirical examples of involving phosphorus

deficiency and submergence tolerance described above. However, even for genes with large effects, detection in small mapping populations is sensitive to H in the experimental system. H should be reported for all QTL mapping experiments, and is an important indicator of the reliability of the results. Roughly speaking, the minimum level of H that must be achieved to detect a QTL accounting for 20% of the genetic variance is 0.5. It is therefore useful to consider how H is estimated, and how it can be maximized in phenotyping experiments.

H is not a constant but is determined by the level of replication within and across experiments. In many experimental systems, including both greenhouse and field trials, there are two important error strata or sources of random variability, which must be taken into account when estimating H: the within- and among-trial errors. H is estimated from a combined analysis of repeated phenotyping experiments as:

$$H = \frac{V_G}{V_G + \frac{V_{GE}}{e} + \frac{V_E}{re}} \quad [1]$$

where V_{GE} is the genotype x experiment (or environment) interaction variance, V_E is the pooled residual error from individual experiments, e is the number of experiments from which genotype means are derived, and r is the number of replicates per experiment. It should be noted that the V_{GE} referred to in this equation is a true error component, not an indicator of specific adaptation to an environment. A random V_{GE} component will usually exist in any series of repeated laboratory, greenhouse, or field trial, even when the most stringent measures have been taken to reduce variability among experiments.

Inspection of Equation 1 indicates that H can be increased by increasing replication levels and repeating experiments. In order to reliably detect large-effect QTL in mapping populations of 100 to 200 DH lines, replication should be sufficient to push H to a minimum of 0.5 to 0.6. H is best thought of as the repeatability of estimates of line means. It is also the expected correlation between means estimated in independent sets of e experiments with r replicates. Only estimates of H derived from two or more experiments permit estimation of V_{GE} , which is required for unbiased estimation of H. H estimates derived from a single field trial or controlled environment are invariably biased upward because genotype and genotype x experiment effects

cannot be distinguished, with the result that V_G and V_{GE} are completely confounded (Comstock and Moll 1963). The potential magnitude of the resulting bias in H can be appreciated by comparing the "true" expectation for H for a single experiment based on variance component estimates from the analysis of a series of similar experiments (Eq. 2) with the expectation for H from the analysis of a single, unrepeatable experiment (Eq. 3, denoted here H'):

$$H' = \frac{V_G}{V_G + V_{GE} + \frac{V_E}{r}} \quad [2]$$

$$H = \frac{V_G + V_{GE}}{V_G + V_{GE} + \frac{V_E}{r}} \quad [3]$$

Because random V_{GE} can be large relative to V_G , particularly in agronomic field trials (e.g. Atlin et al. 2000), upward bias in heritability estimates from single experiments is often very large, and may seriously mislead researchers about the reliability of their QTL phenotyping experiments. Unless there is great confidence in the repeatability of a screening system, phenotyping experiments should be repeated and QTL analyses conducted on the means over experiments, rather than on the individual trials.

Heritability Estimates and Their Implication in MAS

Heritability estimates for drought-related traits can yield important clues about both the existence of large-effect QTL and our ability to detect them. If a high level of "true" H (see Equation 1) can be achieved from individual phenotyping experiments, it is likely that QTL with large effects can be detected in a small mapping experiment. If the examples for phosphorus deficiency and submergence tolerance related above are representative, a very high level of repeatability under repeated screening may also be empirical evidence that such QTL exist.

Several upland rice-mapping populations have been screened for drought-related traits in greenhouse, field, or controlled-environment experiments that were repeated at least twice, permitting realistic estimates of H to be produced. In general, these populations are small (fewer than 150

genotypes), and have been phenotyped at relatively low levels of replication. Two such populations, evaluated for drought-related parameters, are discussed here to illustrate the H levels that may be found for particular traits (Table 3.1.1).

Response of an IR64/Azucena DH Population to Water Stress

A double haploid (DH) population (IR64/Azucena) of 105 lines was evaluated for leaf rolling, leaf drying, relative water content, and yield under stress conditions at the International Rice Research Institute (IRRI) in the dry seasons of 1995 and 1996. Courtois et al. (2001) reported QTL analyses for these experiments. An unbalanced subset of these lines was screened for grain yield under reproductive stage stress in replicated field trials in the dry seasons of 1995, 1996, and 1999. For all traits, data were subjected to a combined variance component analysis via the restricted maximum likelihood (REML) algorithm, according to a linear model recognizing lines, years, and replicates within years as random.

Root Traits in a Population of Recombinant Inbred Lines

In greenhouse trials conducted in 1997 and 1998, 140 recombinant inbred lines (RILs) (Azucena/Bala) were evaluated for root parameters in a large container of soil divided into experimental units separated by glass plates. The trials were conducted under well-watered and drought-stressed regimes. Within each water regime, two plants were grown per experimental unit, and there was no replication within each trial. Data for root length at 35 days after planting under stressed and non-stressed treatments were subjected to a variance component analysis, in which lines and years were considered random. (Data from these experiments were kindly provided by Adam Price).

Osmotic Adjustment in an Advanced Backcross Population

Osmotic adjustment was evaluated in a population (IR62266-42-6-2/4*IR60080-46A) of 144 BC₃-derived lines evaluated in three greenhouse trials, each with three replicates, at IRRI in 1999-2000. Experimental units consisted of a single plant per pot. Variance components were estimated for a model considering lines, trials, replicates within trials, and random effects.

Table 3.1.1. Variance component estimates (proportion of total phenotypic variance) for drought-related traits in three QTL mapping populations.

Trait	Population	Test environment	σ_G^2	σ_{GE}^2	σ_E^2	H for means from 1 trial	H for means from 3 trials
Relative water content	IR64/Azucena	IRRI field trial	3	26	71	0.04	0.10
Leaf roiling	IR64/Azucena	IRRI field trial	58	17	25	0.69	0.87
Leaf drying	IR64/Azucena	IRRI field trial	47	11	42	0.54	0.78
Root length at 35 DAP: stressed	Azucena/Bala	U.K. greenhouse trial	12	88	-	0.12	0.28
Root length at 35 DAP: non-stressed	Azucena/Bala	U.K. greenhouse trial	35	65	-	0.35	0.61
Osmotic adjustment	IR62266-42-6-2/4 *IR60080-46A	IRRI screenhouse trial	14	5	81	0.31	0.57
Grain yield: stressed	IR64/Azucena	IRRI field trial	27	19	54	0.46	0.71

Variance component estimates for these traits are presented in Table 3.1.1, as are heritability estimates for means over 1 and 3 repeated trials. Variance component estimates are presented as a percentage of the total phenotypic variance

$$\sigma_P^2 = \sigma_G^2 + \sigma_{GE}^2 + \sigma_E^2 \quad [4]$$

to facilitate comparisons across traits.

The genotype x experiment interaction variance was large relative to the genetic variance for several traits, including root length and relative water content. Within-trial variances were large relative to the genetic variance for all traits except leaf rolling. Large variances for one or both of these sources caused single-trial H values to be well below the level, permitting reliable detection of any but the largest QTL for all traits except leaf rolling and leaf drying in small mapping populations. Means estimated from at least three trials would be needed to achieve adequate precision for the detection of QTL with moderate effects (accounting for, say, 10% of the genetic variance).

The low H estimate for single trials obtained for most traits is not only evidence that random variability within or across experiments is large; experience shows that it is evidence that QTL with effects large enough to be exploited via MAS are unlikely to be found. As noted above, most instances of successful detection of usable QTL have involved traits for which precise phenotyping systems yield results that are highly repeatable across trials; in other words, they have involved traits for which single-trial repeatability is high. By this standard, leaf rolling and leaf drying are the only traits likely to be affected by large-effect QTL.

It should be noted that H for grain yield under reproductive drought stress was as high as or higher than estimates for most other drought-related characteristics. H for grain yield under reproductive-stage water stress for means from a single trial was higher than for any other drought-related traits except leaf drying and leaf rolling. Few estimates of H for rice grain yield under severe water stress have been reported, but at least one other study indicates that the trait can be measured with high precision in well-conducted field trials. Blum *et al.* (1999) evaluated 100 DH lines from the cross CT9993-5-10-1-M/IR62266-42-6-2) under well-watered conditions and with severe reproductive-stage stress at Bet Dagan, Israel in 1997. The trial was not repeated over seasons, so only H' , the biased heritability estimator described in Eq. [3], is available for this population, but comparison of the estimates from stressed and non-stressed treatments are valid, because they are both subject to this bias. H' estimates were 0.79 and 0.62 for the stressed and non-stressed treatments, respectively, providing further support for the hypothesis that yield evaluation under reproductive drought stress in rice can be conducted with a precision equivalent to that obtained for non-stress trials. The relatively high heritability estimates obtained for reproductively stressed populations indicates that direct phenotypic selection for grain yield under stress will be successful if screening trials are well managed. The high heritability of grain yield under stress relative to other secondary drought-related traits also calls into question whether MAS for these traits is likely to improve the efficiency of breeding for drought tolerance relative to phenotypic selection.

Conclusions

In general, the drought-related traits considered in this report do not appear to be more repeatably measurable than grain yield under stress. In all of the experiments, V_{GE} contributed significantly to the phenotypic variances and within-experiment variances were high. Most H estimates were well below the level required to give adequate power to reliably detect QTL. The low H estimates indicate that it is unlikely that QTL with large effects exist for most of these traits, with the possible exceptions of leaf rolling and leaf drying. To detect QTL with small effects (i.e. 10% of V_G), larger mapping populations must be used than has been the case to date, and experiments routinely achieving $H = 0.7$ or greater must be conducted. This will require substantial replication within and across runs for controlled-environment and greenhouse trials, and across locations and years in field trials. It seems questionable that this effort will be worthwhile for small-effect QTL, unless their correlation with grain yield under stress is very high. Grain yield in carefully designed managed stress environments is a more promising target for breeding efforts aimed at improving drought tolerance in rice.

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3.2 Molecular Dissection of Drought Tolerance in Rice: Association between Physiomorphological Traits and Field Performance

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Abstract

Quantitative trait loci (QTL) linked to plant water relations, phenology, and production traits under irrigated and drought stress conditions in the field were mapped using a doubled-haploid (DH) line population of 154 rice lines. The DH lines were subjected to water stress before anthesis. The DH lines showed significant variation for plant water stress indicators, phenology, biomass, yield, and yield components under stress. A total of 28 putative QTL were identified for various plant water relation and production traits under control and stress conditions, and individually explained 8.5-36.6% of the phenotypic variation. A region on chromosome 4 harbored major QTL for plant height, grain yield, and number of grains per panicle under drought stress. By comparing the coincidence of QTL, we also genetically dissected the nature of association between drought tolerance components such as root traits and osmotic adjustment (OA), and rice production under drought. Root traits had a positive impact on yield under stress.

Introduction

Despite our understanding of the role of physiomorphological traits in drought tolerance, these traits are not selected for in crop improvement programs since

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their phenotypic selection is difficult. Considering this limitation, molecular marker technology is a powerful tool for selecting such traits. Quantitative trait loci (QTL) linked to several drought tolerance component traits have been identified in rice (see Zhang et al. 2001). However, a positive association between variation in such quantitatively inherited traits, and the effects of those traits on plant production under drought, has not yet been established. By comparing the coincidence of QTL for specific traits and QTL for plant production under drought, the significance of a particular constitutive or adaptive trait in improving drought tolerance in rice was tested by the authors.

Materials and Methods

The rice breeding lines, CT9993-5-10-1-M and IR62266-42-6-2 differed consistently for a range of drought tolerance component traits (Babu et al. 2001). A DH line population was developed between CT9993-5-10-1-M (abbreviated as CT9993, an upland *japonica* ecotype possessing a deep and thick root system and low OA) and IR62266-42-6-2 (abbreviated as IR62266, an *indica* ecotype with a shallow root system and high OA) at Centro Internacional de Agricultura Tropical, Colombia and the International Rice Research Institute, Philippines. This population was subjected to phenotypic evaluation and QTL mapping at several collaborating institutes, and data were acquired for root penetration index (RPI), basal root thickness (BRT), root pulling force (RPF), gross root morphology, OA, and plant production under water stress (Blum et al. 1999, Zhang et al. 1999, Kamoshita et al. 2001, Zhang et al. 2001). The total number of DH lines of the population was 220. A subset of 154 DH lines from this population was used to identify the QTL linked to rice performance under drought in the field. The trial was conducted under upland conditions in an experimental field at Tamil Nadu Agricultural University, Coimbatore, India during the 1999 wet season. The DH lines and their parents were evaluated under two water regimes: fully irrigated (non-stress) control and water stress following randomised block design with three replications. Experimental plots were 2 m x 0.6 m size. There was 20 cm and 10 cm spacing between and within rows, respectively. Seeds were hand-dribbled into dry soil at 100 kg ha⁻¹ NPK fertilizers were applied at a rate of 120:40:40 kg ha⁻¹. All the plots were surface irrigated to field capacity once a week, except when water stress was imposed by withholding irrigation to stress plots from 63 to 88 days after sowing (DAS).

Field Measurements

Changes in soil moisture and tolerance were monitored periodically in stress plots using gravimetric measures and penetrometer, respectively. Relative water content (RWC) was determined at midday, 15 days after withholding irrigation in youngest expanded leaf after 4 hours rehydration. Two days later, leaf rolling and drying scores were made at midday on a 1-7 scale. Data on days to heading, plant height, biomass, and grain yield were recorded. Three panicles per DH line per replication were sampled to obtain data on number of grains per panicle, spikelet fertility, and 1000-grain weight. Harvest index was calculated as the ratio of grain weight to total plant dry weight. Relative yield and biomass were calculated as the ratio of yield and biomass under stress to that of control. Drought susceptibility index (DSI) was calculated using the equation of Fischer and Maurer (1978).

Statistical and QTL Analyses

Standard analyses of variance were performed to check the genetic variance among the DH lines for all traits. The broad sense heritabilities (h^2) were then computed from the estimates of genetic (s^2G) and residual (s^2e) variances derived from the expected mean squares of the analysis of variances. Phenotypic correlations among the traits were computed using the genotypic means.

An integrated molecular genetic linkage map consisting of 153 amplified fragment length polymorphisms (AFLPs), 145 restriction fragment length polymorphisms (RFLPs), and 17 microsatellites was earlier constructed with the 154 DH lines (Zhang et al. 2001). Quantitative trait loci linked to various traits were identified using QTLmapper ver.1 software (Wang et al. 1999) with a threshold LOD score of 3.0. Tests for independence of QTL were conducted when two or more QTL of a trait were located on the same chromosome (Paterson et al. 1988).

Results

Variation for Plant Production Traits under Stress

There was a significant genotypic effect for all the traits. The phenotypic means of the population and its parents for the various traits, along with broad-sense heritabilities, are summarized (Table 3.2.1). Water stress was

Table 3.2.1. Trait mean values for CT9993, IR62266 and doubled-haploid (DH) lines.

Trait	CT9993	IR62266	DH lines			h^2
			Mean	Range	S.D.	
Relative water content (%)	94.0	69.3	68.2	29.5- 95.0	12.6	0.49
*Leaf rolling	5.0	6.7	5.7	2.6- 7.0	0.9	0.65
*Leaf drying	4.0	5.7	4.8	1.3- 7.0	1.1	0.71
Days to heading (DAS) - stress	110	121	105	77.0-122.0	7.7	0.70
Days to heading (DAS) - control	98	106	91	74.0-106.0	5.9	0.93
Plant height (cm) - stress	76.1	47.7	61.8	34.1 - 88.1	11.5	0.49
Plant height (cm) - control	75.9	51.9	65.6	38.2- 88.2	11.2	0.79
Grain yield (g m ⁻²) - stress	26.0	13.3	37.4	6.9-108.0	21.0	0.59
Grain yield (g m ⁻²) - control	143.6	85.4	115.5	26.0-235.0	43.2	0.61
Biomass (g m ⁻²) - stress	342.7	233.3	252.6	132.0-469.0	65.0	0.37
Biomass (g m ⁻²) - control	726.9	402.1	485.2	221.0-883.0	139.5	0.60
Spikelet fertility (%) - stress	68.8	55.3	64.7	13.3- 85.7	10.7	0.43
Spikelet fertility (%) - control	82.1	73.8	77.6	37.6- 92.6	9.1	0.69
Grains per panicle - stress	52.0	25.0	45.4	6.3- 83.3	12.9	0.50
Grains per panicle - control	87.7	35.3	59.9	25.3-122.7	17.7	0.76
1000-grain weight (g) - stress	20.6	17.9	20.9	14.9- 25.1	1.9	0.37
1000-grain weight (g) - control	21.7	30.2	22.4	16.3- 30.2	2.5	0.66
Harvest index (%) - stress	7.59	5.70	13.7	3.9- 28.9	5.4	0.60
Harvest index (%) - control	20.6	21.2	23.8	9.2- 47.7	6.1	0.24
Relative biomass (%)	47.1	58.0	52.1	24.5- 95.3	15.1	-
Relative yield (%)	18.1	15.6	32.4	9.2- 89.8	15.9	-
Drought susceptibility index	10.35	11.10	-	1.29- 11.48	2.01	-

*Based on scale 1-7

DAS- Days after sowing

severe, with a continuous stress period of 25 days, from 63 to 88 DAS. During the first 15 days of stress, there was 89% depletion of available soil moisture from field capacity in the 10-20 cm soil layer and the soil strength increased from 0.27 to 3.10 MPa. Mean leaf RWC across the DH lines declined to 68% under stress. The average leaf rolling and drying scores across the DH lines were 5.7 and 4.8, respectively. CT9993 had higher RWC and lower drought scores as compared to IR62266. Mean heading date was delayed by 14 days under stress. Heading date was delayed by 12 and 15 days in CT9993 and IR62266, respectively, under stress. Mean plant height was reduced by 3.8 cm under stress. While CT9993 did not show any reduction, IR62266 showed 4.2 cm reduction in plant height under stress. Water stress caused an average reduction of 48% in biomass and 68% in grain yield. There were 13, 16, and

10% reduction in spikelet fertility, grains per panicle and harvest index, respectively under stress. Drought susceptibility index varied from 1.29 to 11.48 among the DH lines. Broad-sense heritability of leaf rolling, leaf drying and days to heading under stress was high (0.65, 0.71, and 0.70, respectively), while that of RWC, plant height, grain yield, biomass, spikelet fertility, grains per panicle, and 1000-grain weight under stress was low to moderate.

Correlations between Water Stress Indices and Production Traits

The phenotypic correlations between traits showed that parameters of stress indicators were significantly correlated with plant phenology and production traits under stress. Relative water content was negatively correlated with leaf rolling, leaf drying, days to heading, and spikelet fertility under stress ($r = -0.26^{**}$, -0.22^{**} , -0.26^{**} , and -0.17^* , respectively). Leaf rolling and drying were positively correlated with days to heading ($r = 0.25^{**}$ and 0.27^{**} , respectively). Biomass under stress was positively correlated with yield, spikelet fertility, number of grains per panicle, 1000-grain weight, and harvest index under stress ($r = 0.74^{**}$, 0.35^{**} , 0.45^{**} , 0.25^{**} , and 0.40^{**} , respectively). Biomass, spikelet fertility, number of grains per panicle, 1000-grain weight, and harvest index under stress were negatively correlated with DSI ($r = -0.41^{**}$, -0.31^{**} , -0.26^{**} , -0.21^{**} , and -0.68^{**} , respectively).

Quantitative Trait Loci Linked to Rice Performance under Drought

A total of 28 putative QTL were identified for various plant water relation, phenology, and production traits under control and stress conditions (Table 3.2.2). The number of QTL identified for each trait varied from 1 to 3, with the phenotypic variation (R^2) ranging from 8.5 to 36.6%. Quantitative Trait Locus R2170-EM13_1 on chromosome 3 explained the highest phenotypic variation of 36.6% for days to heading under control. Similarly, QTL, ME6_9-RZ602 on chromosome 4 explained highest phenotypic variation of 31.2% for plant height under stress. If we compare QTL locations across traits, QTL for different traits are mapped to similar chromosomal locations. For example, QTL for plant height, yield, and number of grains per panicle under stress are mapped to RG939-RG476-RG214 region on chromosome 4. Quantitative trait locus for harvest index, relative yield, and DSI under stress were mapped to the RG1-ME2_11 region on chromosome 8.

Table 3.2.2. Putative QTL detected by interval mapping for plant water relation and production traits under field conditions in a doubled-haploid line population.

Traits	QTL	Chr# [†]	Interval	LOD	Effect ^{††}	R ^{2†††}
Relative water content	<i>rwc1.1</i>	1	R2417-RM212	6.94	5.74(C)	23.97
	<i>rwc1.2</i>	1	ME2_12-RG532	3.12	3.41 (R)	8.47
Leaf rolling	<i>lr1.1</i>	1	RG109-ME10_14	10.65	0.44(C)	24.69
	<i>lr11.1</i>	11	R1506-C950	6.41	0.35(I)	15.46
	<i>lr11.2</i>	11	ME10_16-EM17_10	5.06	0.32(I)	13.54
Days to heading - stress	<i>dhs3.1</i>	3	R2170-EM13_1	3.92	1.89(I)	9.83
	<i>dhs3.2</i>	3	CDO20-EM11_9	3.77	2.19(I)	13.10
Days to heading - control	<i>dhc3.1</i>	3	R2170-EM13-1	13.16	2.94(I)	36.60
	<i>dhc3.2</i>	3	C563-ME8-1	8.93	2.64(C)	29.51
	<i>dhc3.3</i>	3	EM11_9-RG104	6.56	2.42(I)	21.51
Plant height - stress	<i>phs1.1</i>	1	RG109-ME10_14	12.84	6.31(C)	18.06
	<i>phs4.1</i>	4	RG214-RG476	6.94	4.68(C)	9.92
	<i>phs4.2</i>	4	ME6_9-RZ602	3.72	5.12(I)	31.15
Plant height - control	<i>phc1.1</i>	1	RG109-ME10-4	17.26	5.67(C)	30.66
	<i>phc9.1</i>	9	RM215-RG667	10.32	4.82(I)	22.17
Grain yield - stress	<i>gys1.1</i>	1	EM18_10-ME6_4	7.42	8.58(I)	13.52
	<i>gys1.2</i>	1	ME4J8-EM11-11	9.31	10.96(I)	22.07
	<i>gys4.1</i>	4	RG476-RG939	7.61	10.01(C)	18.41
Biomass - stress	<i>bms12.1</i>	12	ME6_12-G2140	4.68	22.83(C)	11.8
Biomass - control	<i>bmc2.1</i>	2	RZ386-EM14_4	7.63	57.09(I)	25.13
	<i>bmc2.2</i>	2	ME2_7-R1843	6.74	47.76(C)	17.59
	<i>bmc4.1</i>	4	RG620-RG214	4.57	42.12(C)	13.68
Grains per panicle - stress	<i>gpps4.1</i>	4	RG620-RG214	4.17	4.79(C)	14.58
Grains per panicle - control	<i>gppc1.1</i>	1	EM11-11-RG109	5.71	5.99(C)	12.65
	<i>gppc3.1</i>	3	EM11_9-RG104	6.41	7.78(C)	21.34
Harvest index - stress	<i>his8.1</i>	8	G187-ME2_11	3.06	1.59(I)	9.05
Relative yield	<i>ry8.1</i>	8	RG1-G187	5.50	5.30(I)	18.43
Drought susceptibility index	<i>dsi8.1</i>	8	RG1-G187	5.50	0.67(C)	18.43

†: chromosome number; ††: letters I and C in parentheses indicate that positive or favorable alleles for the effects are from IR62266 and CT9993, respectively; †††: relative contributions of the QTL to the phenotypic variation: units as in Table 3.2.1

Discussion

Association between Drought Tolerance Components and Rice Performance

Root Traits

Although the QTL regulating plant production under stress are scattered throughout the genome, certain chromosomal locations assumed significance. For example, the region RG939-RG476-RG214 on chromosome 4 was found to be important in terms of drought tolerance in rice, since it regulates plant height, yield, and number of grains per panicle under stress. When comparing the locations of these QTL, we found that this genomic region also harbored QTL for RPI, BRT, thickness, and dry weight of penetrated roots under simulated soil hardpans (Zhang et al. 2001), deep roots per tiller, and deep root thickness in a greenhouse study (Kamoshita et al. 2001), RPF and number of panicles under rainfed condition in the field in Thailand (Zhang et al. 1999) in this population (Fig. 3.2.1). This region was earlier found to regulate root thickness in two other rice populations, IR64/Azucena DH lines and C039/Moroberekan RI lines (Zheng et al. 2000). Further, RG214 was linked to drought avoidance in the field (Champoux et al. 1995) in C039/Moroberekan RI lines. When QTL for different traits were linked, the favorable alleles for both the traits came from the same parent. CT9993, the *japonica* accession, contributed the favorable alleles for plant growth and production traits under stress and also for the root related drought tolerance traits. The considerable overlap found between the QTL for plant production under stress and root traits suggests the presence of genes with pleiotropic effects/linkage on the investigated traits.

The overlapping of QTL for different traits should be associated with a correlation of the phenotypic data. Plant production under stress in this study was correlated with root traits (Table 3.2.3). Leaf drying was negatively correlated with deep root thickness ($r = -0.22^*$). Biomass and yield under stress were positively correlated with all the root-related traits. Similarly, grains per panicle and harvest index under stress were positively correlated with several root-related traits. DSI was negatively correlated with most root traits. Thus, the positive association between the level of phenotypic correlation and the linkage of QTL for root traits and rice yield under drought in the field is verified in this study.

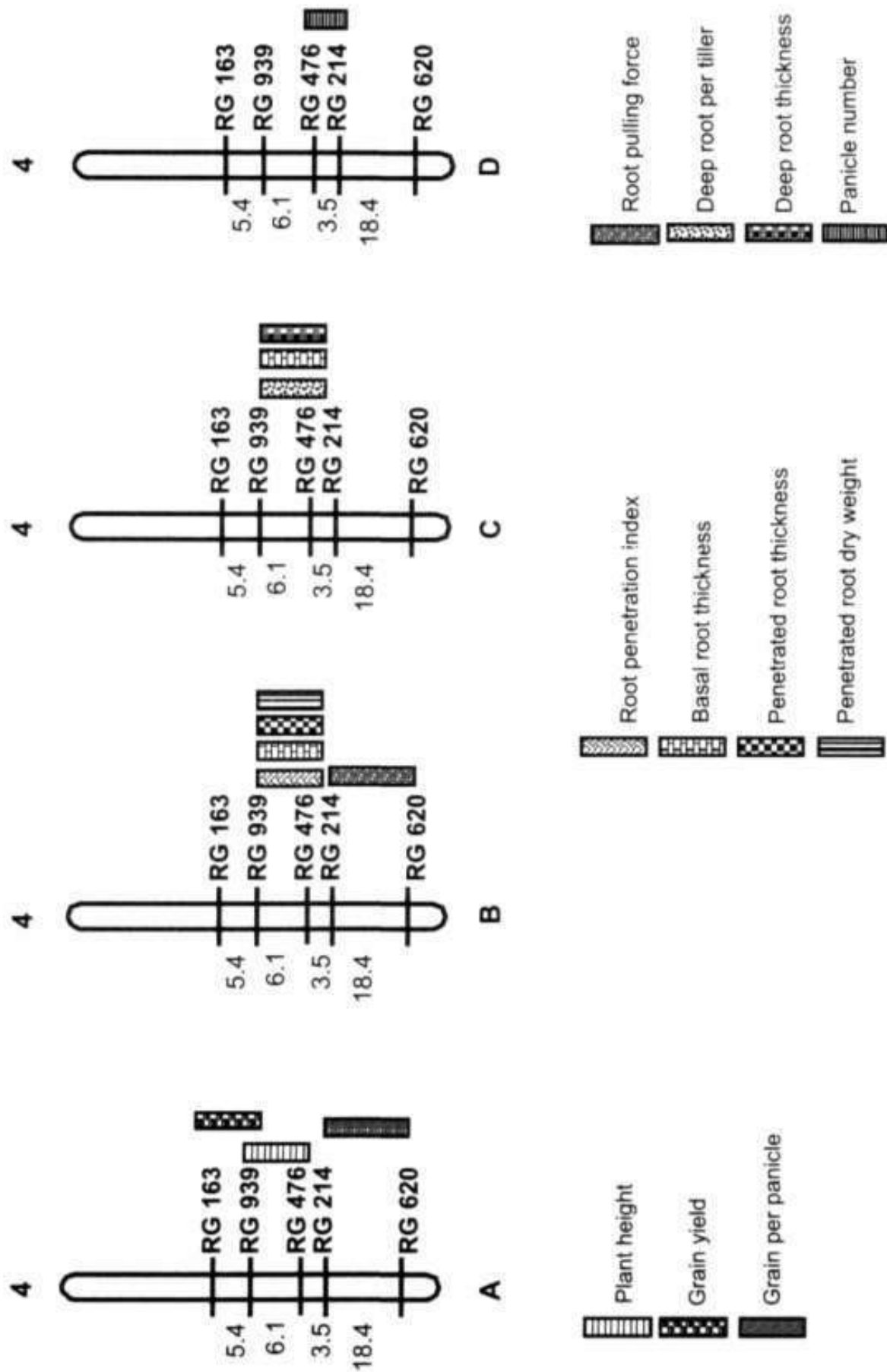


Figure 3.2.1. A genomic region of rice chromosome 4 showing overlapping of QTL identified for plant production traits under drought stress in the field as identified in this study (A), drought tolerance components identified by Zhang et al. (2001) (B), root morphological traits (Kamoshita et al. 2001) (C) and panicle number under rainfed conditions in Thailand (Zhang et al. 1999) (D), in this DH population of rice.

Table 3.2.3. Correlation coefficients among plant water relation and production traits under drought in the field and drought tolerance components in a DH line population of rice.

	OA	RPI	BRT	RPF	DPRT	DRDW	RDEPTH
RWC	0.19*	0.00	0.11	-0.09	-0.11	-0.10	-0.02
LD	0.02	-0.06	0.00	0.06	-0.22*	0.02	-0.04
BM	-0.01	0.23**	0.32**	0.17*	0.31"	0.28**	0.23**
GY	-0.03	0.18*	0.34**	0.17*	0.28"	0.22*	0.25**
SF	-0.06	0.20*	0.02	-0.01	0.08	0.12	0.14
GPP	0.05	0.19*	0.36**	0.23**	0.03	0.13	0.06
HI	0.04	0.12	0.24**	0.16*	0.20*	0.16	0.26**
DSI	0.03	-0.19*	-0.13	-0.21**	-0.21*	-0.18*	-0.23**

OA- osmotic adjustment, RPI- root penetration index, BRT- basal root thickness, RPF- root pulling force, DPRT- deep root thickness, DRDW- deep root dry weight, RDEPTH- root depth, RWC- relative water content, LD- leaf drying, BM- biomass, GY- grain yield, SF- spikelet fertility, GPP- grains per panicle, HI- harvest index, DSI- yield drought susceptibility index.

** and * indicate the significant difference at 0.01 and 0.05 level, respectively.

Osmotic Adjustment

The QTL region, RG1-G187-ME2_11 on chromosome 8, that regulates harvest index, relative yield, and DSI under stress in the present study was located in the same genomic region as QTL for OA (Zhang et al. 2001); canopy temperature and days to heading under drought in Bet Dagan, Israel; and days to 50% flowering under rainfed conditions in Thailand (Zhang et al. 1999) in this population. However, no QTL was mapped for yield under stress to this region in this study. Further, yield under stress was not correlated with capacity for OA in this study (Table 3.2.3). Similar results were reported earlier (Zhang et al. 1999a).

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3.3 Target Traits for QTL Analysis and Marker-assisted Selection Strategy for Drought Tolerance in Rice

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Abstract

Drought tolerance, manifested by a plant or crop community, is a cumulative effect of several component traits. Each trait, governed by single or polygenes, follows a characteristic inheritance pattern, interacting with the environment in the process. Genes governing these traits, and traits themselves, interact with one another either positively or negatively to affect grain yield. As in the case of dominant or recessive monogenes, each locus governing a polygenic trait manifests its effect by enhancing or depressing the phenotype.

As selecting for components of drought tolerance in breeding material is difficult, closely segregating, indirect phenotypic or molecular marker tags seem ideally suited to molecular marker-assisted breeding for drought tolerance. In major crops like rice, pearl millet, maize, and sorghum, dense molecular maps have offered the advantage of easy locating of several loci governing quantitative traits associated with drought tolerance and ascertaining their relative importance to the manifestation of trait and expected breeding outcome. It has also helped understand the inter-trait relationships and has contributed towards devising molecular breeding strategy.

Introduction

Increasing drought tolerance of major crops will have a significant impact on global food production as the irrigated habitats are reflecting the stress and strain of high-input agriculture in the form of yield plateaux. Quantum jumps in productivity from irrigated habitats are difficult to expect, as huge investments in enlarging irrigated land area, use of inorganic fertilizers, and plant protection chemicals are either not possible or not desirable for

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sustainability and environmental concerns. In a low-input agricultural system farmers of rainfed lowland rice, a fragile ecosystem, cannot afford high-cost external inputs.

Traits Associated with Drought Tolerance

A wide range of genetic and phenotypic variability for several components of drought tolerance have been well documented in most major crops over the past few decades. In spite of this impressive array of variability for each component, establishing grain yield advantage in stressed habitats, is difficult due to negative associations between (some) components in pairs or groups with grain yield, the final economic product. For a breeder any trait, individually or in combination with other traits, that would directly or indirectly be associated with enhanced plant survival, improved yield (with or without stability), and/or increased economic yield constitutes potential target(s) for study and selection. It is thus imperative that utility of trait(s) for enhancing drought tolerance must be manifested as enhanced plant survival and "better than normal" grain- and dry matter yield under conditions of drought stress when assessed at the level of whole plant and crop community. Finally, the magnitude of expression of each trait and its ability to blend with other causal- or causally-related traits will contribute to its ultimate utility in plant-breeding programs.

Practical and Usable Low-Moisture Stress Tolerance

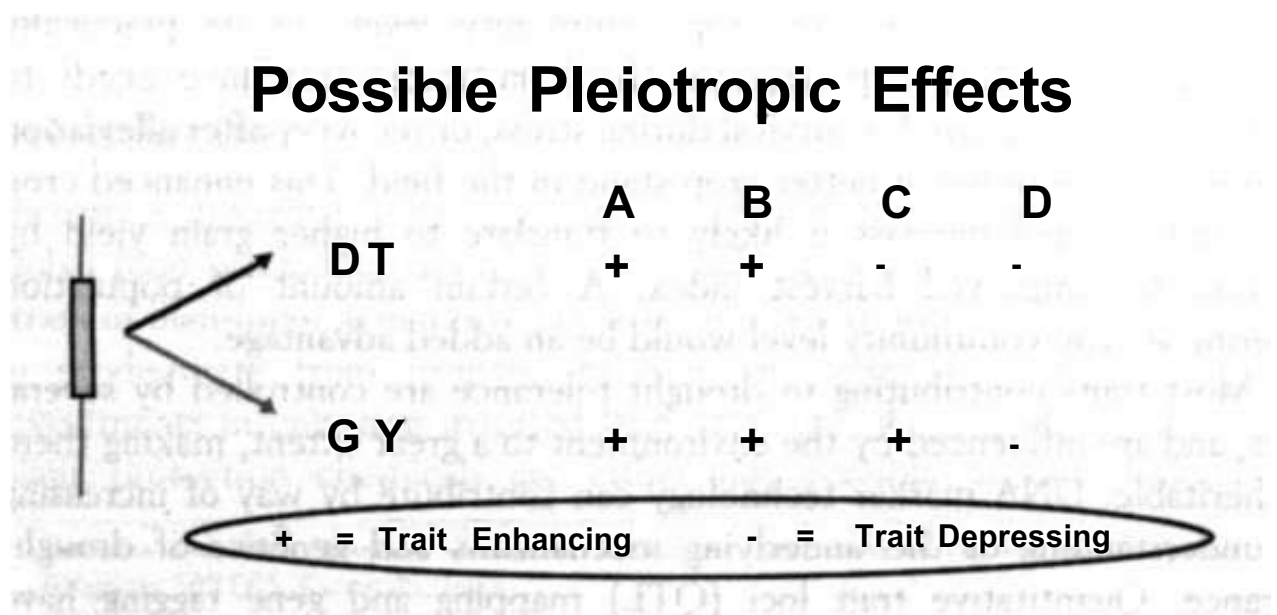
Tolerance to low-moisture stress can be identified and studied at cellular, tissue, organ, whole plant, or crop community level. To be practically applicable in breeding, any response that constitutes resistance needs to manifest as an advantage for survival during stress, or recovery after alleviation of stress, thus resulting in better crop stand in the field. This enhanced crop stand and accrued biomass is likely to translate to higher grain yield by selection for improved harvest index. A certain amount of population buffering at crop community level would be an added advantage.

Most traits contributing to drought tolerance are controlled by several genes, and are influenced by the environment to a great extent, making them less heritable. DNA marker technology can contribute by way of increasing our understanding of the underlying mechanisms and genetics of drought tolerance. Quantitative trait loci (QTL) mapping and gene tagging have

enabled dissection of drought tolerance into components that are easier to study and comprehend. Plant breeders seek molecular markers closely associated with genes (and their alleles), controlling enhanced drought tolerance and grain yield under drought stress conditions. Genes for each QTL could be present on the same chromosome, showing varying levels of linkage, or present on different chromosomes and showing independent assortment. Ideally, the molecular marker should cosegregate with the trait(s), have a favorable influence on trait(s) that happened to co-map to that locus, and still have no negative influence on other loci controlling the target trait (or any other desirable attribute) as shown in Figure 3.3.1. Thus, if two traits are under pleiotropic control, with a type B and C interaction, they cannot be used in breeding. On the other hand type A (ideal situation) and D (worst case scenario) are to be selected for and against, respectively. Closely linked PCR-based markers are expected to be of immense utility in such situations, as they will permit scanning of the desirable loci among germplasm accessions and permit selection of the best allele for a given trait.

Many examples of identification of the genetic map locations of QTL are available for rice, sorghum, maize, pearl millet, wheat, and barley. While results of tagging QTL governing morphological, physiological, phenological, and biochemical component traits have been widely reported, studies aimed at addressing tricky genetic and biological phenomena like the distinctions

Figure 3.3.1 Mapping quantitative trait loci associated with drought tolerance.



between tight linkage and pleiotropy, coupling phase and repulsion phase linkage, intergenic and intragenic interactions, and environmental sensitivity of genes are far less common and seldom adequate.

Fine-tuned investigations into QTL depend on appropriate genetic materials involving chromosomal substitution lines, which could be in transient or genetically stable generations. These are invaluable in a detailed search for tightly linked molecular markers and for validation across germplasm to assess for wide applicability in real-life breeding situations. Transgressive segregant (transgressant) doubled haploid lines have been used in a backcrossing program aimed at development of near-isogenic lines. Chromosomal substitutions are followed using MapPlotter (Shen 1999) for graphical genotyping. Phenotyping for root morphology- and productivity-related traits simultaneously allows identification of pairs of near-isogenic lines specific to each major locus associated with the target trait. Each transgressant is being advanced to produce pairs of substitution lines using the strategy of heterogeneous inbred families (Tuinstra et al. 1998).

Of the several characters associated with drought resistance, root morphological traits are among the important ones (Blum 2001) that are likely to confer the maximum advantage, as they are associated with acquisition of moisture, nutrients, and transmitting drought perception signals. The authors evaluated accessions of rice of diverse origin by using PVC pipes (1 m long and 18 cm wide) using soil as the medium (Shashidhar et al. 1999). While traditional accessions of *indica* rices and tropical *japonicas* possess very deep roots, improved varieties bred for irrigated habitats and high grain yield are known to possess very shallow roots (Latha 1996). Several root morphological, and physiological traits have been tagged to molecular markers using a double haploid mapping population of rice. The map locations of several traits congregated to the same locus (Shashidhar, H.E., unpublished data, Fig. 3.3.2, Table 3.3.1) and showed consistency at some chromosomal regions with the works of Ray et al. (1995), Yadav et al. (1999), Zhang et al. 1999, Venuprasad et al. (1999), and Champoux et al. (1995).

Drought tolerance, manifested as maintenance of turgor, better crop stand, and recovery after alleviation of stress, needs to reflect in terms of improved productivity under moisture-limited conditions to be of some succor to the farmers. This entails simultaneous selection for traits associated with drought tolerance and grain yield. In 1980 Blum stated that breeding for drought tolerance could be easily accomplished just by selecting for low grain

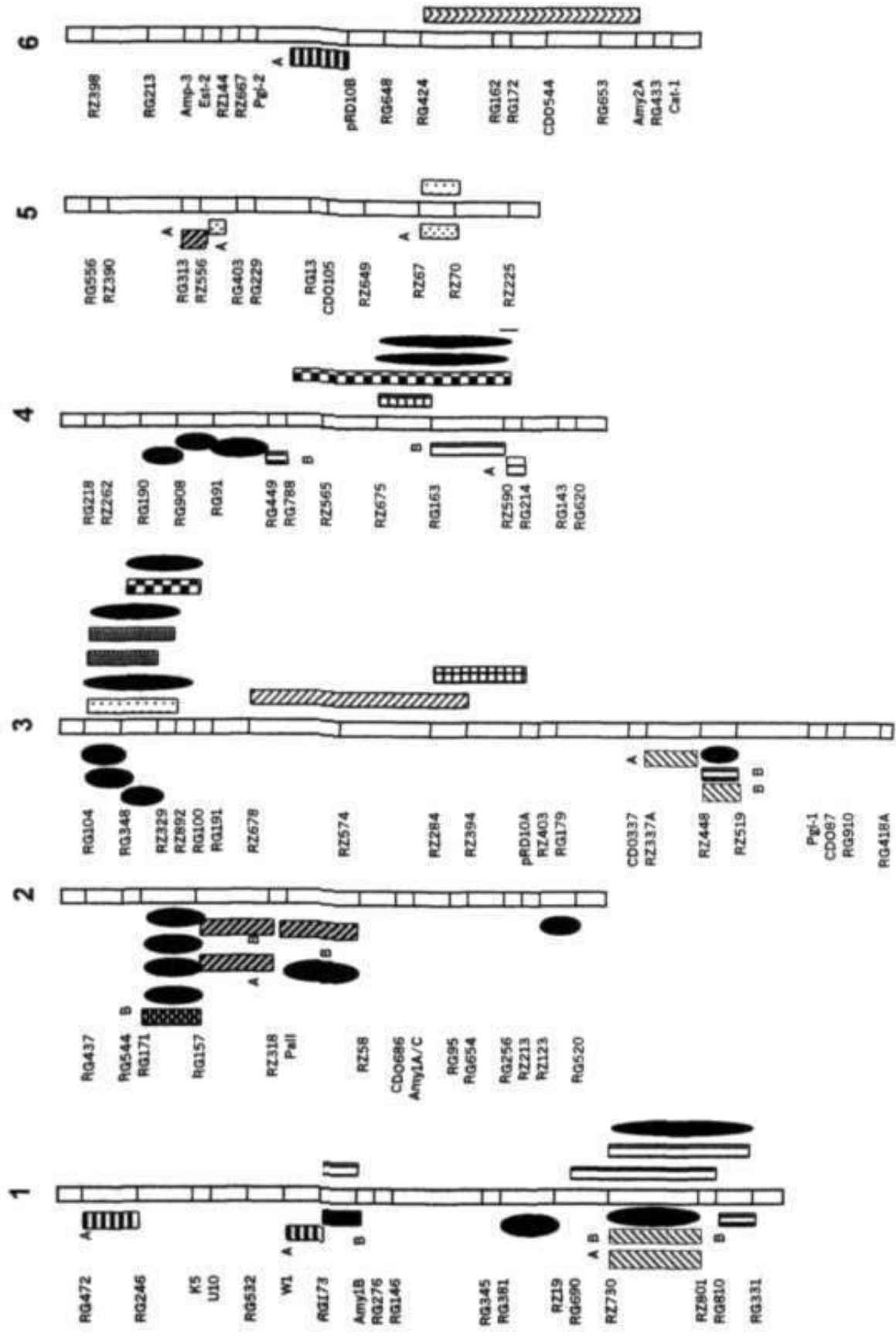


Figure 3.3.2. Putative QTL associated with grain yield related traits (right side of the chromosome) and root morphological traits (left side of the chromosome) studied in two different moisture condition in an IR64/Azucena mapping population. (Chromosome 1 not shown as there were no QTL for traits studied).

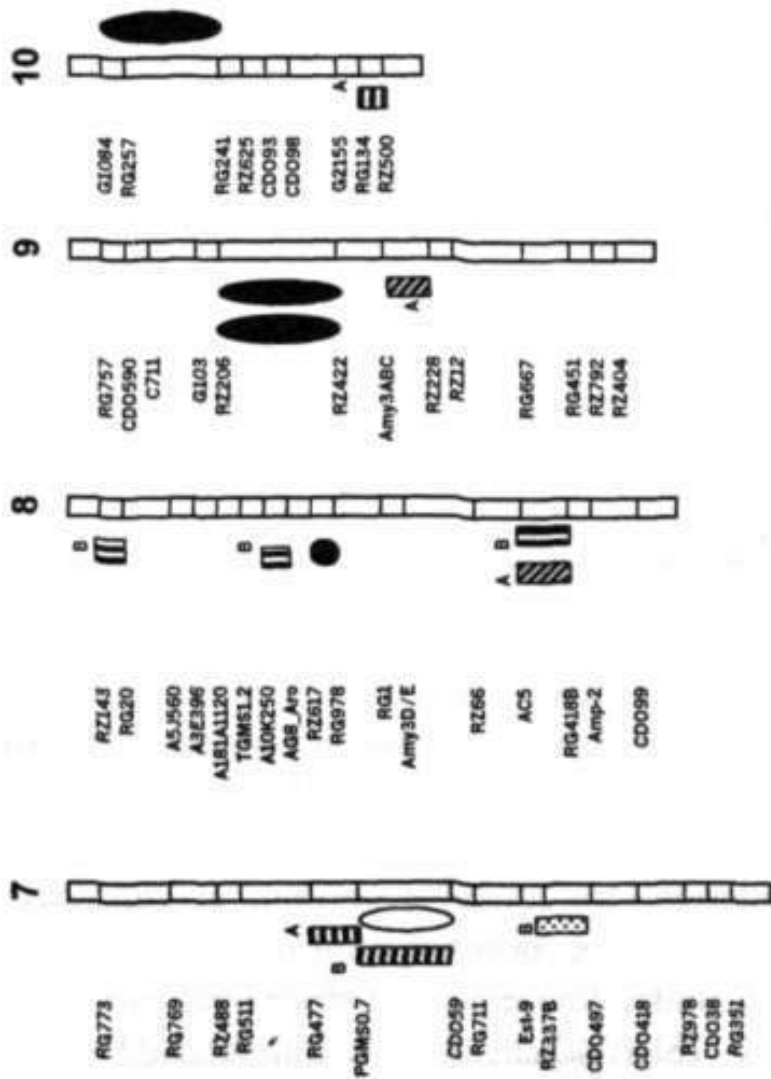


Figure 3.3.2. Continued..

Table 3.3.1. Molecular markers linked to QTL identified by interval analysis in nonstress (NS), stress (S) and 'mean' (M) environments using IR64/Azucena DH population of rice (LOD>2.20).

Trait (units)	Moisture Environment	Flanking Markers	Chromosome number	% Variance explained	Additivity#	LOD Score
DFL (d)	NS	RZ892-RG104	3	24.7	2.53	5.27
		RZ67-RZ70	5	13.1	1.86	2.27
	M	RG100-RG104	3	19.9	2.69	3.21
DAM (d)	NS	RZ329-RG104	3	18.7	2.14	4.02
	S	RZ892-RG104	3	14.9	3.72	2.48
	M	RZ892-RG104	3	20.9	2.74	3.58
		G1084-RG141	10	13.3	2.06	2.33
PHT (cm)	NS	RG173- <i>AMY1B</i>	1	12.3	-9.42	2.51
		RG690-RG801	1	20.9	8.36	3.38
	S	RZ730-RG331	1	22	5.90	3.12
	M	RZ730-RG331	1	23.1	6.71	2.91
NOT (#)	S	RZ394-RZ678	3	16.1	-0.66	2.47
PDT(#)	NS	RG424- <i>AMY2A</i>	6	17.1	-0.62	2.96
PL (cm)	NS	RZ565-RZ675	4	12.5	0.87	2.20
	S	<i>pRD 10a</i> -RZ284	3	14.5	1.07	2.29
GY(g.p ⁻¹)	NS	RG100-RG348	3	15.1	2.31	3.06
		RG788-RZ590	4	19.2	2.62	3.30
	M	RG100-RG348	3	15.6	1.27	2.65
		RZ675-RZ590	4	18.3	1.36	2.87
ADM (g)	M	RZ675-RZ590	4	15.6	2.01	2.32
		CDO544- <i>AMY2A</i>	6	15.9	1.94	2.39

yield. As field evaluation of lines for productivity-related traits and traits associated with drought tolerance, such as root morphology and osmotic adjustment pose logistic problems, it is imperative that the two sets of traits be assessed independently to understand interrelationships. In a field evaluation of DH lines of mapping population, traditional accession, in contrasting moisture regimes, we found desirable root morphological traits had positive association with grain yield only under low-moisture stress and not under adequately watered conditions (Shashidhar, H.E., unpublished data). Quantitative trait loci tagging of the traits using a dense molecular map

revealed that the map positions of grain yield-related traits were different from those of root morphological traits. One of the interpretations of the result is that in the population studied, the traits were neither tightly linked nor pleiotropic to one another and this brightens the prospects of combining the two sets of traits together (Venuprasad, in press). Breeding strategy for drought tolerance would involve generating recombinants, selecting for the rare segregants using molecular markers, and energy balance between traits contributing to survival and productivity-linked traits.

In spite of the wide array of variability for components related to drought tolerance in germplasm, it might not be desirable, for most traits, to select for the extreme values such as longest roots and highest level of osmotic adjustment. The plant type envisaged and the intrinsic variability that is characteristic of the habitat would dictate the magnitude of each component desired in the ultimate plant designed. This is necessary, as each trait manifestation which would contribute towards enhancing drought tolerance, is associated with a "cost" to the plant thus depressing grain yield to a small extent (Schulze 1988, Hall 2001). Biological yield that can also be assessed as dry matter, at any stage of crop growth, especially under moisture stress situation, reflects the plant's ability to have harnessed edaphic and atmospheric resources and face biotic constraints (if any). Biological yield has been found to show very strong correlation with the ability of a genotype to extract moisture (Venuprasad 1999).

Marker-Assisted Selection Strategy

If molecular markers are to be useful in breeding, they need to cosegregate with the trait. This needs closely linked markers, preferably based on the polymerase chain reaction (PCR). These markers could be integrated into breeding programs. We have identified RAPD and SSR markers for maximum root length in rice (Shashidhar et al 2000). The RAPD band has been mapped relative to existing markers with a LOD of 3.0. Single marker analysis revealed that it was associated with maximum root length with F value of 0.01. The SSR marker on the other hand cosegregated with maximum root length with a LOD score of 3.2 and accounted for 24% of variability (Fig. 3.3.3). These have been found to amplify the deep root-specific band in parents of other mapping populations and straight varieties. Both these markers behave in a co-dominant manner.

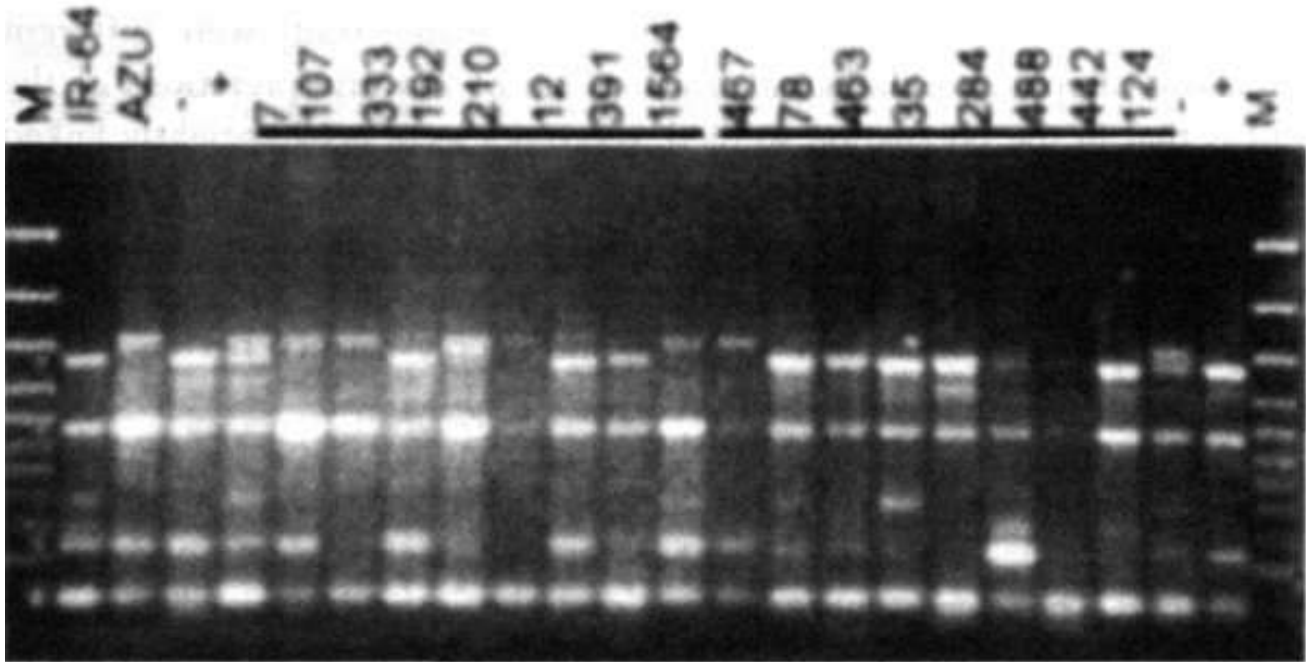


Figure 3.3.3. RAPD marker associated with maximum root length in rice.

Marker-assisted selection (MAS) strategies were discussed with reference to breeding for drought tolerance in rice and pearl millet (Hash et al. 2000a, b). The accomplishments and proposed strategies in maize (Veldboom and Lee 1996; Ribaut and Betran 1999), pearl millet (Yadav et al. 1999, 2000; Hash and Bramel-Cox 2000; Hash et al., 2000a, b), and sorghum (Tuinstra et al. 1998; Subudhi and Nguyen 2000; Tao et al. 2000), were described.

Breeding populations based on local gene pools (to harness local adaptability), and improved lines (to access genes for improved productivity) need to be developed for each habitat. Innovative selection strategies including selection to be made in the target habitat for drought tolerance (which is very difficult to reproduce in experimental stations) and traits associated with improved production potential (which would most likely be manifested only under unstressed conditions) need to be integrated into a single program. Participatory plant breeding or participatory varietal selection strategy will augur well for such a situation. The variability across the target habitat with reference to timing, intensity, and other aspects will thus be considered in the selection process. The molecular markers cosegregating with traits associated with drought tolerance could be incorporated in the selection process.

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3.4 Molecular Breeding for Improving Drought Tolerance in Rainfed Lowland rice in North and Northeast Thailand

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Late season drought commonly occurs in drought-prone rice areas of North and Northeast Thailand, causing a significant reduction in yield. Currently, improvement of drought tolerance cultivars using conventional selection has made little progress due to genetic complexity of traits related to drought tolerance and G x E interactions. Physiological and morphological breeding approaches have been proposed to improve the efficiency of breeding programs, by using traits that offer drought tolerance as a selection criterion if their values are closely associated with grain yield or its components in the target environments. However, this approach requires large numbers of accurate measurements in order to precisely determine genetic variation. Selection for lines possessing the traits of interest may thus be impractical for mass selection. On the other hand, the use of molecular techniques by marker-assisted selection (MAS) may be useful for mass selection screening if the location of quantitative trait loci (QTL) of traits related to drought tolerance can be identified. Marker-assisted selection can then be integrated into the breeding program to help rice breeders achieve their goals in a short period of time.

The Rice Research Institute (Thailand) is currently conducting a breeding project for drought tolerance in collaboration with the National Center for Genetic Engineering and Biotechnology (BIOTEC-Thailand). The first objective is to identify QTL in order to develop MAS scheme for underlying traits related to drought tolerance and yield performance under water deficit at flowering stage. The second objective is to introgress these QTL into commercial rice cultivars adapted to rainfed lowland areas in North and

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Northeast Thailand. This project is supported by the Rockefeller Foundation and has just begun in the wet season 2000. The characters being examined are leaf water potential, osmotic adjustment, spikelet sterility, delayed flowering, yields under well water and water deficit conditions, and drought response index (DRI).

Two populations, double haploid (DH) population (CT9993-5-10-1-M x IR62266-42-6-2) and near-isogenic lines (NILs) (Azucene x IR64), will be examined in this work to achieve the first objective. Field experiments are being conducted at three sites, Ubon Rice Research Center and Chum Phae Rice Experimental Station in the northeast and Phrae Rice Research Center in the North. There are three systems of water deficit trials to be used: a line-source sprinkler, late season drought stress, and a slow water deficit development.

Quantitative trait loci for all characters mentioned above will be identified in mapping population by BIOTEC (Thailand). Stable QTL for osmotic adjustment (OA) and leaf water potential (LWP) will be introgressed into Thai commercial varieties via MAS. BC2 will be evaluated across the target environments.

**Session 4: Field Screening and
Genetic Improvement of
Drought Tolerance in Other Crops**

4.1 Field Screening for Drought Tolerance - Principles and Illustrations

Francis R Bidinger¹

Abstract

Establishing a screening procedure for genetic differences in drought tolerance involves 1) practical decisions on the objectives of such a screening program, 2) the selection of environment(s) and stress occurrence(s) to be targeted in the program, and 3) the design and operation of field physical facilities and experimental methods to apply a uniform, repeatable drought stress. This paper considers these points from a conceptual and a practical viewpoint.

Drought tolerance can be approached on various plant organizational levels, from crop yield stability under stress, through responses to stress indicative of tolerance, to the biological mechanisms that underlie these responses, to the genes and alleles governing the presence or expression of the responses/mechanisms. Defining stress tolerance at each level has specific advantages and disadvantages for designing a field-screening program. Work on pearl millet has mainly focused on the crop tolerance response level, targeting the relative ability of genotypes to maintain grain numbers per panicle and seed filling in terminal stress environments.

Target environments and target stress occurrences for a screening program must be established from the analysis of historical climate data. Water budgeting is probably the minimum level, but opportunities to use crop simulation modeling for this purpose are improving. Establishing screening systems with environmental conditions representative of the target environment is difficult, involving a major tradeoff between providing representative day length, vapor pressure, and temperature conditions, and easily managing soil water/rainfall. In contrast, duplicating target environment moisture patterns in non-target environments is easier, but G x E effects can be a problem.

The effectiveness of a drought screening procedure is best measured by the genetic heritabilities achieved for target traits, whether the focus is

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conventional or marker-assisted plant breeding. Managing drought screening nurseries therefore requires careful analysis of likely sources of nongenetic variation among plots, replications, and repeated experiments, and seeing that these are minimized. These include 1) the choice of site for screening, 2) the physical management of both water-related and non water-related sources of variation in crop growth within and across experiments, 3) the choice of experimental design and the effective use of blocking to remove expected sources of nonmanageable variation, and 4) the efficient collection and management of data. These considerations are illustrated here with examples from the pearl millet drought screening system used at ICRISAT.

Introduction

Accurate field phenotyping of mapping populations, for traits as complex as drought tolerance, is almost certainly the limiting factor in our ability to detect and evaluate molecular markers for such traits. The creation and genotyping of mapping populations is often the more expensive part of the overall effort, but its ultimate success depends much more on the effectiveness of the phenotyping procedure in detecting repeatable, highly heritable differences among recombinant lines, that permit the identification of robust quantitative trait loci (QTL). Drought tolerance is a particularly difficult topic for molecular mapping as it is not possible to define or measure tolerance with the same clarity or precision as it is for disease resistance or for morphological or physiological traits, nor is it easy to manage experimental drought environments with a high level of control and repeatability. Therefore, extra effort is needed in the conceptualization, design, and management of phenotyping programs for drought tolerance, to maximize the chances of identifying QTL that will be useful in the future improvement of tolerance in the target crop and in the target environment. This paper reviews some of these considerations in 1) developing a functional definition of drought tolerance to use in a screening program, 2) designing screening procedures to focus effectively on the target environment and its major stress problem(s), and 3) managing the screening experiments to minimize problems in detecting heritable differences in tolerance. General considerations will be illustrated by examples from the screening program for terminal drought tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br.] at ICRISAT.

Defining Drought Tolerance

Drought tolerance has been defined in many ways in the past; but not all of these are likely to be equally useful for a program with the ultimate goal of genetic improvement of crop yield, or the stability of crop yield, under drought stress. It is possible to group various approaches to defining stress tolerance into the following four hierarchical classes, each of which has its own implications for use in a screening program.

A stable grain yield despite the occurrence of stress. Although a more stable yield is the ultimate objective of stress research, and while the presence of desirable traits, mechanisms, or QTL should result in a more stable yield, yield under stress is probably too complex a phenomenon to use as a variable for evaluating stress tolerance per se, as it represents genotype response to the total of the environmental factors to which it has been exposed over the course of the entire season. In addition, grain yield has predictably very large environmental (E) and genotype x environmental (G x E) effects, and consequent modest across-environment heritabilities, which reduce its value as a screening/selection criterion.

The maintenance of normal developmental and growth processes under stress (such as maintenance of normal water status, developmental events, and leaf area). Focusing screening on such processes has the advantage of better focusing on unambiguous expressions of field resistance/susceptibility, rather than on yield itself. At the same time, it is often relatively straightforward to link the maintenance of normal growth processes under stress to more stable yields. On the other hand, the field quantification of such responses may be considerably more demanding than quantifying yield differences, and their expression, and therefore heritability, may also be affected by G x E interactions.

The biological mechanisms underlying these favorable responses under stress. Associating drought tolerance with the existence or expression of specific biological mechanisms (e.g. maintenance of plant water status or cell turgor) can help greatly in defining the focus of field or controlled environment screening and in establishing screening protocols which allow better management of E and G x E influences. However, a focus on underlying mechanisms is likely to be at the cost of the linkages to final grain yield, and to increased measurement costs, thereby complicating conventional and molecular breeding for tolerance.

The loci or alleles that underlie these biological mechanisms. Focusing on genes coding for basic mechanisms can (theoretically at least) greatly simplify the problem of breeding for drought tolerance to one of simply selecting for established DNA markers, without the effects of E and G x E interactions that complicate phenotypic selection. However, it is very likely that adaptive responses to stress are multigenic, and that the expression and consequences (if not the presence) of QTL for stress tolerance are still subject to G x E influences. More experience with QTL as selection criteria for stress tolerance is needed before it will be possible to confidently equate stress tolerance to the presence of selected QTL.

A useful, applicable criterion for stress tolerance, and ultimately a useful selection criterion, should have several attributes, which may not always be fully compatible in a single definition:

- There must be a clear, strong linkage between drought tolerance and higher or more stable grain yield in the target stress environment.
- The across-stress-environment heritability of tolerance should ideally be higher than that of grain yield itself.
- The expression of tolerance must be readily measurable, with adequate replication in both time and space, of the numbers of genotypes necessary in contemporary phenotyping/breeding programs.

These requirements tend to favor specific whole plant or crop responses to stress that are clearly linked to yield maintenance, and which can be readily and repeatedly measured on large numbers of genotypes, such as the anthesis-silking interval used as an indicator of differential susceptibility to stress at flowering in maize (Bolanos and Edmeades 1996). More basic physiological and chemical mechanisms, by and large, are not sufficiently strongly linked to yield maintenance under stress, and have major sampling and measurement limitations for large populations, that make them less attractive as functional definitions of drought tolerance.

Defining Terminal Stress Tolerance in Pearl Millet

An analysis of factors associated with differential ability to maintain grain yields under terminal stress in pearl millet has led us to identify panicle harvest index or PNHI (the ratio of grain to total panicle weight, on a plot basis - line 1, Table 4.1.1) as an indicator of genetic tolerance/ susceptibility to such stress (Fussell et al. 1991). Stress beginning at different times during the flowering

Table 4.1.1. Effects of increasing severity of terminal drought stress on pearl millet panicle yield components and panicle harvest index (hypothetical data).

Drought severity and time of onset	Rachis, glumes, etc. (g)	Grains per panicle (no.)	Single grain mass (g)	Total grain mass (g)	Total panicle mass(g)	Panicle harvest index (%)
Non-stress	5.0	1500	.0100	15.0	20.0	75
Mild, Late onset	5.0	1500	.0085 (-15%)	12.8	17.8	72
Moderate, late onset	5.0	1500	.0070 (-30%)	10.5	15.5	68
Moderate, mid onset	5.0	1275 (-15%)	.0070 (-30%)	8.9	13.9	64
Severe, mid onset	5.0	1275 (-15%)	.0055 (-45%)	7.0	12.0	58
Severe, early onset	5.0	1050 (-30%)	.0055 (-45%)	5.8	10.8	53
Severe, pre-flowering onset	3.5	600 (-30%)	.0040 (-60%)	2.4 (-60%)	5.9	42

and grain filling periods affects the various panicle yield components formed during these periods in predictable ways. For example, a stress beginning late in the grain filling period will affect mainly individual grain mass; a 15% reduction in individual grain mass will reduce total panicle grain mass from 15.0 to 12.8 g, total panicle mass from 20.0 to 17.8 g, and PNHI from 75% to 72% (Table 4.1.1, line 2). Similarly, a 30% reduction in individual grain mass will reduce PNHI from 75 to 68% (Table 4.1.1, line 3). A stress beginning earlier will reduce both grain number and individual grain mass, with greater effects on PNHI (Table 4.1.1, lines 3, 4, and 5). In this fashion, PNHI is a simple but effective measurement for quantifying the known effects of stress during flowering and grain filling.

Different levels of genetic tolerance, expressed as differential ability to maintain both grain numbers and grain filling under stress, are effectively captured by differences in PNHI (Table 4.1.2). For example, a tolerant genotype will more effectively maintain both grain number and individual grain mass, than will an intermediate or susceptible one (compare lines 3, 4,

Table 4.1.2. Consequences of different levels of terminal stress tolerance on panicle components and panicle harvest index (hypothetical data).

Genotype level of tolerance	Rachis, glumes, etc. (g)	Grains per panicle (no.)	Single grain mass (g)	Total grain mass (g)	Total panicle mass (g)	Panicle harvest index (%)
Non-stress	5.0	1500	.0100	15.0	20.0	75
Escape - early flowering	5.0	1500	.0085 (-15%)	12.8	17.8	72
Tolerant	5.0	1350 (-10%)	.0085 (-15%)	11.5	16.5	70
Inter- Mediate	5.0	1200 (-20%)	.0070 (-30%)	8.4	13.4	6
Susceptible	5.0	1200 (-20%)	.0050 (-50%)	6.0	11.0	5

and 5 in Table 4.1.2), which is clearly reflected in the differences in PNHI. Because PNHI integrates the effects of stress on both grain number and grain filling, it is less subject to compensatory tradeoffs between individual yield components, and is better related to yield-based estimates of tolerance/susceptibility to terminal drought stress than are the individual components. Panicle harvest index is, however, influenced by differences in drought escape (i.e. by differences in the severity of stress actually experienced by different genotypes), so valid comparisons can be made only between genotypes with similar flowering times.

Panicle harvest index has been successfully evaluated as a selection criterion for terminal stress tolerance in pearl millet in both variety and hybrid parent breeding (Bidinger et al. 2000) and it is currently being used as one of the traits for which QTL are being identified from a mapping population made from parents that differ in the ability to maintain PNHI under stress. PNHI, however, is readily and inexpensively measured in field experiments, and can be readily used as a direct selection criterion. The main potential benefit to identifying QTL for PNHI will be in allowing rapid, marker-assisted backcross transfer of improved tolerance of terminal stress to otherwise elite lines and varieties, without the requirement for extensive field screening.

Selection of a Screening Environment/Method

Experimental procedures to screen for drought tolerance, however this is defined, need to be effective in identifying heritable genetic variation *for the specific target environment and the target stress (es) in this environment*. They thus need to reliably provide stresses of the timing, severity, and duration characteristic of those stresses common in the target environment. Quantifying the nature of the stress (es) in the target environment requires an analysis of long term climatic data; using, as a minimum, a water balance model approach which integrates rainfall, plant-available water in the soil, potential evaporative demand, and crop coefficient (Frere and Popov 1979). Crop simulation modeling can provide a much more rigorous analysis; if an appropriate crop model and long term weather data sets are available (Muchow et al. 1999). The better the description of the variation in the occurrence of stress in the target environment, the better targeted the screening is likely to be.

Screening environments can be either natural growing environments, chosen/managed to maximize the frequency of stress under natural environmental conditions, or specially managed stress environments in which the emphasis is primarily on a providing a controlled, repeatable stress. Whether or not the screening environment needs to exactly duplicate the overall target environment depends partly on the way in which drought tolerance is to be assessed. If the screening is targeting a yield-based definition of tolerance, then the environmental conditions of the screening environment which affect yield need to duplicate those of the target environment. For example, if daylength in the target and screening environments differs to a degree sufficient to affect phenology, then drought escape, which can play a large role in the determination of yield under stress, will operate differently in the screening and target environments. Under such conditions, it is better to use a variant of the natural target environment (rain shadow sites, shallow soil fields, late sowing) where stress is likely. However, if the intent is to evaluate more basic stress responses or tolerance mechanisms, it may be feasible to use non-natural growing environments such as a dry season or more arid locations where the occurrence and severity of stress can be controlled through management of irrigation or sowing date.

Managed stress environments have definite advantages in terms of control and repeatability of stress, with consequent advantages in control of G x E interactions and improved heritabilities of tolerance-related

observations. Managed stress environments can also be used to exploit repeatable genotype x stress interactions to improve specific adaptation to defined stresses in the target environment. There are two options for using managed stress environments: 1) artificially creating stress in a normal growing season, and 2) managing water availability in the dry season. The first option has the definite advantage of avoiding genotype x season interactions, which can affect genotype response to stress, but excluding water to create stress in a normal growing season can be costly/difficult. Using rainout shelters, covering the surface of soils to encourage runoff, etc. are feasible for small, critical experiments, but less so for large-scale screening exercises for most field crops. Managing water in the dry season or a dry location has the advantages of scale, reliability, and economy of screening, but may require verifying that the expression of tolerance is not affected by genotype x season interactions. Most field screening is done under managed stress environments, but there is often inadequate assessment of the repeatability of genetic differences observed in the dry season, in the target environment itself.

Whatever the screening environment selected, the screening protocol designed needs to achieve the following objectives:

Application of a Uniform Moisture Stress

Unless all genotypes in the screen are exposed to a similar stress, the measured differences among them are as or more likely to reflect differences in stress experienced, than differences in stress tolerance/susceptibility (Blum, this volume). The screening procedure thus must assure uniform water application rates, uniform soil water storage/plant-available water content, and a uniform rate of potential water use. Some of this is a matter of good experimental management, but choice of field, especially soil texture and depth, and design of water application systems can also make large differences. Sprinkler irrigation, for example, is convenient but seldom uniform.

Application of Repeatable Moisture Stress

Uniformity across experiments is as critical as uniformity within experiments in obtaining broad sense heritabilities of sufficient magnitude to use in either direct selection for tolerance, or in the identification of tolerance QTL. Repeatability over experiments requires a screening environment with stable potential evaporation, a regular, dedicated field screening facility, and well

established field and crop management systems, to minimize G x E interaction effects on tolerance expression.

Effective Differentiation between Genotypes

To effectively distinguish differences among genotypes requires that stress is of a sufficient severity to obtain statistically significant differences among genotypes for the measurements of stress tolerance to be made, but not so severe that genotype differences are expressed. It also requires that differences among genotypes due to differential stress exposure (stress escape), rather than to differential stress tolerance/susceptibility, be minimized. Achieving both of these objectives will require some initial experimentation; particularly where there are significant differences in phenology among test materials.

Screening Environment for Terminal Stress Tolerance in Pearl Millet

The main growing area in the northwest (NW) Indian states of Rajasthan, Gujarat, and Haryana is the target environment for ICRISAT work on stress tolerance in pearl millet. This area has a short (75 to 90 day) growing season with a total seasonal rainfall between 250 and 500 mm, in a generally arid to dry semi-arid climate. Soils are mainly sandy, with low to moderate levels of plant-available water content. Growing season temperatures (mean maximum $\sim 33^\circ$ and mean minimum $\sim 25^\circ$) and potential evaporation rates are high ($\geq 6 \text{ mm day}^{-1}$). An analysis of the frequency of occurrence of drought stress, based on a five-day soil water budget, for a transect across central and western Rajasthan indicated that post-flowering stress, either alone or in combination with preflowering stress, is a very common feature of the environment (Table 4.1.3, van Oosterom et al. 1996). In the two drier sites (Bikaner and Banner) terminal drought occurred between 75 and 80% of the years: between 15 and 30% percent of the years alone, and in 50-60% of the years in combination with preflowering drought. ICRISAT millet research has thus focused on terminal drought tolerance, as terminal stress is clearly a common feature of the target environment and is the most damaging to grain yield, as the crop has few adjustment mechanisms available to it in contrast to the situation with preflowering drought stress (Mahalakshmi et al. 1987).

Table 4.1.3. Distribution of years with various combinations of severe pre- and post-flowering drought stress at four locations in Rajasthan. Values in parentheses are frequencies (%). Results are based on water balance studies, using long-term daily rainfall data (van Oosterom et. al. 1996).

	Severe drought stress class			
	No	Yes	No	Yes
Preflowering	No	No	Yes	Yes
Postflowering	No	No	Yes	Yes
Ajmer	63 (72)	3 (3)	19(22)	2 (2)
Jodhpur	26 (31)	8(10)	33 (39)	17(20)
Bikaner	6 (7)	13 (16)	13 (16)	51(61)
Barmer	5 (9)	7(13)	16 (29)	28(50)

Managed irrigation has been used during the dry season at Patancheru for the majority of the screening work, although key trials are regularly planted in the target area as well. The main reason for this is the requirement for very high-quality trial management on a large scale (4-6 ha yr⁻¹), which has been difficult to achieve on collaborators' research stations in NW India. The use of irrigation in the dry season allows effective (and repeatable) management of the timing and severity of the stress. Temperatures and vapor pressure deficits during March/April, when the stress is applied, are representative of those during drought periods in NW India. We know however, that we have genotype x season interactions for actual grain yield with landrace material from NW India, possibly because of differences in early season temperatures, and differences in day length, between the dry season at Patancheru (17° N) and the normal season in NW India (23-28° N). We believe that genotype x season interaction for PNHI and its components is not a serious problem, where flowering, and hence drought escape, is not influenced by genotype x season interactions for phenology.

Management of Screening Nurseries

Effective screening for genotype differences in drought tolerance/susceptibility requires a high degree of care in the design and management of the trials to obtain precise data and to maximize the heritability of the selected measurements of drought tolerance. This is particularly critical in field experiments designed to identify QTL for tolerance, as the strength of QTL for target traits depends directly on the heritability of these traits achieved in

the experiment. The more the measure of drought tolerance is influenced by local environmental variation, the greater is the need to control such variation. Effective management of experiments to control variation requires a number of components:

Field Screening Facility

The screening facility must be capable of applying a uniform stress to a large set of genotypes. This means that the soil of the field must have uniform plant-available water content, that the irrigation system chosen must be able to apply water uniformly, and that the location of the field be such that it has a spatially uniform rate of potential evapotranspiration (ET). Any deviation from these three requirements (such as variation in soil texture or depth, nonuniform irrigation water application, or inadequate fetch or local windbreaks) will result in gradients (at best) or nonlinear heterogeneity (at worst) in the timing or severity of the stress applied. In addition, it is necessary to be able to repeat stress environment (timing, severity, and duration) over experiments, to confirm genotype differences in tolerance and to maximize across-environment heritability of tolerance estimates. To do this, it is generally necessary to have a dedicated field for screening, in a stable water use environment, and to use consistent, well-established crop, soil, and water management practices.

Statistical Design

Despite maximum care in the choice and management of a field screening facility, there will still be experimental error: soils are not commonly uniform in depth or texture; most irrigation systems have inherent gradients in water application. It is necessary to understand the sources of experimental error in a screening procedure, and to use appropriate statistical designs and field blocking to remove as much of the known sources of error as possible. Unbalanced lattice or alpha designs, which allow a high degree of blocking within replication, can be very useful to adjust for both primary (replication) and secondary (within-replication blocks) gradients in soil water holding capacity, water application patterns, etc. Small blocks also provide greater flexibility in field layout that larger replications do not, and provide useful ability to adjust for the effects of time in the collection of data where this is a major confounding factor (plant water potential or water content).

Field/Crop Management

In addition to variation in factors affecting water availability, differences in crop growth prior to the application of stress are often major confounding factors in the assessment of stress tolerance, as these result in differences in ability to access soil water, in canopy transpiration rate, or in inter-plot competition. It is necessary to improve field management practices to eliminate, as much as possible, differences in plant stands, in fertilizer application rate, in pre-stress water application and drainage, and in pest and disease incidence. Management of the final irrigation prior to initiating the stress is a particularly critical factor. This should be designed to completely fill the soil profile to eliminate differences in soil water storage due to the effects of previous irrigation, or differences in water use among genotypes, and to then rapidly drain excess water to prevent local waterlogging. Finally, the experimenter should be prepared to learn from past problems and to adjust management practices to minimize these.

Data Management

Finally, the screening system needs to be organized to record, manage, and verify large volumes of data from screening experiments. Data collection should be done electronically wherever possible; simple and inexpensive equipment is available for recording scores, weights, and measures; in the case of weights, these can be directly linked to electronic balances, so all that needs to actually be entered is the plot identity. This reduces both time and errors in recording data. Also, quick and efficient procedures for checking the completeness and accuracy of data are easy to establish with modern spreadsheet/analytical software, which will detect outliers and missing plots, and calculate means, ranges, and basic statistics. Finally, linking spreadsheets to analysis packages can allow the scientist rapid access to analyzed data to check heritabilities of measurements and means for control entries.

Management of Pearl Millet Screening Nurseries

Field Screening Facility

For field screening, only a designated six hectare field with a shallow and relatively uniform soil profile is used, which contains enough plant-available water for about 6 days of full ET during April, when pan evaporation rates

average 8-10 mm day⁻¹. As a part of the development of this field for surface irrigation, the A and B horizons of the original soil (50-75 cm depth) were removed, the gravelly subsoil material graded to a uniform slope of 1.5%, and the surface soil spread evenly over the graded subsoil. Thus the major source of heterogeneity in the original field - the variable depth of soil to the C horizon, and the consequent variable amount of plant-available water - has been largely removed.

Sprinkler irrigation is used to supply water to the crop before flowering, adjusting the amounts of water applied to meet increases in transpiration demand as the season progresses, as millet is sensitive to low soil oxygen tensions that occur following surface (furrow) irrigation during cooler times of the year. Sprinkler lines are placed 14.4 m (24 crop rows) apart, with each sprinkler line in the center of 4 border rows, so that leakage from the sprinkler lines does not affect test plots. Final irrigation before the onset of the stress is done by furrow, to completely fill the soil profile.

The time of planting of the nurseries is standardized to have the crop flower and fill grain during the period of maximum evaporative demand, and irrigation is managed to achieve a 50-60 % reduction in yield for a severe stress and a 30-40% reduction for a moderate stress. Standard crop management procedures (described below) are followed to obtain uniform preflowering crop growth and initiate the stress (es) at fixed crop developmental stage(s). This latter is necessary as differences in temperatures during the earlier, cooler part of the growing season can affect time to flowering, even though a common planting time across years assures a similar daylength each year.

Statistical Design

Incomplete block or alpha designs are generally used in the majority of screening experiments, to provide for as much adjustment capability to local variation in stress intensity as possible. Small blocks of between 6 and 9 plots are used (18-27 m²/block), with the total number of blocks variable, depending upon the numbers of entries in the trial. It is generally found that the effect of such blocking is statistically significant, despite the general precautions taken in managing experimental crops.

The sprinkler irrigation system used provides standard 20 row experimental 90-m long strips between the lines (Fig. 4.1.1). We replicate along the 90-m axis to adjust for differences in water application due either to decreasing pressure in the sprinkler line before flowering, or to differences in

← Sprinkler line and furrow direction →							
1					46	Block	Replication
2					47		
3					48		
4	Single Plot				49		
5					50		
6					51		
7					52		
8					53		
9					54		
← Sprinkler line and furrow direction →							

Figure 4.1.1. A example of plot block, and replication arrangement within one experimental strip between two irrigation lines, from a phenotyping experiment conducted on 162 mapped F2 - derived F4 lines. Each block consists of 9 plots (3 plots wide x 3 plots long); each replication consists of 18 such blocks (6 blocks per strip x 3 strips); and one stress environment consists of 3 replications (across 3 such strips).

time for water infiltration along the 90-m axis during the final prestress surface irrigation. The blocks are then arranged at right angles to the sprinkler lines (and the 90-m axis) to adjust for differences in water application between the sprinkler lines before the stress, or to miscellaneous local variation (Fig. 4.1.1). The ICRISAT statistician is currently evaluating the effectiveness of spatial adjustment techniques to further reduce effects of both inherent and management-induced variation.

Field/Crop Management

A number of ways to improve the uniformity of crop growth prior to the initiation of the stress have been learned by experience. 1) The field is land planed every 2 to 3 years to remove local surface irregularities that result in collection of excess irrigation water and reduced crop growth. 2) Fertilizer is banded into the ridges with a precision applicator, rather than broadcasting it, to assure that all seedlings have equal access to nutrients. 3) Light sprinkler irrigation is provided prior to sowing, to moisten the surface soil and improve control over the depth of seed placement. 4) Oversowing is done with a precision planter and seedlings thinned about 10 days after emergence to achieve uniform plant stands. 5) Sprinkler irrigation is used in the early crop stages, rather than furrow irrigation, to prevent excess water application and reduced crop growth. 6) Sprinkler irrigation is provided at the time of

secondary root initiation to assure that these roots penetrate the soil rapidly and completely. 7) Weed management is practiced during the entire year in the screening field to prevent the buildup of weed seed, and cultivation is done early and as often as necessary to remove weed seedlings in early stages before they can establish. 8) Prophylactic pest and disease control is applied whenever a problem is suspected (for example a soil insecticide is banded with the seed to control wireworms, when following a groundnut crop).

At the time of initiation of the stress, furrow irrigation is used to be sure that the full soil profile is wetted. The furrows are filled rapidly, one strip at a time, to have a sufficient head of water for this purpose. Water is held in the furrows for 4 hours and then drained rapidly to prevent waterlogging. All irrigation operations are managed by the researchers themselves to assure that irrigation is done as precisely and uniformly as possible.

Data Management

Hand-held data collection devices (Tandy portable computers and Omnidata polycorders) are used to record all information taken in the field or the laboratory. This includes flowering dates, plant and panicle counts, plot scores for various criteria, and outputs from instruments without microprocessor storage. All of the balances are linked to one or more of the same instruments so that the weight is automatically recorded by depressing the enter key, following the manual entry of the plot numbers. For this purpose plot numbers are never repeated within a season, so that it is not necessary to identify the experiment, location, etc. in the data entry.

Data are downloaded to a personal computer twice a day (noon and evening) to prevent loss of data stored on data collection devices. Because unique plot numbers are used, it is easy to sort data (such as grain weights) in a spreadsheet from more than one experiment and, by ordering plots in ascending order, to quickly determine if any plots have been missed or if there are any duplicate plot numbers entered. Means and standard deviations are calculated for all variables with either Excel or SAS to establish expected ranges of data values, and possible outliers are searched for using the delete and print option in SAS.

Rapid, same day analysis of data can be done if required, as all variable names are standardized, transformation routines to calculate derived variables, conversion of plot values to unit area values, etc. are standard, and

analysis models/statements written for each experiment. Finally one staff member handles all data analysis and archiving, after the technicians check it for missing values.

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4.2 Field and Laboratory Screening for Drought Tolerance in Sorghum

N Seetharama and V Mahalakshmi¹

Sorghum is generally thought to possess several excellent drought-tolerant characteristics. However, the progress made in improving drought tolerance of modern cultivars is still very limited, and that too was mainly achieved through empirical screening. Despite the surge in physiological research on drought tolerance and the contributing plant traits during the seventies and eighties, their direct application in breeding is insignificant. The usefulness of simpler traits such as accumulation of a stress-induced metabolite can be tested by techniques such as genetic transformation with relevant candidate genes for over-expression, or with antisense versions of such genes for suppression. However, most of the traits that may be used in breeding in the near future are complex as they relate to overall crop development, yield formation, and performance under stress. While such traits are good candidates for using DNA marker-assisted selection, the key to successfully identify markers lies in our ability to phenotype large mapping populations accurately and consistently under a range of target environments. Unfortunately, such techniques, especially field-based ones, are yet to be fully developed.

In this paper, we discuss progress made with field screening of large sets of germplasm accessions or populations in managed nurseries, and the problems faced in both screening and breeding for drought tolerance. The problems include complex interactions of morpho-physiological traits of test entries with stress occurring at different stages of crop growth, and other edaphic, climatic, crop management, and biotic stress factors. Considering both the complexity and the number of traits contributing to drought tolerance, one needs to deal with operational traits that are intermediate in complexity and integrated in effect. Examples of the above are traits related to better stand establishment, slower leaf senescence during grain-filling ('staygreen') or root growth, and water extraction during drought. Thus, using appropriate combinations of managed field-nurseries, semi-controlled

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environments, and field experimental designs, it should be possible to phenotype large populations for desired component trait of drought tolerance. In all cases (and especially so in the case of laboratory or glasshouse screening) from the beginning, the quantitative relationships between final performance and expression level of adaptive traits need to be considered as they are not always linear. When such a capacity is demonstrated, sorghum can be a model crop for application of biotechnological tools for improvement for drought tolerance.

4.3 Breeding for Drought Tolerance in Maize: From Theory to Practice

M Banziger¹, J M Ribaut, J Bolanos, and G O Edmeades

Most maize in the developing world is grown under rainfed conditions and an estimated 24 million tons of maize are lost annually to drought. This paper gives a brief overview of the breeding approach taken by El Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), better known as the International Maize and Wheat Improvement Center, 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. These 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, and associated quantitative trait loci have been identified. Little change was found in water uptake and water use efficiency. Challenges to molecular approaches lay in 1) identifying the genes underlying known drought-adaptive traits, 2) exploring additional traits that confer drought tolerance, and 3) cost-effectively deploying molecular techniques that improve the drought tolerance in adapted germplasm.

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4.4 Genetic Improvement of Drought Tolerance in Chickpea at ICRISAT

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Abstract

More than 90% of the global chickpea-growing area is rainfed. Chickpea is grown mostly as a post-rainy season crop on soil moisture conserved from the preceding rainy season. The crop is therefore, often exposed to terminal drought and heat stress. Yield losses in chickpea due to terminal drought range from 20% to more than 50%. Large effects of climate and soil moisture on chickpea yield, and strong crop x soil x climate interactions were observed in field experiments in India. Research on management of drought has therefore been a high priority theme in the ICRISAT research portfolio.

Controlled terminal drought conditions were created in field experiments at ICRISAT (Patancheru: 18° N, 78° E) and more than 2000 chickpea germplasm and elite breeding material were screened in post-rainy seasons for many years. From the world collection of chickpea germplasm at ICRISAT, a subset was created on the basis of phenology (days to flowering and maturity) to minimize confounding effects of drought tolerance and escape from drought in a given set of test genotypes. Drought tolerance indices were computed using appropriate statistical methods, with yield and days to flowering as variables.

The drought-tolerant genotypes thus identified were characterized, and root and shoot traits associated with tolerance were identified. A nondestructive method to screen for root system at early crop growth stages was developed. A causal relationship between morphological (root and shoot) traits and maintenance of high midday shoot water potential was established. A functional trait of rate of seed filling was also identified. These traits were used for making drought-tolerant selections in the segregating populations of

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the conventional chickpea improvement program. The same traits were also used to characterize Recombinant Inbred Lines (RILs) for identifying molecular markers of drought tolerance. Chickpea varieties enhanced for drought tolerance with stable and high yield were developed.

Field methods of screening chickpea for drought tolerance, and drought-tolerant sources identified at ICRISAT, were verified by ICRISAT's partners in the National Agricultural Research Systems (NARS) in India and also in Iran and Ethiopia.

Introduction

Crops grown on farmers' fields are often exposed to various kinds of biotic and abiotic stresses, causing huge losses of available genetic- and realizable- yield potential. Losses in chickpea yield due to drought alone range from 20 to 50% (Saxena et al. 1993). Progress made in chickpea drought research shows that it is realistic to expect to recover at least half of these losses through a holistic and integrated management of drought, which would contribute to a substantial increase in global chickpea production. One of the important components of the integrated management of drought is the development of drought-tolerant varieties, using conventional or biotechnological tools.

The first step in this direction is the identification of drought-tolerant germplasm to initiate a systematic crop improvement program for developing drought-tolerant varieties. To achieve this first objective it is essential to develop methods of field screening that are representative of the target drought conditions, and are simple, rapid, reliable, and reproducible.

The second step is to characterize the drought-tolerant sources for identification of morphological (root and shoot) and functional (process-based) traits of drought tolerance. These traits could be used in conventional trait-based (ideotype) breeding programs, and for the identification of molecular markers of drought tolerance for use in marker-assisted breeding programs.

The third step is to validate the traits and response in yield of the varieties enhanced for drought tolerance traits in simulated drought environments and also in target drought-prone areas in on-farm experiments.

Development Of Field Screening Methods

Essential factors that were considered while developing the field screening method for drought tolerance were that the:

- Method(s) developed should be simple and reproducible.
- Experimental drought be similar in intensity and duration as in the target area.
- Onset of drought in experiments was the same as in on-farm conditions in relation to crop developmental stage.

Simplification of screening methods is considered essential to encourage routine use of the methods developed in conventional breeding programs.

Characteristics Of Terminal Drought In Chickpea

The soil and climate drought (evapotranspiration demand of the atmosphere) as it prevails in two contrasting chickpea-growing locations in India, ICRISAT center (Patancheru 18° N, 78° E) and at Hisar (30° N and 75° E) in northern India were studied and described (Saxena 1987). In order to characterize the annual variation in the natural occurrence of terminal drought, simulated soil moisture profiles during the chickpea-growing seasons were studied at ICRISAT-Patancheru. (Johansen et al. 1994).

It was evident from the data reported that the onset of terminal drought depended upon the receding of monsoon rainfall. However, a moderate to severe intensity of drought always sets in by the time flowering commences in rainfed chickpea crops at ICRISAT, even in the short duration, early season varieties. Therefore, to avoid variations that could be introduced by the uncertain end of the rainy season, the experiments were irrigated to minimize the effects of transient drought until the time of flowering. Drought treatments were then imposed by withholding irrigation to treatment plots. This was done so that the simulated drought treatments in the target environment are similar in characteristics to natural drought. It would also ensure that the method could be reproduced across experiments in a given year and between experiments in different years at one location.

Application of Drought Treatments

Two methods are commonly used in chickpea drought research at ICRISAT for creating simulated drought conditions. One is by imposing non-stress (control, frequently irrigated with known amounts of water) and terminal drought (withholding water coinciding with flowering time) (Saxena 1987). Irrigation was applied by surface flooding with gated irrigation pipes or by an overhead method using perforated irrigation pipes. The second method creates a gradient of drought by using a line-source sprinkler irrigation method (Johansen et al. 1994). Field experiments were conducted on both Alfisol (more severe drought) and Vertisol (moderate drought) to screen against severe and moderate intensities of drought. Yield reduction due to drought in field experiments with simulated drought generally ranged between 30-50% across Alfisol and Vertisol. This was similar in magnitude to yield reduction generally observed in farmers' fields and in chickpea experiments at many places in India.

In both the methods, the amount of soil moisture during the crop-growing season is monitored using the neutron probe method and the gravimetric method. These measurements are made to relate genotypic differences in response to drought between years and experiments.

Precise control of experimentally managed drought environments is critical in field drought screening experiments, particularly when empirical selection criteria such as yield or shoot mass production are used. To protect these experiments from uncertain rains, manually moveable rainout shelters, designed and developed at ICRISAT, (Chauhan et al. 1997) were used.

Design of Field Experiments

In the first method (Saxena 1987), the control (non-drought) and drought treatments were applied to the main plots in a split-plot design. The test genotypes were allocated randomly in the subplots. In the preliminary screening when more than 500 test entries were evaluated in each test, the test entries were allocated in augmented design with three check entries in fixed position in each block. In the second method, in which a gradient of drought is applied (Johansen et al. 1994) the field experimental design was a strip plot, where the irrigation treatments were fixed but the varieties were randomized, and the design was in a replication.

The two methods of screening were found to be very effective in identifying drought-tolerant sources in chickpea. It was encouraging to find that the drought-tolerant traits identified by the first methods (Saxena 1987) were verified by the second method (Johansen et al. 1994). The first method of screening was also verified by the partners of the Indian chickpea drought research network coordinated by the Indian Institute of Pulses Research (IIPR), Kanpur, India (ICRISAT 1996).

Identification of Sources of Drought Tolerance

Genotypic differences in drought tolerance were compared by computing drought tolerance indices (Saxena 1987, Johansen et al. 1994). More than 2000 chickpea germplasm available in the ICRISAT gene bank (originating from all the important chickpea-growing countries in the world, many of them from drought-prone regions in Asia and Africa), ICRISAT elite chickpea breeding material, and released chickpea varieties were screened for drought tolerance at ICRISAT. These were screened using the two field methods described in the section above.

Promising drought-tolerant chickpea germplasm were identified (ICRISAT 1990), and those used in the drought improvement program are listed in Saxena et al. (2000). The chickpea germplasm ICC 4958 was registered as the most promising source of drought tolerance (Saxena et al 1993). The drought tolerance of ICC 4958 was confirmed in many field trials at ICRISAT and by the Indian NARS partners (ICRISAT 1996). It also proved to be the most drought-tolerant of spring-planted chickpea in the Mediterranean climate at ICARDA (ICARDA 1989) and in autumn sowings in Ethiopia (Geletu Bejiga, personal communication). The other germplasm that has been used in the breeding program at ICRISAT is ICC 5680 (Saxena et al. 1997).

Characterization of Drought-Tolerant Sources

The drought-tolerant sources identified were characterized in field experiments. Morphological (shoot and root) and functional (rapid seed filling determined by large seed size) traits associated with drought tolerance were identified (Saxena et al. 2000., Table 1, Legumes Program, ICRISAT. 1993.). Recombinant Inbred Lines (RILs) of a wide chickpea cross (ICC 4958 x *Cicer reticulatum*) provided by ICRISAT's collaborator from Washington State

University (WSU), USA, were phenotyped for root traits (Saxena et al. 2000). Identification of quantitative trait loci (QTL) for the large root system of ICC 4958 for developing a marker-assisted selection technique is currently in progress.

A large root system was introgressed for greater acquisition of available soil moisture, and smaller leaves with fewer pinnules, to reduce transpirational water loss. These two traits were together expected to enhance water use efficiency (WUE). However, it would be unrealistic to expect large jumps in yield with the drought-tolerant varieties, as the increase in yield would always be in proportion to the fraction of additional soil moisture extracted or water saved. However, a modest increase in yield with greater yield stability under drought-prone conditions would be a realistic expectation.

Non-Destructive Measurements of Root Size

A non-destructive method of screening for larger root systems was developed in order to make trait-based selections for root characteristics in segregating populations, and at the same time advance generations of selected plants. Plants were grown in pots using a sand + soil + nutrients system. Around 40 days after sowing, soil from the pots was washed out carefully without damaging the roots. Root size of the progeny was scored visually in relation to that of parents (ICRISAT 1988) and root volume was also measured quantitatively by the water displacement method. Plants selected on the basis of root system were repotted and generations were advanced.

Enhancement of Chickpea Germplasm for Drought Tolerance

For genetic enhancement of drought tolerance, the two traits (the large root system of ICC 4958 and the fewer pinnule trait of ICC 5680) were combined through backcrossing at ICRISAT. This resulted in the development of nine varieties (ICCV 98901 to ICCV 98907) that combine these two traits. (Saxena et al. 2000, Table 3)

Causal Relationship between Traits and Crop Response

The large root system in ICC 4958 appeared to be effective in greater extraction of available soil moisture. However, the total water use in ICC 4958 was similar to Annigeri, a control variety, in field experiments conducted at ICRISAT. The real advantage of the large root system of ICC 4958 seems to be in its rapid and greater extraction of soil moisture in the early crop growth period (Krishnamurthy et al. 1996), coinciding with flowering and early seed filling stages of crop growth. The large seed size in ICC 4958 (30 g 100-seed⁻¹) confers on it the advantage of rapid and early seed filling compared to Annigeri (19 g 100-seed⁻¹), thereby accumulating most of the seed mass before the onset of severe terminal drought (Saxena and Johansen 1989, (Saxena et al. 1997). The fewer pinnule trait in ICC 5680 (Saxena et al. 1997) indeed reduced the transpirational loss of water by 30% compared to ICC 4958 in experiments conducted in controlled environment facilities at ICRISAT (Saxena 2000). Recombinants with traits of ICC 4958 and ICC 5680 maintained high mid-day leaf relative water content (RWC 80%) compared to the parents (RWC 74%) in field trials conducted at ICRISAT (Saxena 2000).

Genetic Improvement of Drought Tolerance

At ICRISAT, an ideotype approach for genetic improvement of drought tolerance in chickpea was proposed and followed (Saxena and Johansen 1990a, and b; Saxena and Johansen 1989, Saxena et al. 1997). A three-way cross was made using ICC 4958 (drought-tolerant parent), Annigeri (a high-yielding parent), and ICC 12237 (a wilt and root rot resistant parent). Following this, generations were advanced using a diversified bulk method of breeding and nine selections each of yield- and root-traits were made. Yield-based selections were effective in producing varieties with high yield and trait-based selections produced varieties with greater degree of drought tolerance (Saxena et al. 1995). Yields of root-based selections were low because selection for yield was deferred to the F8 generation and a high selection pressure on root traits alone in early generations (F4 to F6) could have eliminated recombinants with large root system and high yield. Concurrent selection for drought-tolerant traits, disease resistance, and yield in early segregating generations is, therefore, essential to develop multiple stress

tolerant and high-yielding varieties. The varieties ICCVs 94916-4, 94916-8, 94920-3, 94924-2, and 94924-3 are promising drought-tolerant, fusarium wilt resistant lines with high yield (Saxena et al. 2000).

A backcross program was initiated at ICRISAT to incorporate drought-tolerant traits in agronomically superior cultivars and for genetic enhancement of drought tolerance through combining or pyramiding drought-tolerant traits. The two drought-tolerant traits identified, large root and fewer pinnules, have been combined successfully. A few of these recombinants showed a greater degree of drought tolerance, and a high mid-day RWC and yield similar to the high-yielding parent (Saxena 2000).

The fewer pinnule leaf trait has also been incorporated into other agronomically useful genetic backgrounds, e.g. the tall types, cold (chilling) tolerant, high yield, and also in chickpea genotypes with a range in flowering duration (Saxena et al. 2000).

Conclusions

Genetic improvement of drought tolerance in chickpea does not seem to be an unrealistic or elusive goal, as has been in the past. Necessary tools (for characterizing drought environments, screening methods, sources of drought tolerance, traits of drought tolerance, and methods of rapid advancement of generations) are now available to chickpea researchers to embark upon a focused program on genetic enhancement of drought tolerance, through conventional breeding methods. In future, we can expect the progress to be even faster with marker-assisted selection techniques for traits that would further enhance the efficiency of breeding for the complex trait of drought tolerance. Drought-tolerant chickpea varieties already developed at ICRISAT and the segregating populations available for drought-tolerant traits (Saxena et al. 2000) could be used to initiate such programs and build upon the information and material already available.

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4.5 Experiences in Field Screening for Drought Tolerance in Pigeonpea

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Abstract

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is predominantly grown under rainfed conditions and is subjected to drought at one or the other stage during its growth cycle. The newly developed short-duration plant types are relatively more sensitive to drought stress than the traditional long-duration plant types due to their shallow rooting, and require improvement in their tolerance. Significant differences were found for drought tolerance between extra-short-duration pigeonpea (ESDP) and short-duration pigeonpea (SDP) lines screened using line-source sprinkler irrigation technique. The drought sensitivity varied as SDP hybrids > SDP lines > ESDP lines. Pigeonpea hybrids ICPH 8 and ICPH 9 were particularly more tolerant to drought, which appears to be related to hybrid vigor in their root system, enabling them to acquire more moisture from deeper soil profiles. The screening results were generally in agreement with the multi-location testing of SDP and ESDP lines and hybrids in different moisture environments. A limited screening under rainout shelters during the rainy season showed appreciable differences in drought tolerance. Rapid trait-based screening using leaf area retention for tolerance to drought at the preflowering stage in SDP, and osmotic adjustments for tolerance to drought at flowering stage in ESDP lines, were also feasible. ESDP line ICPL 88039 was found to be tolerant to drought at the flowering stage and is undergoing extensive on-farm testing in different drought-prone areas.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important grain legume grown under rainfed conditions in the semi-arid tropical and subtropical regions. The

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traditional medium and long-duration cultivars and landraces of the crop that mature in 6-9 months have enjoyed the reputation of being drought tolerant. These develop deep root systems and overcome drought by tapping water held in the deeper soil layers (Chauhan 1993). The need for developing drought-tolerant pigeonpea cultivars became compelling with the development of short-duration pigeonpea (SDP) and extra-short-duration pigeonpea (ESDP) cultivars whose productivity depend on the amount and distribution of rainfall (Sinha 1981). Their shallow roots (Chauhan 1993) render their productivity most susceptible to drought. Large gaps in the rainy season are not unusual in the semi-arid regions, when ESDP and SDP may be forced to grow with limited ability to extract water due to their shallow root system. Considering the greater flexibility to cropping systems and diversification of rice-wheat systems that ESDP and SDP cultivars provide, research on improving their drought tolerance was considered a worthy investment by ICRISAT, which has a world mandate for pigeonpea improvement. However, since little work on screening for drought tolerance had been done previously at ICRISAT and elsewhere, an understanding of major drought patterns, drought sensitive stages, and adaptation/development of appropriate screening methods to identify drought-tolerant cultivars were considered important.

Identification of Drought Patterns of Target Environments

Throughout the semi-arid regions of India, where much of the pigeonpea is grown, the amount and distribution of rainfall is erratic. However, based on long-term rainfall pattern, it is generally possible to broadly characterize drought patterns of a given environment by calculating probability of dry period followed by wet period or vice-versa from the long-term rainfall data (Virmani et al. 1982). This assessment is helpful for developing genotypes for target environments or for identifying environments with similar drought patterns. For example, for the pigeonpea-growing region of northern Andhra Pradesh where ICRISAT is located, the probability of drought coinciding with flowering stage is in 4 out of 10 years. Similarly, the probability of drought coinciding with the preflowering stage is 5 out of 10 years. In the rain-shadow areas of Maharashtra, drought at the pod-filling stage is expected in 4 out of 10 years. In the short-rainy season in Sri Lanka, where pigeonpea is just being introduced, the probability of protracted drought coinciding with the flowering and pod-filling stages is high.

Drought-Sensitive Stages

Intermittent drought can coincide with any of the growth stages of a crop and are generally difficult to predict on a year-to-year basis, although a broad pattern could be determined for a particular environment as indicated above. Drought responses of both ESD (Nam et al. 2001) and SD pigeonpea (Lopez et al. 1996) have been examined under rainout shelters. For ESDP and SDP, the flowering stage was found to be the most sensitive to drought followed by the preflowering stage (Fig. 4.5.1). A similar pattern has earlier been reported for SDP (Lopez et al. 1996). Thus, in spite of being a perennial crop, the behavior of pigeonpea is essentially similar to that of other annual tropical legumes such as groundnut (Rao et al. 1985). In fact, pigeonpea being a perennial crop, there could be a greater likelihood to reduce reproductive growth under stress than for annual crops which may have a greater tendency to complete their life cycle to allow seed formation for the next generation.

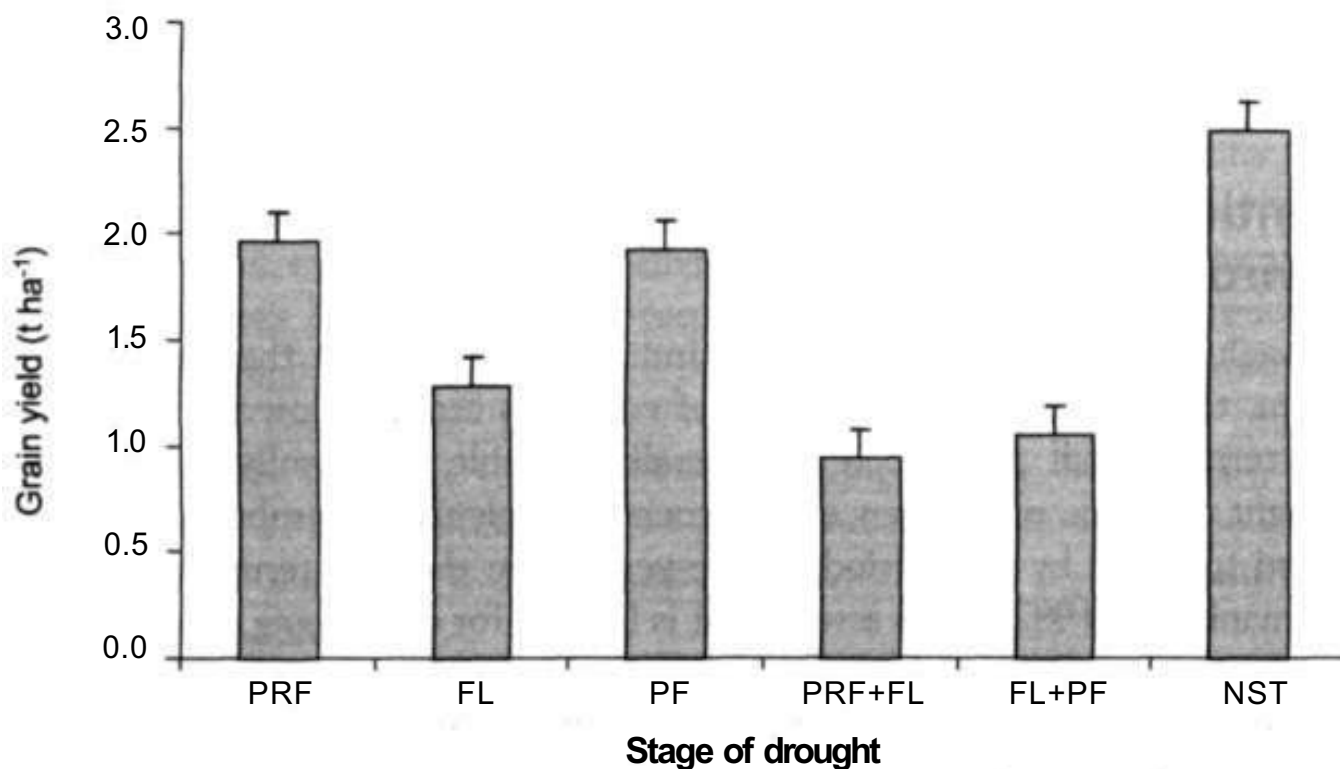


Figure 4.5.1. Effect of timing of drought stress on grain yield of extra-short-duration pigeonpea lines during the 1991 and 1992 rainy season. PRF = preflowering stress, FL = flowering, PF = pod-filling; PRF + FL = preflowering + flowering; FL + PFL = flowering + pod-filling, NST = No stress control.

Screening for Drought Tolerance

Line Source Sprinkler Method

Identification of drought-tolerant pigeonpea lines was taken up during the rainy seasons of 1986 and 1987 using a line-source-sprinkler technique developed by Hanks et al. (1976) and further standardized at ICRISAT (Rao et al. 1985). In both years, long breaks in rains occurred around the reproductive stage, which facilitated imposition of drought treatments. Thirty ESDP and SDP advanced breeding lines in the first year and 40 in the second year were sown across a moisture gradient created by line source sprinklers at a spacing of 30 cm row-to-row and 10 cm plant-to-plant spacing. There were four 15 m long adjacent rows genotype⁻¹ covering the entire length of the moisture gradient on each side of the sprinkler line. There were four replications. Some of the promising lines included in the first season were repeated in the second season. Irrigation was applied to match the cumulative evaporative demand when it was least windy and the amount of water applied was measured in catch cans kept at different places. Total dry matter and yield data were recorded from central two rows from 10 sectors of 1.5 m length, each representing different parts of moisture gradients.

The responses to applied water varied from curvilinear (Fig. 4.5.2) to linear (Fig. 4.5.3). The curvilinear response was due to sensitivity of lines (largely of ESDP) to excess water near the line and water deficit at the distal end. The drought sensitivity in general varied as follows: SDP hybrid > SDP line > ESDP line. Among pigeonpea hybrids, ICPH 8 and ICPH 9 were particularly more drought tolerant. It appears that pigeonpea hybrids have greater drought tolerance due to hybrid vigor not only in shoot biomass but also in their root system, which helps them to extract moisture from deeper layers. The limited observations made indicate that hybrid ICPH 8 had 27% more root mass than its best parent. The greater susceptibility of ESDP lines to drought is consistent with this observation because they may have less root development due to their shorter growth duration.

The greater drought tolerance of hybrids ICPH 8 was well supported in multilocation trials grown with and without irrigation, in which both hybrids out-yielded ESDP and SDP lines in most environments (Chauhan et al. 1998) (Fig. 4.5.3). Interestingly, pigeonpea hybrids did well in both irrigated and rainfed environments, indicating that there was no yield penalty associated with the drought tolerance of hybrids.

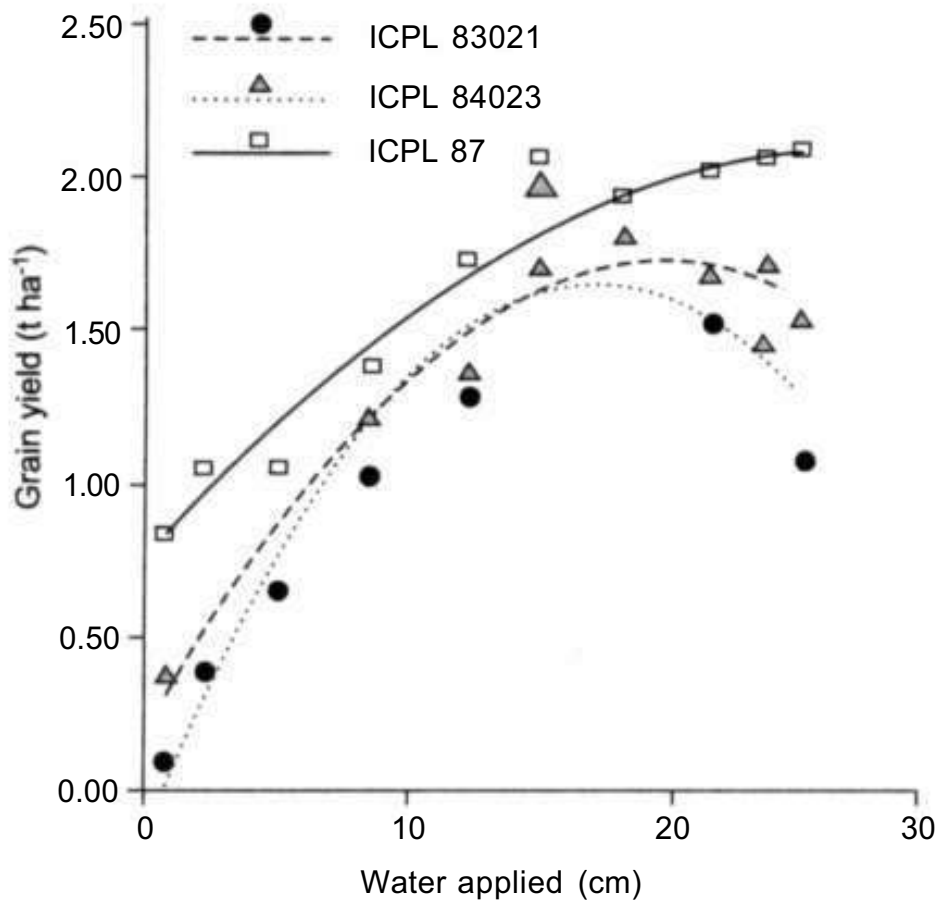


Figure 4.5.2. Effect of different amounts of water applied through a line source sprinkler system on grain yield of two extra-short (ICPL 83021 and ICPL 84023) and a short duration pigeonpea (ICPL 87) genotypes.

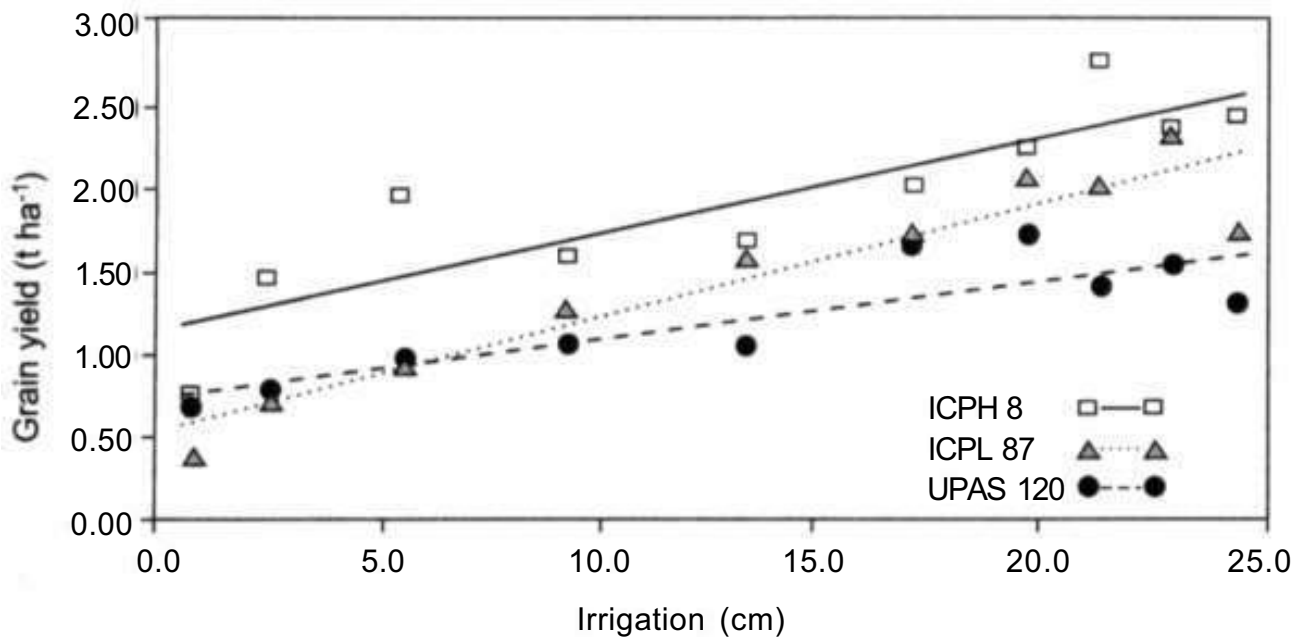


Figure 4.5.3. Grain yield response of ICPH 8, ICPL 87, and UPAS 120 to different levels of irrigation (cm) applied.

Screening under Rainout Shelters

Except for the two years 1986 and 1987, further screening for drought tolerance using the line source sprinklers method has not been successful due to unexpectedly good rainfall that continued for about 10 years in a row. The off-season screenings for drought tolerance have been routinely carried out for all other tropical mandate crops of ICRISAT during this period. However, this could not be done for pigeonpea, because it reacts strongly to changes in photoperiod and temperatures it encounters in the off-season, and puts up little growth.

In the intervening years, the creation of the rainout shelter facility at ICRISAT has permitted identification of drought-sensitive stages in pigeonpea as indicated above through the performance of a limited number of genotypes.

Thirty-two ESDP and SDP lines were screened for flowering stage drought and appreciable differences were recorded. The relative differences among lines were measured using the drought susceptibility index (DSI) as proposed by Fisher and Maurer (1978) after ruling out the fact that the yield performance under stress was not related to days to 50% flowering ($R^2 = 0.003$) and yield potential under irrigated conditions ($R^2 = 0.034$). The OSI was calculated as:

$$DSI = ((I_{yield} - Rainfed_{yield}) / I_{yield}) - ((Mean I_{yield} - Mean Rainfed_{yield}) / Mean I_{yield})$$

The lower the DSI, the greater was the tolerance of the line for drought. The top five lines (susceptible) and bottom five lines (tolerant) with respect to

Table 4.5.1. Effect of moisture stress on drought-susceptible (top 5) and drought-tolerant (bottom 5) lines.

Line	Df	No-stress	Stress	DSI
94005	59	2.99	1.33	1.56
97111	64	2.09	1.07	1.37
94008	64	2.43	1.32	1.29
85012	65	2.35	1.30	1.26
89002	61	1.91	1.08	1.22
UPAS 120	63	1.86	1.42	0.67
88039	53	2.09	1.60	0.66
93097	50	1.95	1.54	0.59
92047	66	2.24	1.77	0.59
94009	63	2.08	1.77	0.42

DSI from this screening are shown in Table 4.5.1. Lines UPAS 120 (an SDP cultivar released for cultivation in North India) and ICPL 88039 showed greater drought tolerance in this screening. ICPL 88039 has performed well under rainfed conditions in different environments. The drought screening under rainout shelter, though reliable, has limitations of space and the fact that pigeonpea cannot be grown year after year at the same place. To overcome the latter problem, rainout shelters that can be moved to different places have been designed (Chauhan et al. 1997).

Traits for Rapid Screening for Drought Tolerance

A number of drought tolerance mechanisms seem to confer tolerance to drought at different growth stages in pigeonpea. These include leaf area retention related to tolerance to preflowering stage drought in SDP (Lopez et al. 1997), maintenance of radiation use efficiency (Nam et al. 1998), and osmotic adjustments (Subbarao et al. 2000). The positive relationship of leaf area retention and tolerance to drought at the preflowering stage stress offers an opportunity to make visual selections of SDP lines that retain greater leaf area under drought in the early stages. For greater tolerance to drought at flowering and pod-filling stages, genotypic differences in osmotic adjustment soon after flowering could be utilized to identify drought-tolerant lines (Subbarao et al. 2000).

Conclusions

The limited work on drought physiology of ESDP and SDP has identified drought-sensitive stages and shown genotypic differences in drought tolerance. The most important of these findings is the identification of drought tolerance associated with hybrid vigor, and the drought-tolerant ESDP line ICPL 88039. Other ESDP and SDP lines that have shown even greater tolerance to drought require further testing. A number of limitations of screening methods used for pigeonpea have been identified, which need to be resolved for making further progress in drought research on pigeonpea. The line source method has limitations for pigeonpea and cannot be applied in field conditions in most years. Rain-out shelters have limitations of space and the fact that pigeonpea cannot be grown repeatedly. To overcome this problem, manually operated rainout shelters may be used, which can be moved to different places (Chauhan et al. 1997). The screening and breeding for

drought tolerance can be more rapidly done if an environment that can reliably develop drought stress, and in which pigeonpea can attain normal growth, can be identified. Long-term weather analysis suggests that such weather occurs in the dry zone of Sri Lanka during short-rainy season (Chauhan et al. 1999). There could be more such locations and identification of these would be helpful in facilitating multilocation testing of drought-tolerant genotypes.

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4.6 Field Screening for Drought Tolerance in Groundnut

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Drought is a major abiotic stress affecting yield and quality of rainfed groundnut worldwide. Yield losses due to drought are highly variable in nature depending on its timing, intensity, and duration, coupled with other location-specific environmental factors such as irradiance and temperature. The effects of drought on groundnut are manifested in several ways, affecting both quantity and quality of the crop. Water deficits, depending on the timing of occurrence, can cause significant reduction in yield by affecting physiological processes such as nitrogen fixation, photosynthesis, and calcium uptake by developing pods. The end-of-season drought can predispose the crop to aflatoxin contamination, which can severely affect the economic value of the crop. The importance of genetic enhancement for improved adaptation to water-limited conditions and efficient water use has long been recognized by ICRISAT.

Drought Patterns and Genetic Options

The extreme variability of the nature of drought has made it difficult to define plant attributes required for improved performance under drought, consequently limiting plant-breeding efforts to enhance drought tolerance in groundnut. The most frequently encountered drought patterns can be grouped into three types i.e., early-season drought, mid-season drought, and end-of-season drought. Genetic options for improvements in drought tolerance vary with most drought patterns experienced in a given environment.

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Early-Season Drought

Once the crop is established, early-season drought does not have much effect on groundnut. As a matter of fact, a 20-25 day moisture stress early in the season and its subsequent release by applying irrigation is encouraged, as it induces heavy and uniform flowering, leading to increased groundnut productivity.

Mid-Season Drought

Mid-season droughts affect the most vulnerable stages (pegging, and pod and seed development) of plant growth in groundnut. A poor relationship between the yield potential (achieved under adequate water availability) and the sensitivity of genotypes to mid-season drought suggested the possibility of identifying/or developing genotypes with high yield potential and relatively low sensitivity to mid-season droughts.

End-of-Season Drought

End-of-season drought affects the seed development most. It also predisposes the produce to aflatoxin contamination. Genotypic yield accounts for 90% of the variation in pod yield sensitivity to water deficit during the seed filling stage. Where the growing season is short and terminal drought predominates, matching of phenological development of a cultivar with the period of soil moisture availability is an important drought escape strategy to minimize the impact of drought stress on crop production. Using the concept of thermal time and staggered harvesting, ICRISAT has made considerable progress in shortening crop duration of groundnut without unduly penalizing realized yield. However, it is still necessary to screen genotypes in a given maturity group for tolerance to end-of-season drought because of two reasons: 1) to identify genotypes with reasonable pod yields and better vegetative growth (as groundnut haulms are valuable fodder in most semi-arid environments), and 2) to identify genotypes with resistance to *Aspergillus flavus* infection and aflatoxin production.

Development of Drought Genotypes at ICRISAT

Empirical Approach

Most of the drought tolerance breeding activity at ICRISAT Center, Patancheru, is conducted during the postrainy season (Nov-April), when there is least interference from the rains. ICRISAT adopted a holistic approach in screening and selecting groundnut genotypes with superior performance under two most critical droughts i.e., mid-season and end-of-season. For the development of genotypes with superior yield performance under drought conditions, germplasm and segregating populations are evaluated/selected in the postrainy season under simulated drought conditions. In addition, the advanced breeding lines are also evaluated under rainfed conditions in the rainy season (June-October).

Germplasm Screening

Using a line-source sprinkler irrigation system, germplasm lines are screened for early-season and mid-season drought in the field. Based on harvest index (HI) and biomass production, germplasm lines are selected for resistance to different kinds of drought. Several lines with superior performance under different kinds of drought (ICG# 3086, 3141, 2738, and 1163, and ICGV# 91151, 94127, 92209, and 91109 for mid-season drought; ICG 2213, ICGS 76, ICGV# 90226, 91074, 91185, 91192, 92004, 92022, 92023, 92028, 92029, and 92033 among others for end-of-season drought) are now available for use in breeding programs.

Development of Breeding Materials

Under imposed mid-season (withholding irrigation from 40-80 days after sowing) and end-of-season (withholding irrigation from 80 days after sowing until harvest) droughts, the selection in segregating populations is based on high pod and seed yields. In advanced breeding lines in replicated trials, yields under imposed drought conditions and normal (no moisture stress) conditions are considered. Following this approach, several drought-tolerant advanced breeding lines have been developed and distributed to national programs in the form of international drought tolerance groundnut varietal trials. Many of these lines have now been released as cultivars in different countries. In India, these include ICGS # 11, 37, 44, and 76, and ICG (FDRS) 10 and in Indonesia, ICGV 86021, released as Terapah.

Notwithstanding these success stories, the empirical approach to drought tolerance breeding remains resource-extensive and tardy. Because of larger genotype (G) x environment (E) interaction for seed yield in groundnut, its heritability is low. Unfortunately, the phenotypic model for yield provides little understanding of biological significance and reasons for G x E interactions. However, the empirical breeding approach continues because so far there are no tools to obtain better information about genotypic traits contributing to yield under drought conditions in a large-scale breeding program.

Physiological Approach

In recent years, there has been significant improvement in the understanding of the physiological basis of genotypic response to drought in groundnut. The traits contributing to superior performance under drought conditions in groundnut have been identified and substantial genetic variation observed in them. These include HI, total amount of water transpired (T), and transpiration efficiency (TE, defined as amount of dry matter produced per unit amount of water transpired). However, there are substantial difficulties in accurately measuring these physiological traits in the large numbers of plants/populations needed for selection programs.

Earlier studies indicated that TE and HI were negatively correlated. However, a more strategic and comprehensive selection program, funded by the Australian Centre for International Agricultural Research (ACIAR), involving collaboration among Indian Council for Agricultural Research (ICAR), Queensland Department of Primary Industries (QDPI), and ICRISAT has been implemented to identify genotypes with high levels of the physiological traits in the vast germplasm pool at ICRISAT. These results suggested that the negative association between TE and HI, observed in earlier experiments, could be broken and there was scope for selecting for and combining TE and HI traits concurrently to improve yield performance. It was also apparent that high levels of at least two out of the three physiological traits were necessary for superior performance of a genotype. Interestingly, genotypes involving parents selected from drought screening at ICRISAT (e.g. ICGS# 44 and 76, ICGV# 86754, and 87354) had superior yield performance because of higher TE and HI or all the three traits, while for the other cultivars, the dominant contribution to the yield was from T and/or HI.

This analysis indicated scope for developing new cultivars by pyramiding the traits or identifying the deficient trait(s) in the popular cultivars so that the parental selection and genetic enhancement can be focused to improve levels of the deficient trait in the required agronomic background. It was interesting to note that the yield performance of some of these selected genotypes was superior even under irrigated conditions, suggesting that the physiological traits such as TE and HI could be used as selection criteria for crop improvement under irrigated conditions as well.

Use of Indirect Selection Tools

Recent studies have identified surrogate traits, such as carbon isotope discrimination in leaf (D) and specific leaf area (SLA), which are associated with TE in groundnut. Furthermore, SLA, which is a crude but easily measurable parameter, can be used as a rapid and inexpensive selection criterion for high TE.

Screening of groundnut germplasm for SLA indicated significant variability within and between taxonomic groups. It was interesting to note that the genotypes belonging to variety *hypogaea* (Virginia bunch and runner types) had a lower mean SLA than those of variety *fastigiata* (Valencia and Spanish types) suggesting a likelihood of higher TE. However, the former had lower partitioning ability than the latter. There is new evidence that the groundnut genotypes having lower SLA (high TE) showed more stability in dry matter production under drought. It has recently been shown that a handheld portable SPAD chlorophyll meter can be used effectively following necessary protocols for rapid assessment of SLA and specific leaf nitrogen. This would facilitate screening of large numbers of segregating populations in the field.

An ongoing ACIAR-funded ICAR-QDPI-ICRISAT collaborative project is currently assessing the value of indirect selection tools in improving the efficiency of selection in large-scale groundnut breeding programs in India and Australia.

Session 5: Marker-Assisted Breeding in Other Crops

5.1 DNA Marker Technology as a Tool for Genetic Enhancement of Drought Tolerance at ICRISAT

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Abstract

Recent developments in plant genomics research have renewed interest in targeted breeding for enhanced drought tolerance with particular reference to the application of molecular tools to enhance crop water productivity. However, there is an urgent need for corresponding progress in the understanding of the function, and interaction of genes underlying the physiological mechanisms of drought. It is in fact, well accepted that the complexity of the drought syndrome can only be tackled through a holistic approach, integrating physiological and molecular genetic dissection of the tolerance traits with detailed agronomic analysis of soil, crop, and environment. ICRISAT and other international research institutes dealing with crop productivity under arid and semi-arid conditions have achieved substantial success and impacts during the past two decades in terms of crop genetic improvement. All ICRISAT cereals and legumes mandate crops (pearl millet, sorghum, chickpea, groundnut, and pigeonpea) are generally known for their relative ability to produce grain and biomass in the semi-arid tropics (SAT). However, the current challenge is to generate high and sustainable levels of crop productivity under rainfed and marginal conditions, in order to support the rapid population growth in the semi-arid tropics. To face this challenge, ICRISAT has recently placed a high priority on biotechnology-assisted germplasm enhancement using multidisciplinary teams that embrace the application of genomics to boost the utilization of genetic resources in plant breeding programs, with a major focus on drought-tolerant crop improvement.

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Introduction

The potential value of genetic markers, linkage maps, and indirect selection in plant breeding has been known for over 80 years. However, there are relatively few morphological markers available, many of which are affected by the environment. Thus, dramatic advances in the use of genetic markers have only become possible with the development of DNA markers. During the last 20 years there has been rapid progress to develop ever more abundant and elegant marker systems able to tag a range of important agronomic traits. Throughout this time DNA marker technology has promised to dramatically enhance the efficiency of plant breeding as molecular biology has already revolutionized research in the life sciences. Yet it is only very recently that advances in automated technology have presented the precision, convenience, speed, and level of throughput that can finally offer relevance to modern plant breeding programs.

The use of DNA markers for indirect selection offers greatest potential gains for quantitative traits with low heritability as these are the most difficult characters to work with in the field through conventional breeding. However, developing marker-assisted selection systems for this type of trait is also most difficult. This is largely due to the effects of genotype-by-environment (G x E) interaction and epistasis. Precise phenotypic evaluation in several locations and seasons is essential to measure these effects and estimate the relative contribution and stability of component quantitative trait loci (QTL). In addition, the use of large mapping populations is a critically important factor to facilitate the dissection of quantitative traits such as drought tolerance. The techniques here, which have allowed plant breeders and physiologists to deal with complex phenotypes, are increasingly important in the new field of molecular breeding.

Drought stress is a complex syndrome involving several climatic, edaphic, and agronomic factors, characterized by three major varying parameters, i.e. timing, duration and intensity. Recent breakthroughs in genomics research have fostered a renewed interest in targeted breeding for enhanced drought tolerance with particular reference to the application of molecular tools to enhance crop water productivity (Bruce et al. 2002). However, these rapid advances in facilitating technology for molecular breeding have highlighted an urgent need for parallel progress in the understanding of the function and interaction of genes underlying the physiological mechanisms of drought. It is now well accepted that the

complexity of the drought syndrome can only be tackled through a holistic approach (CIMMYT 2000), integrating physiological and molecular genetic dissection of the tolerance traits with detailed agronomic analysis of soil, crop and environment.

ICRISAT has achieved substantial success and impacts during the past two decades in terms of genetic improvement of its five mandate crops (pearl millet, sorghum, chickpea, groundnut, and pigeonpea). These crops are all generally known for their relative ability to withstand water-limited conditions to produce grain and biomass in the SAT. However, the current challenge is to generate high but stable and sustainable levels of crop productivity under rainfed and marginal conditions, in order to support the rapid population growth in the semi-arid tropics. ICRISAT has placed major priority on biotechnology-assisted germplasm enhancement using multidisciplinary teams that embrace the application of genomics to enhance the utilization of genetic resources in plant breeding programs. In this way biotechnology is used as a functional bridge between germplasm collections and breeding programs to release the value of genetic resources with particular reference to drought tolerance, pest resistance, disease resistance, and quality components of foods and feeds.

Justification for Marker-Assisted Selection and Molecular Breeding

Molecular approaches have rapidly proved to be powerful tools for indirect selection and for studying the organization and behavior of plant genomes (Dear 1997). The primary resource for plant breeding programs is the genetic variability available within germplasm closely related to the crop of interest. However, the success of crop improvement programs is highly reliant on the power and efficiency of the methodologies with which this genetic variability is manipulated. DNA marker technologies offer plant breeders the potential of making genetic progress more precisely and more rapidly than through conventional breeding based solely on phenotypic selection. Thus, in the private sector, huge investments have been made in molecular breeding technologies for indirect selection of traits that are too difficult or expensive to precisely score using traditional approaches, and to reduce linkage drag during introgression and backcross breeding. At ICRISAT marker-assisted breeding is not approached in the context of a single point intervention or replacement of

conventional breeding. Instead, the Institute looks to develop new paradigms in crop improvement where the value of DNA markers, wide crosses, and transgenics are released in a synergistic way in combination with the most advanced approaches to conventional breeding.

Genetic markers also offer the possibility of addressing previously unattainable goals. This is now equally true for both temperate and tropical crops. In particular, progress in model systems offers the possibility of supporting both substantial and rapid developments in tropical crop improvement, which would not be conceivable through traditional methods.

Scientific Constraints to Molecular Breeding

There are now many types of markers available from hybridization-based assays such as restriction fragment length polymorphism (RFLP), through polymerase chain reaction (PCR)-based assays such as those based on microsatellites or amplified fragment length polymorphism (AFLP), to sequence-based markers such as expressed sequence tag (EST) and single nucleotide polymorphism (SNP). Each class of marker has a specific set of advantages and disadvantages that make it more or less suitable to a particular application, as summarized in Table 5.1.1. Restriction fragment length polymorphism, although used extensively in early mapping studies, particularly of cereal crops, is not amenable to routine applications in molecular breeding (Rafalski and Tingey 1993). RFLP marker analysis is too labor intensive and has too low throughput potential for routine screening of breeding populations. However, with the development of the PCR-based techniques there are now many other assays that have significant potential for molecular breeding (for further information see <http://www.nal.usda.gov/pgdic/tutorial/lesson4.htm>).

For traits as complex as drought tolerance, the success of QTL mapping is largely conditioned by the accuracy and precision of the phenotyping procedure. In order to develop robust QTL markers of value to plant breeders, it is essential that repeatable, highly heritable differences can be detected between recombinant lines of a mapping population tested in multiple environments across several seasons.

Table 5.1.1. Major classes of DNA markers for molecular breeding.

Marker	Abbreviation	Details
Restriction fragment length polymorphism	RFLP	<ul style="list-style-type: none">• Requires hybridization of probe DNA with sampled plant DNA.• Provides high quality data, it has a severely restricted throughput potential.• RFLP analysis retains significant value for linking genomes and genes across species and genera that can be used in translating knowledge of drought tolerance in model species for the benefit of lesser-studied crops.
Random amplified polymorphic DNA	RAPD	<ul style="list-style-type: none">• The first of a new generation of markers based on the PCR.• Uses arbitrary primers to initiate amplification of random pieces of the sampled plant DNA.• Requires no knowledge of the genome to be screened.• Highly inconsistent across different populations, experimental protocols, and laboratories.
Simple sequence repeat length polymorphism	SSR	<ul style="list-style-type: none">• Provides high quality, consistent results• The markers are still expensive to develop, as they require extensive sequence data from the species of interest.
Amplified fragment length polymorphism	AFLP	<ul style="list-style-type: none">• The sample DNA is enzymatically restricted into small fragments (as with RFLP analysis) and a selection of fragments are visualized following PCR amplification.• Provides a great quantity of marker information, but is not particularly well suited to high throughput marker-assisted selection.
Expressed sequence tag	EST	<ul style="list-style-type: none">• Development of these is dependent on extensive sequence data of regions of the genome that are expressed.• Once developed, they provide high quality, consistent results. Because they are limited to expressed regions of the genome, the markers can be directly associated with functional genes.• EST markers may be most useful in mapping drought tolerance as the functional information they embody may be of considerable value to the physiological dissection process.
Single nucleotide polymorphism	SNP	<ul style="list-style-type: none">• The vast majority of differences between individuals are point mutations due to single nucleotide polymorphisms.• Thus, there are a vast number of potential SNP markers in all species.• Considerable amounts of sequence data are required to develop SNP markers.• Their great advantage lies in the potential to screen them using truly high throughput approaches.

Evaluating the Cost-Benefit Ratio of DNA-Marker Assisted Breeding

Despite a vast array of publications reporting the identification of DNA markers for specific agronomic traits, there is a particular paucity of reports evaluating the application of such markers in breeding programs. A few reports from the model cereal crop systems (Dreher et al. 2000) and simulation studies (Ribaut and Hoisington 1998) begin to provide some insight for marker-assisted selection practitioners who have been reviewed in detail elsewhere (Crouch 2001). The comparative cost-benefit analysis of marker-accelerated backcross versus conventional breeding has not yet been addressed. However, many commercial breeding companies have invested in marker technology on the basis of the expected decrease in time and cost, and increase in precision, particularly for marker-accelerated introgression of pest, disease, and quality traits from exotic germplasm. These advantages are even expected to be even higher in the case of drought tolerance, given the high complexity of the traits and the limited success achieved through traditional breeding.

With increasing emphasis on molecular marker technology, the proportion of costs allocated to labor decreases while the proportion for equipment and reagents increases. However, the unit costs of marker analysis can be dramatically reduced through the implementation of automated high throughput technologies and complementary approaches combining different types of assays.

Finally, it is important to acknowledge that this type of cost-benefit analysis from public sector researchers only concerns the application phase. In reality, of course, marker development costs may be very substantial. This is particularly relevant for molecular breeding of complex traits such as drought tolerance, where appropriate mapping of the underlying loci requires replicated multiocational evaluation of very large populations using labor-intensive and expensive approaches to achieve accurate and precise phenotyping.

Achieving New Breeding Goals through DNA Marker Application

Where breeding goals cannot be achieved through traditional approaches, there is considerable scope for the use of almost any type of molecular marker,

including isozymes and RFLR. This approach has been successfully used at ICRISAT for the backcross transfer of QTL for downy mildew resistance in pearl millet [Witcombe and Hash 2000]. Here, a limited number of RFLP probes have been used directly for marker-assisted selection to improve disease resistance in both parent lines of a popular hybrid variety. Despite the labor-intensive nature of this approach and the resultant limitation of population size in a given generation, good progress has been made and field evaluation of the finished projects is under way just four years after initiation of the project. Based on this success, molecular breeding of pearl millet at ICRISAT is currently being transferred to semi-automated analysis based on recently developed microsatellite markers.

Molecular Breeding for Enhanced Drought Tolerance at ICRISAT

Drought is an important constraint to production in many tropical regions. However, drought tolerance is a highly complex character and unpredictable environmental conditions and the time consuming and expensive nature of assessing component traits confound its precise evaluation. In addition, the efficiency of selection is lower under drought conditions than well-watered conditions, due to a decrease in the heritability of grain yield under stress. Nevertheless, several molecular markers and QTL have been mapped for several components of drought resistance in various crops (overviewed by Nguyen, 2001). Furthermore, many laboratories are currently testing the value of marker-assisted selection for the genetic enhancement of drought tolerance.

Sorghum and Pearl Millet

Sorghum is one of the most extensively adapted crop plants, being grown from -35°S to 45°N, from sea level to 2000 meters, and under crop season rainfall of 300-2000 mm. This diverse cropping range has led to an equally diverse range of utilization, and in turn, quality preferences for foods and feeds. For these reasons, sorghum-breeding programs are very sensitive to the introduction of new diversity, while in the legumes there is less difference in yield potential between landraces and varieties. Thus, to sorghum breeders, landraces are effectively exotic germplasm as regards their likely effect on the breeding program.

Drought is an overwhelming constraint to sorghum production in Asia and Africa but there are very different types of drought in different parts of Africa. The variable moisture environment during the cropping season can have effect from seedling establishment to grain filling and thereby severely impact grain yield for food and feed plus biomass production for fodder. Although many sources of drought tolerance are available, it is widely considered by plant breeders that most sources of biotic tolerance and abiotic resistance are associated with loss of yield potential. Yet, these deleterious effects may be due to tenacious linkage drag, which cannot be easily broken by conventional breeding. In this scenario, the application of molecular markers is a fully justified tool to speed up and refine breeding progress. Stay green (delayed senescence) is considered an important adaptive trait for postflowering drought stress. On this basis, ICRISAT is currently following marker-assisted selection approaches to rapidly introgress two different sources of this trait through backcross breeding into landraces and cultivars adapted to target cropping zones in Africa, Asia, and South America. This molecular breeding program is using two sources of stay green: (1) B35, which has a high level of stay green expression but in a background not preferred by sorghum breeders, and (2) E36, which has a lower level of staygreen expression but is in an acceptable background for sorghum breeders.

Drought tolerance in pearl millet is a major breeding priority but there is limited variability and low heritability. Not surprisingly, new varieties tend to have lower levels of drought tolerance than landraces as breeders struggle to maintain a positive selection pressure for this trait. However, there is much discussion over how best to evaluate drought tolerance in this crop. Some suggest that dissection into components of drought tolerance will not generate tools of value for plant breeders. Nevertheless, the unpredictability of drought stress pressures in traditional breeding programs fully justifies the use of marker-assisted selection for this trait.

Several mapping populations have been developed using RFLP skeleton mapping, trait phenotyping (Hash and Witcombe 1994), and QTL mapping of terminal drought (Yadav et al. 2001). Test crosses of mapping population progenies, derived from inbred pollinators and from seed parents differing in their response to terminal drought, were evaluated in a range of managed terminal drought-stress environments to identify individual QTL associated with drought tolerance. A number of QTL associated with drought tolerance of grain yield and its agronomic and physiological components have been reported (Yadav et al., 2001). Some of the identified QTL 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 QTL contributed to increased drought tolerance either through their effect on increased maintenance of growth, or harvest index, or both, in terminal drought-stress environments. Programs for marker-assisted backcross transfer of the identified QTL into the elite parent of these mapping populations have been initiated for the improvement of pearl millet productivity in water-limited environments (Yadav et al. 2001). The development of near-isogenic lines will also provide an ideal opportunity to further test the effect of the identified QTL and to dissect the associated physiological mechanisms involved in terminal drought.

Chickpea, Groundnut, and Pigeonpea

Global yield losses due to drought have been estimated at 6.7 M tonnes in groundnut, 3.7 M tonnes in chickpea and 1.8 M tonnes in pigeonpea. Yet it appears that a substantial proportion of these yield losses can be recovered through crop improvement efforts combined with good agronomic practices (Subbarao et al. 1995).

There is a relatively low level of genetic diversity amongst cultivated chickpea. Thus, initial maps in this crop genus focused on interspecific crosses. However, the first intraspecific map has recently been completed, and the size of this population at ICRISAT is being extended beyond 750 individuals in order to carry out detailed QTL mapping of complex agronomic traits.

Many morphological traits, such as prolific and deep root systems, fewer pinnules per leaf, and rapid rate of partitioning and double podding may contribute to drought tolerance in chickpea (Saxena, 2001). In addition, early maturity represents an escape mechanism for terminal drought stress, the genetic basis of which has already been mapped (Kumar and van Rheenen, 2000). Extensive physiology studies have indicated that the prolific root system of the breeding line ICC 4958 was highly efficient at extracting soil water, thereby conferring terminal drought tolerance. However, measuring root traits in breeding trials is time consuming and expensive while being significantly affected by the environment. Thus, a recombinant inbred line (RIL) population (Annigeri x ICC4958) of over 250 individuals has been developed for mapping root traits. Root growth parameters (root volume, root

length, and root dry weight) amongst the RIL population were studied in greenhouse and field grown plants.

Microsatellite (SSR) markers are the assay of choice for molecular breeders due to the high level of polymorphism they usually detect and the co-dominant data they provide. Over one hundred SSR markers have shown polymorphism between the parental genotypes (Annigeri and ICC4958) using silver stained polyacrylamide gel electrophoresis (PAGE) analysis. These SSR markers are being optimized for preliminary mapping of root traits, using high throughput methods including liquid handling robotics, multiplexed fluorescent PCR, and automated capillary electrophoresis.

In this way it is proposed to dissect key components of drought tolerance in chickpea and individually map the QTL underlying each component in order to build a holistic approach to the molecular breeding of this highly complex trait.

In groundnut, drought tolerance and resistance to aflatoxin contamination are intimately connected, thereby creating an additional level of complexity. However, there appears to be substantial potential for genetic improvement of water use efficiency in groundnut, as significant genotypic variations have been reported for the amount of water transpired and the transpiration efficiency (Wright et al. 1994). Thus, RIL populations are being developed for mapping components of drought tolerance in groundnut.

Until recently, there have been no microsatellite markers available in pigeonpea. However, a collaborative project between ICRISAT and Birmingham University is now developing SSR markers that have already been used in the first high throughput project at ICRISAT, to assess the molecular diversity of one thousand pigeonpea accessions from across India. Meanwhile, a number of components of drought tolerance have been characterized in pigeonpea, including early vigor, leaf area maintenance, root and shoot growth rate, and developmental plasticity (Johansen 2001).

Integrated Strategy for Drought Tolerance Research at ICRISAT

Drought triggers a wide range of morphological, physiological, and molecular responses in plants (Bohnert et al. 1995), which makes the identification of relevant mechanisms challenging. The temporal variability in amount and distribution of available moisture from year to year results in high interaction

of genotype x environment. The same putative drought tolerance traits have different probabilities for expression in different years and environments. Under these conditions, breeding improved genotypes for the arid and semi-arid tropics based on grain yield is extremely complex. Facing the rapid expansion of global drought problems, little and slow progress has been achieved in the genetic enhancement of crop drought tolerance through conventional breeding approaches (Turner 1997).

The multiplicity and plurigenic characters of drought tolerance traits demand a multidisciplinary research approach integrating marker-assisted breeding, simulation modeling, physiology, and molecular genetics to increase the efficiency of crop enhancement programs for drought-prone environments. It is now becoming critical that a strategic approach for the prioritization of crop drought tolerance research should include:

1. Accurate characterization of target drought-prone environments.
2. Precision of screening tools and protocols for consistent phenotypic description.
3. Physiological dissection of crop water relations and water use efficiency.
4. Use of comparative and functional genomics tools to elucidate drought tolerance traits and mechanisms.
5. Evaluation and refinement of procedures for mapping drought tolerance.
6. High throughput marker screening of breeding lines and populations.
7. Development of new paradigms for breeding of drought tolerance.

Developing the Applied Genomics Laboratory at ICRISAT

At ICRISAT the potential of a wide range of biotechnologies to assist the breeding of the mandated crops are being investigated. A major focus of this work is on the use of DNA markers for assisting the breeding of traits that are difficult, expensive, or slow to screen through traditional means. On this basis, nearly US \$ 1 million were invested during 2000-2001 to establish a state-of-the-art high throughput genomics facility, based on a high level of automation to ensure consistency, reduce unit costs, and achieve a throughput appropriate to real challenges in breeding programs and germplasm collections. ICRISAT is targeting a throughput of one million samples per year during the establishment phase of this facility (2002-05). The application of these technologies for the molecular breeding of drought tolerance is a major priority.

High throughput genomics technologies developed for sequencing whole genomes are highly appropriate tools for molecular breeding. Thus, with liquid handling robotics and 16 and 96-capillary electrophoresis platforms, ICRISAT is now in a position to make the transition from molecular biology research to large-scale diagnostics for germplasm enhancement and plant breeding.

Biotechnology-Assisted Germplasm Enhancement

ICRISAT has extensive biotechnology capacity for wide crosses, applied genomics, and transgenics formulated around substantial breeding programs and germplasm collections. On this basis, ICRISAT's molecular breeding facilities provide extensive opportunities for conducting centralized marker-assisted germplasm enhancement for traits of importance in Asia, Africa, and Latin America.

Through \$1.2 million funding from the Asian Development Bank, ICRISAT is working with the national programs of Bangladesh, China, India, Pakistan, and Vietnam to bolster national capacity in molecular breeding. A similar approach will be developed with the Institute's NARS partners in Latin America. Meanwhile, ICRISAT is actively investigating means of decentralizing routine DNA marker screening in order to facilitate testing and refinement of technologies for immediate application in multilocational breeding sites in West (Mali, Niger, Nigeria), East (Kenya), and Southern (Zimbabwe) Africa.

In addition to the efforts with national programs, ICRISAT is strongly committed to developing mechanisms for intensive collaboration with the private sector. Already two crop-based consortiums of commercial seed companies fund a substantial portion of the conventional breeding programs for millet and sorghum in India. For the next phase of partnerships with the *private* sector, ICRISAT has established an incubator Science Park for joint ventures with consortiums of breeding companies and biotechnology start-up companies.

Future Outlook

After three decades of promises, knowledge and facility technologies are becoming available that allow biotechnologists to tackle the marker-assisted selection of complex traits. Meanwhile, entirely new technology platforms such as microarrays promise another leap in the ability to simultaneously screen a large number of genomic points. These technologies have already

forged the new research area of functional genomics and it is to be expected that in due course they will be refined for genomic investigations and molecular breeding applications. Furthermore, developments in bioinformatics promise to foster progress towards knowledge-led plant breeding in ways and degrees never previously possible.

Application of Progress in Model Plants to Crop Systems

The relationship between major cereal crop genomes has been elucidated and aligned in comparative concentric circles with rice, as the smallest genome, at the center. At ICRISAT the focus is on five crops, most of which are of minor importance to industrialized countries, and are thus relatively little studied. However, comparative (or synteny) mapping offers the potential to achieve leapfrog progress in crops by directly using progress made in the model species such as *Arabidopsis*, soybean, and *Medicago* for the legumes plus rice and maize for the cereal crops.

Plus-Minus Tests

These approaches rely on the development of PCR assays that produce only one amplification product from one genotype and no amplification product from the alternative genotype at that locus. PCR products can then be processed manually or assayed fluorometrically for rapid identification of desired genotypes. Complete automation of this approach can be achieved through the use of reporter systems coupled with fluorescence reading thermocyclers. However, ultimately breeders would like to see plus-minus assay kits that can be easily used in the field.

Complex Traits

Many traits of agronomic and economic importance exhibit continuous variation due to an underlying array of QTL. The rate-limiting factor for developing effective marker-assisted selections systems for these characters remains the confounding effect of genotype x environment interaction and epistasis. This has resulted in a rapidly evolving array of computational methods aimed at addressing this issue. To date there is a wide range of publications in this area using simulation studies, but very few based on empirical data. This is partly due to the very large population sizes that are required for effectively mapping complex traits. The development and

widespread adoption of automated high throughput instrumentation will undoubtedly result in rapid advances in empirical developments for QTL mapping.

Solutions through Genetically Modified Crops

Progress in genetic transformation has been slower than expected. Although transgenic crops now cover vast areas in many countries, the traits involved are generally of simple genetic basis and highly targeted in their effect. It is likely that this will continue to be so, until gene expression of plurigenic traits is much better understood, and ability to action simultaneous multiple transformation events with a high level of efficiency and low cost is substantially enhanced.

New Partnerships

Modern plant breeding is evolving into a highly complex, multifaceted, high technology business. Even multinational corporations are realizing that it is neither cost effective nor time efficient to have in-house capacity in all elements of this process. Modern plant breeding is increasingly characterized by public-private sector partnerships. ICRISAT's location in India and its regional mandate for South Asia give the institute a strategic advantage in building effective public-private sector partnerships. These partnerships, which involve national and international centers, advanced research institutions, nongovernmental organizations (NGOs), and commercial companies, can harness the power of high throughput genomics and bioinformatics within an arena of intense private sector activity in the areas of plant breeding, biotechnology, IT, and bioinformatics. This represents an ideal scenario for testing the value of agribusiness incubator Science Parks for stimulating agricultural and agribusiness development in the semi-arid tropics.

New Paradigms

In this new era, successful plant breeding programs will be characterized by dynamic, holistic approaches led by functional multidisciplinary teams, often including both public and private sector partners. The success of this strategy will certainly depend on the ability to close the phenotype gap existing between DNA sequencing and phenotypic exploitation in farmer's fields. The gap will be narrowed by a multidisciplinary approach, in a series of steps that will reveal the

functions of genes and their connections with phenotypes and potential applications. As this area becomes increasingly complex, it will be the human factor of creating functional multidisciplinary teams often separated by vast distances and distinct disciplines, that will determine much of the future success in this field. A high level of synergy between team members will be a vital element in product-led innovation and problem solving for commercial success. In particular, the role of computational methods (including biometrics and bioinformatics) will be the leading force behind realizing the full potential of DNA marker-assisted approaches, as indeed it is for the entire genomics revolution. Only through the application of holistic approaches will crop productivity keep pace with the demands of a rapidly increasing global population.

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5.2 Phenotyping for QTL Mapping

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Abstract

The reliability of QTL mapping results depends in a major way on the achieved level of accuracy and precision of field phenotyping of mapping population individuals. The accuracy of phenotyping determines how realistic the QTL mapping results are. Increased precision of phenotyping increases heritability which, in turn, increases the statistical power of QTL detection. Using appropriate incomplete block designs and biometric analysis techniques that effectively account for extraneous variation in the field can increase the accuracy and precision of field phenotyping. Randomization of mapping population individuals to field plots, although it should be faithfully followed, may not alone guarantee bias-free phenotyping. This is due to the use of typically closely laid out small plot sizes in field phenotyping, which induces spatial correlation among observations from nearby plots, introducing bias in phenotyping. Based on our experience, we strongly recommend using spatial statistical methods to account for this spatial correlation in order to achieve bias-free and precise phenotyping.

Introduction

Mapping of quantitative trait loci (QTL) is predicated on detecting a significant statistical association between the phenotypes and the marker-genotypes of individuals of a mapping population. Data on both phenotypic value or performance and marker-genotyping of mapping population individuals are therefore required. Phenotyping data contain information on segregation and genetic effects of QTL. Marker-genotyping data provide information on site-specific segregation of putative QTL on the genome. The level of accuracy and precision of both the phenotyping and the marker-

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genotyping data determines the level of reliability of results from QTL mapping. It is therefore important, to achieve a high level of reliability of QTL mapping results, so that highly accurate and precise phenotyping and marker-genotyping data are generated using efficient phenotyping and genotyping protocols. This chapter presents a discussion of phenotyping protocols to enhance the reliability and relevance of QTL mapping results.

An efficient suite of phenotyping protocols is one that, *for the targeted environmental conditions*, delivers highly accurate and precise assessment of phenotypic value or performance of mapping population individuals for the agro-economic traits of interest. Given a random/representative sample of n_g mapping population individuals to be phenotyped, with n_g *chosen to be large enough* (≈ 500) *to achieve high statistical power for QTL detection*, the required components of the suite of phenotyping protocols are:

- A representative sample of n_e environments and their optimal location;
- Number of replications n_r per individual in each environment;
- An experimental field with $n = n_r n_e$ plots in each environment;
- An experimental design to effectively account for extraneous variation in experimental field; and
- Appropriate biometric techniques for efficient analysis of data.

Before any discussion on these issues, it is useful to first clarify the meaning of the key concepts of *precision* and *accuracy* of estimates.

Accuracy and Precision

The basic phenotypic data required for QTL analyses are the estimates of phenotypic performance of individuals in single environments and/or across environments. The total uncertainty/error in the estimated phenotypic performance m of any individual, with m being its corresponding true, unknown phenotypic performance, is quantified by its *mean square error* $MSE(m) = E(m-\hat{m})^2 = E\{m-E(m)\}^2 + \{E(m)-m\}^2$ where E is the average value. The term $E\{m-E(m)\}^2$ is the *error variance* of m , the square root of which is the standard error (SE). The second term represents the square of the *bias* in m . There are thus two component errors that make up the total error in m - the SE that measures the *imprecision* in m , and the bias that measures the *inaccuracy* in m . The smaller the SE, the higher the precision of m . The smaller the bias, the more accurate m is as an estimate of m . It is the accuracy

of m that is the more critical factor for realistic QTL mapping results. In contrast, a smaller SE increases the heritability of the trait, which in turn enhances the power of QTL detection.

The suite of phenotyping protocols to be used should be designed so as to minimize both SE and bias in m . It is often taken for granted that the physical act of randomization of individuals to field plots will provide unbiased/accurate estimates of m . While this argument is mathematically valid, it provides no guarantee of achieving bias-free estimates. The randomization argument only allows us to proceed with data analysis *as if* the phenotypic expression of an individual in a given field plot is independent of the phenotypic expression of other individuals falling on other field plots in the experimental area. This is highly unlikely to be the case for typical small plot phenotyping trials for QTL mapping, however refined the randomization scheme may be. Due to the small size of closely laid out plots, there is a distinct possibility, despite randomization, of the phenotypic expressions of individuals in nearby plots affecting each other and hence being biased. Nevertheless, randomization should still be followed to avoid personal bias, but data analysis, where appropriate, should consider accounting for the possibility of correlated phenotypic expression of individuals in nearby plots in order to obtain maximally bias-free estimates of m .

Number of Environments and Replications

With n_g individuals, each phenotyped with n_r replications in each of the n_e environments, the error variance of the observed average phenotypic performance m of an individual is given by

$$s_m^2 = (s_{GE}^2/n_e) + \{s_e^2/(n_e n_r)\} \quad (1);$$

where s_{GE}^2 and s_e^2 are, respectively, the genotype-environment interaction variance and the pooled error variance. Increasing n_r only reduces the second term, while increasing n_e affects a reduction in both terms on the right-hand side of (1). For a fixed manageable number of plots, the maximal reduction in s_m^2 is theoretically achieved with $n_r=1$, with a corresponding increase in n_e . At least $n_e=3$ environments should be considered, selected in a manner so as to represent one intermediate and two extreme points on the scale of expected variation in the targeted set of environmental conditions. Though $n_r=1$ is optimal, it is advisable to take $n_r=2$ so as to get an internal estimate of error

variance in each environment and to enable estimation of s_{GE}^2 , which is important in obtaining a more realistic multienvironment estimate of trait heritability $h^2 = s_G^2/(s_G^2+s_m^2)$.

Experimental Design

A statistically sound design for an experiment requires three basic ingredients - *replication* and *randomization* of individuals, and *local control* of error arising from interplot variation. These ingredients, when properly used, have three benefits: they allow separation of signal from noise, needed to obtain unbiased estimates of differences in phenotypic performance m of individuals; they maximize the signal-to-noise ratio; and they deliver a valid and unbiased estimation of level of noise/uncertainty in results. The signal is the true differences among individuals and the noise/error is due to interplot variation.

Replication is necessary to obtain an internal estimate of experimental error variance and to permit separation of s_{GE}^2 from error variance. Multiple observations from within a plot do not constitute replication. Randomization provides statistical validity to results and protection from bias. Local control of error can be physically achieved by proper blocking of plots in a manner that maximizes interblock and minimizes intrablock variation. It is the physical device of blocking that, if properly carried out, helps reduce experimental error. However, no matter how effective the blocking is, there is always some variation left uncontrolled within blocks. As the experiment progresses with time, it is also possible that the continuously changing nature of extraneous environmental factors will induce additional intrablock variations in field phenotyping experiments, if the block size is large. It is therefore safer to use blocks of not more than 8-10 plots. Orientation of the blocks, as far as possible, should be perpendicular to the expected gradient in the experimental field, glasshouse bench, etc.

Replication versus Blocking

Replication simply indicates the number of plots (n_r) assigned to an individual. The basic function of replication is to deliver an estimate of error mean square s_e^2 ; it is not a device to reduce s_e^2 . Increased n_r only helps in obtaining a better estimate of s_e^2 . In attempting to reduce SE of a mean $SE_m = \sqrt{(s_e^2/n_r)}$, the first thought that comes to mind is to increase n_r . This increases the cost of experimentation. In contrast, blocking is a physical method to reduce s_e^2 .

Rarely is enough thought given to reducing SE_m by reducing s_e^2 , by attempting effective physical blocking of plots in terms of proper orientation and size. Attempts should be made, where appropriate, to use covariance/spatial analysis techniques for a possible additional reduction in s_e^2 .

Generalized lattice (also known as alpha) designs are suitable for phenotyping large numbers of individuals. They are more flexible than lattice designs that need n_g to be necessarily a perfect square of some integral number. Alpha designs also offer greater convenience for management of the experiment and better choices to conform to expected interplot variation in the field. For $n_g=300$ individuals, some possible design choices are: 3 x 100, 5 x 60, 6 x 50, 10 x 30, and 15 x 20, where the first number is the block size and the second number is the number of (incomplete) blocks per replication. Alpha designs may also allow better use of available experimental area, as small blocks allow much more flexibility in the layout of an experiment than do larger blocks or classical replications.

Biometric Analysis of Data

Having generated and entered the data, we are often eager to quickly get our results. While this is natural, haste may result in the waste of time and effort, because a *sequential plan for data analysis* has not been properly thought out. A plan for data analysis, *made before undertaking actual data analysis*, should consist of the following six steps:

- *First*, screen and validate the data for correctness.
- *Second*, bring the data into a format required by the software to be used for analysis.
- *Third*, understand the treatment and block-structure of the data.
- *Fourth*, understand the nature of the data (discrete, continuous, percentages, etc.).
- *Fifth*, determine the nature of experimental and environmental factors - fixed or random.
- *Sixth*, build an analysis model according to the structure and nature of data and the nature of experimental and environmental factors.

It is only at the end of the sixth step that the actual data analysis can be effectively and confidently begun.

In the context of phenotyping for QTL mapping, the effects of mapping population individuals should be considered as random, with their average

phenotypic values derived as best linear unbiased predictions (BLUPs) using restricted maximum likelihood (ReML). The BLUPs differ from the usual (generalized) least square means in that the former show a smaller range among the phenotypic values of the individuals than the latter. BLUPs are expected to represent more realistic differences among individuals' phenotypic values as extreme phenotypic values, which might arise by chance from a fortuitous interplay of external factors, are forced to shrink towards the general mean. Treating individuals' effects as random is also consistent with the fact that the mapping population individuals constitute a representative sample from the underlying mapping population. Considering the effects of individuals as random is anyway necessary for a valid estimation of genetic variance and heritability, which is required to assess the prospects for marker-assisted selection. The effects of (incomplete) blocks should be treated as random. The effects of environments should be considered as random if more than eight environments are used for phenotyping, and fixed if less than eight environments are used. As a result of genotype effects being random and environment effects being fixed/random, the effects of genotype-environment interactions (GEI) will be random. Analysis can be done for each environment separately or jointly across-environments, depending on the approach to be used for QTL analyses. The ReML BLUPs of individuals and of GEI from an across-environments analysis, however, may be more appropriate to use for QTL mapping, as this will more objectively allow detecting QTL for GEI also (Yan et al. 1998). This is because an across-environments analysis will properly separate the effects of individuals from those of GEI.

Effect of Alpha Blocking and Spatial Analysis on Heritability and QTL Mapping

As a result of effective blocking a decrease in error variance, and therefore an increase in heritability of a trait, can be expected. An increase in heritability, as noted earlier, effects an increase in the power of QTL mapping. Table 5.2.1 presents results of two analyses, one based on excluding and other on including the effects of blocks in a 9x18 alpha design trial on pearl millet at Patancheru conducted in 2000. Accounting for block effects (a) consistently resulted in an increase in heritability, (b) the number of detected QTL either remained the same or increased, and (c) LOD scores and R^2 values of detected QTL generally increased.

Table 5.2.1. Effect of Alpha-blocking (9 plots/block x 18 blocks/replication) on phenotyping of 160 testcrossed pearl millet mapping populations in irrigated control and early-onset terminal drought stress environments, Patancheru dry season, 2000. REML analyses were done with and without the block effect (+ and - block) in the model. Numbers of QTL detected are those with LOD scores > 2.0, from a combined model to obtain cumulative LOD score and R² values using interval mapping in MAPMAKER.

Variable	Plot mean heritability (%)		Number of QTL detected		Cumulative LOD (R ²) scores of detected putative QTL	
	-block	+ block	-block	+ block	-block	+ block
Irrigated control						
Days to flowering	72.9	75.9	4	4	15.8 (41.1)	21.7 (51.2)
Stover yield rrr ²	56.2	57.3	2	4	7.9 (24.7)	13.1 (36.3)
Grain yield m ⁻²	31.4	37.5	1	1	4.3 (13.8)	4.7 (15.2)
Biomass yield m ⁻²	44.2	46.9	2	2	8.1 (26.4)	8.3 (26.8)
Harvest index	63.9	67.6	3	5	17.7 (45.2)	26.2 (57.7)
Early-onset stress						
Days to flowering	72.0	79.3	3	4	17.7 (45.2)	22.2 (53.5)
Stover yield rrr ²	54.7	57.3	3	4	11.6 (33.7)	12.2 (36.0)
Grain yield m ⁻²	53.4	58.9	4	4	17.2 (46.7)	17.3 (48.7)
Biomass yield m ⁻²	39.7	43.8	2	4	6.0 (19.0)	9.3 (29.1)
Harvest index	68.6	71.2	5	5	27.2 (59.3)	26.2 (57.7)

In another detailed study of the effects of spatial analysis on QTL mapping results in pearl millet with 149 F₂ intercross mapping population individuals laid out in an alpha design, it was found that strong spatial variability existed along rows and/or columns of the trial fields. Spatial adjustment substantially decreased the error variance and bias, and increased heritability, the latter up to 100% for days to flowering at Nagaur in Rajasthan. Relative ranking of spatially adjusted means for all traits analyzed was substantially different from unadjusted alpha-design-based means. Use of spatially adjusted means substantially increased LOD scores of detected QTL, the increase being as much as 100% for days to flowering QTL at Nagaur. This was accompanied by a correspondingly proportionate increase in R² values of detected QTL. A number of additional QTL of large effects, not detectable in analysis based on unadjusted alpha means, were also detected. Spatial analysis, compared to alpha blocking, provided more realistic and powerful QTL

mapping resulted, from use of bias-free and more precise estimates of phenotypic values.

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Session 6: Conducting and Management of Field Experiments on Drought

6.1 Elements of Soil Water and Related Field-Based Measurements

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Soil and water are the two fundamental resources of our agriculture and our natural environment. Agricultural scientists, farmers, and other producers generally require knowledge about the water status of the soil in order to increase and sustain food and feed production. The variable amount of water contained in a unit mass or volume of soil and the energy state of water in the soil are important factors affecting the plant growth. Numerous other soil properties depend very strongly upon water content. Included among these are mechanical properties such as consistency, plasticity, strength, compactibility, penetrability, stickiness, and the frequency of field traffic (agricultural implements, people, and animals). In clayey soils, swelling and shrinkage associated with addition and extraction of water change the overall specific volume (or bulk density) of the soil as well as its pore size distribution. Soil water content also governs the air content and gas exchange of the soil, thus affecting the root respiration, the activity of microorganisms, and the chemical state of the soil. Frequently asked questions about soil water are: How dry or wet is the soil? How much moisture can the soil hold and supply to plants to support normal growth and maintain or improved yields?

Moisture content is the basic measurement required to answer these questions and there are several direct and indirect methods for its measurement. Since the soil water content varies continuously with time and space, a systematic measurement under field conditions is required. This paper presents some of the field techniques available for measuring soil water, such as gravimetric, neutron probe, and time domain reflectometry (TDR) methods. The various aspects of all three measurement techniques have been discussed in detail, including the operating principle, procedure, equipment needed, calculations, technical data, specifications, and accuracy. The other aspects of measurement techniques such as cost and time saving, the degree to which the sample collected represents the experimental area, reliability, handling, mobility, flexibility, and measuring range are also covered.

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6.2 Creating Managed Drought Stress Environment in Field Experiments: Methods of Water Control and Management

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The semi-arid tropics present a unique set of climatic features to those involved in agricultural research and development. Crops are grown in a wide range of varying environments. Lack of moisture is the key limiting factor to stabilized and improved agriculture in the SAT. In recent years, there has been a growing appreciation of these climatic constraints, and attempts are being made to understand the complexities and find a way out to lessen and overcome the impact of variable rainfall. The first step in understanding the effects of variable rainfall is to understand the impact of drought on plant response of various crop genotypes and to determine methodologies of screening for drought-tolerant genotypes. Plant responses such as plant morphology, phenology, and functional traits can be better understood by simulating the drought stress environment. This paper describes and discusses various methods such as restricting water application at certain crop stages, use of rainout shelters to prevent rain for a period during plant growth, application of line-source sprinklers as a tool for creating variable water application, and various traditional water application methods such as furrow, perfo, and sprinkler irrigation. Finally, the role of drip irrigation methodology is examined as a method to create a drought environment for the study of plant responses.

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Session 7: Synthesis and Overview of the Workshop on Crop Drought Research

7.1 Synthesis and Overview of the Workshop on Crop Drought Research

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A cause of major concern in the world today is that global water resources, which are not only limited but also fragile, are dwindling at an alarming rate in many developing countries. This would adversely affect food, fodder, and feed production and have an impact on all aspects of human society and animal life. Severe competition for the finite water resources among the major stakeholders (agriculture, industry, urbanization, health, and environment) has already commenced and is expected to intensify in the current decade. It is estimated that the irrigated agriculture sector worldwide currently uses more than 80% of the total available water. Only a small proportion, less than 20%, is used by all other stakeholders together.

The demand from other stakeholders for more water has been increasing consistently over the years and pressures will soon mount that the use of water in agriculture be reduced by at least 15-20%. The paradox of the situation is that agricultural production would need to be increased far above present levels achieved to meet the future increase in demand for food and feed and agricultural raw material for industries.

Accomplishing this difficult task without diverting more water towards agriculture calls for the most efficient possible use of available water in increasing agricultural production through integrated options for the management of agricultural drought. The major thrust has to be on agronomic and genetic management options for conserving rainfall as soil moisture and surface water, and efficient use of conserved rainwater. Genetic improvement of crops for drought tolerance, therefore, should be a high priority theme of research for development in the next two decades. Expectations from such research will be the availability of drought-tolerant varieties of important food crops, to be delivered in a defined, short-term time frame for on-farm evaluations. The challenge to agricultural research scientists in this decade is to develop varieties that can adapt to water limiting environments in a time frame of 4-5 years.

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This workshop has brought into sharp focus the progress made in genetic improvement of crops for drought tolerance and has highlighted the fact that it is realistic to expect that this task, though difficult, is achievable with concerted and coordinated action plans on genetic management of drought.

The papers presented and discussed have generated a good working knowledge base on a broad spectrum of themes. These include diagnostic, strategic, applied, and adaptive aspects of drought research, the aim being to expedite genetic improvement and adaptation of food grain crops, cereals (rice, maize, sorghum, and pearl millet), and legumes (groundnut, chickpea, and pigeonpea), to water limiting environments. Discussions focused on important questions such as the use of targeted and simulated drought environments, principles and methods of field/laboratory/glasshouse screening, genetic resources for drought tolerance, and strategies for developing crop varieties, particularly rice, adapted to drought-prone conditions.

The progress made was documented and lacunae were identified in the current knowledge that would hamper further progress and the development of a robust genetic management technology for drought-tolerant varieties adapted to specific target drought conditions. Discussion in the workshop affirmed the supposition that the genetic management option will be economical, easy to disseminate, and readily acceptable by resource-poor farmers who primarily rely on rainfall to cultivate their staple food crops.

It was felt that development of drought-tolerant rice varieties, apart from increasing and stabilizing production of upland or rainfed lowland rice, would also help in the shift of land area from flooded or lowland cultivation of rice (requiring large inputs of water) to aerobic rice. This would contribute to conservation of water normally used for agriculture and its diversion to other uses. The information presented in this book would be useful to planners and managers in preparing strategies on economizing the use of the scarce resource of global water and increasing agricultural productivity per unit (mm) of rainfall and supplemental irrigation.

Target Drought Environments

A common observation made was that target drought environments for each crop differed widely. This was not surprising, because this variation is related

to the wide variation in climate (quantity and distribution of rainfall, temperature and rate of evaporation), the physicochemical characteristics of the soil, the depth of soil profile, and interaction of these factors with the crop in different agroecoregions. To manage such diverse environment it was felt necessary to understand the crop x climate x soil interactions and characterize these in quantitative terms. There are examples of such efforts in which the annual soil moisture profile and atmospheric drought (evapotranspirative demand of air) were prepared for the rice-wheat production system for countries in the Indo-Gangetic plains (Bangladesh, India, Nepal, and Pakistan) (Subbarao et al.,2001).

Using the tools of geographic information system (GIS) and global positioning system (GPS) it is possible to cluster target areas that are broadly similar in drought characteristics (the iso- or homo-drought climes). Networks could then be created to develop varieties for major iso-drought climes and common screening facilities developed at one or more locations to meet the requirements of the target area. Varieties developed through such a coordinated effort would be specifically adapted to each of the target iso-drought climes in a given crop.

Creating Simulated Drought Treatments

A point of major concern is that the simulated drought environments in which screening for drought tolerance is conducted are often not very well defined and perhaps also not reproducible with regard to the magnitude and duration of stress. The simulated drought conditions discussed in the workshop thus varied widely from properly defined drought conditions to undefined drought conditions, except that these were two contrastingly different drought treatments.

It was recognized that important factors that interfere with experiments on drought are:

1. Interference from rainfall.
2. Difficulties in creating representative and repeatable drought treatments similar in characteristics to target drought conditions, with uniform application of irrigation water.
3. Minimizing sources that increase error variance in relation to variance due to treatments and their interactions in experiments.

Preventing Interference from Rain

This is a crucial factor in the successful conducting of an experiment of high and accepted quality on drought. Two approaches are available to overcome or minimize the effect of this interference - one the use of rainout shelters and the other dry season (postrainy or out of main season) screening.

Using Rainout Shelters

There was a good level of awareness among the drought researchers on the use of rainout shelters in minimizing the effect of rainfall in the rainy season experiments and also in areas where there are relatively more rainy days in the dry season. Various available options for rainout shelters(see cover) that ranged from very expensive high tech automatic rainout shelters to manual rainout shelters that were developed at ICRISAT were demonstrated to the participants during the workshop. Some partners in the Indian national program had used local technology to develop low-cost, reasonably effective rainout shelters. An important constraint in the use of rainout shelters is that it is expensive to cover large areas under the shelters. However, considering the importance of this research, necessary resources should be obtained to set up such facilities. The infrastructure, once created, can be used as a long-term facility for many years and by a number of research groups.

Dry Season Screenings

If it is not feasible to access rainout shelters, the only other option available is to avoid effects of intermittent rains by conducting screening in the dry season. The relevance, value, and application of results obtained in dry season screenings under high evaporative demand (water vapor pressure deficit) conditions to performance in rainy season screening was discussed. It was argued that photoperiod x temperature interactions on phenological differences can be managed by grouping the genotypes based on the main season data on phenology and by staggering planting to coincide the time of flowering in each phenology group. A suggestion was made that if the ranking for phenology does not change markedly between the two seasons, and if the structure of yield (such as number of tillers and other yield components) matches satisfactorily between the two seasons, it is possible to use the results of the dry season as a proxy for the normal rainy season.

Creating Simulated Drought Environments

Very often during the workshop, mention of drought and non-drought treatments was made in vague and qualitative context. In contrast, most of the plant responses to drought in growth, yield, and plant functions were determined very precisely and in quantitative terms. This large difference in precision of measurement of the two types of parameters are being correlated makes comparison less meaningful. It is imperative that this lacuna is plugged and drought is also defined in very precise and quantitative terms.

Controlled Application of Irrigation

Controlled and measured application of irrigation water is very important in creating reproducible and defined drought conditions; this is, however, very often neglected while conducting experiments on drought. The amount of water applied throughout the period of the experiment must be measured. Depending upon the resources available a range of methods of irrigation such as surface irrigation (through gated pipes or by flooding), overhead sprinklers, overhead droplet application with perforated pipes, and drip irrigation could be used. Each method has its own advantages and disadvantages in terms of uniformity of application.

Monitoring Soil Moisture and Climate Drought

Whatever the method of irrigation, the magnitude of drought can be defined by quantifying the amount of moisture in the soil profile with periodic measurement during the crop growing period. This fact was recognized and emphasized. These data are essential for making comparisons in the performance of varieties across experiments at a site and also between sites, years, and countries.

Genetic Improvement of Drought Tolerance

Traits of Drought Tolerance

Although it was recognized that drought-tolerant varieties have evolved with different traits, the trait most often specified was that of roots. A deeper root system with enhanced water uptake capacity was considered synonymous

with drought tolerance in many crops and is accepted as an undisputed trait of drought tolerance. It has been recently demonstrated in China that the rice hybrids derived from *indica* x *japonica* crosses had always a larger root system in upland than in lowland crops.

There are many other morphological traits, e.g., the anthesis to silking interval (ASI) in maize, the stay green trait in sorghum, small leaves and rapid rate of seed filling in chickpea, leaf rolling and waxy coating in rice, and others that are functional, such as osmotic adjustment, that seem to be related to drought tolerance. For making rapid progress in a cost-effective way it was discussed that instead of all researchers working on all traits, research groups could work on one or two traits that are most important to their crop and target conditions. Once success is achieved, the same could be applied to other crops. Precise identification of traits is important both in conventional trait-based breeding and in identifying the genetic markers related to a trait. In marker-assisted selection QTL that have large effects would be more valuable. It was reported that in pearl millet one such large QTL with seven linkage groups, that seems to regulate many responses under stress conditions, has been identified. Its impact on grain and straw yield in on-farm conditions is being evaluated.

Causal Relationships between Traits and Functions

Before a given trait is used to enhance drought tolerance in a variety, the end product, the causal relationship between traits, the related plant function (mechanism or process), and its relationship with yield under drought needs to be established. Sometimes combining two drought-tolerant traits may not produce the additive effect of further enhancing drought tolerance. For example, the deep root trait has been shown to be effective in maintaining a high plant water status in water limiting conditions. The osmotic adjustment trait is expressed when there is a plant water deficit. If these two traits are combined, then the osmotic adjustment will not express if the root trait is effective and has a dominant effect. However, the two together may confer some advantage under brief spells of severe intermittent drought. A better understanding of interaction between the processes regulated by different traits is therefore essential. It is necessary to ascertain that the introgressed trait is expressed into the target genetic background and increases yield in target drought conditions.

Development of isogenic or near-isogenic lines for various drought tolerance traits, although time-consuming, would be valuable in the study of causal relationships between traits and plant function or response and their use in improvement of drought tolerance and yield. The intensity of the trait required, e.g. the root system size, may also depend upon the availability of soil moisture, the resource that it is tapping.

There is very limited data reported on the causal relationship between deep roots and improved plant water status (such as high relative water content, turgid leaves, and absence of leaf rolling). More data generation is required to strengthen the causal relationship between the most important traits, and their relationship with plant function and yield. This aspect needs to receive a very high priority in drought physiology research.

Conventional Breeding Approaches

Examples were presented of introgression of root trait in rice and in chickpea through conventional methods of backcrossing for improvement of drought tolerance. Although these were not near isogenic lines, they retained nearly 90-95% of the characteristics of the recurrent parent, and of the desired introgressed root system added to it. Simple and effective methods for root screening that help in screening for the trait in segregating populations were described. The desired root system has been incorporated in varieties and, with focused efforts and attention, such varieties that are enhanced for root trait and drought tolerance could be made available in 4-5 years' time for evaluation under on-farm conditions. It was emphasized that these varieties should be evaluated in the kind of target drought conditions for which these have been developed.

Marker-Assisted Breeding

Many participants felt that marker-assisted breeding should be applied for incorporation of traits that cannot be easily manipulated with conventional methods of breeding. In certain situations where the desired phenotype can be easily scored or selected for or against, the progress made could be faster and cheaper with phenotyping and conventional breeding methods rather than by using markers.

Attention was drawn to ongoing concerted efforts for marker assisted breeding for drought tolerance in rice, at the same time retaining other

available biotic and abiotic stress tolerant traits, combined with visual selection for yield. The question of identification of QTL for high grain yield under drought was debated. Participants wondered how it would be different from direct selection for yield. Since heritability for grain yield is low, particularly in drought conditions, reservations were expressed about the usefulness of the QTL for grain yield that would be rendered useless if these interact with drought environments.

A point made in favor of using secondary traits or QTL rather than selection for yield itself was that many yield-based breeding programs are often as costly as or even costlier compared to marker-assisted breeding programs, and yet do not help in achieving the objective. Since a good awareness about appropriate phenotyping has been generated as a result of this workshop, this could be used in refining trait-based breeding programs for greater efficiency and economy in achieving targeted outputs using the genetic marker approach.

A high priority should be awarded to developing a better understanding of the traits that control yield and their interaction with the environment in order to minimize the effects of genotype x environment interaction on yield. It would be difficult to find examples of crop varieties that have no (zero) interaction with drought environments, but efforts could be made to minimize these interactions.

The current knowledge in drought tolerance research and development shows that hoping for a universal variety for all drought-prone environments would be like chasing a mirage, resulting in loss or waste of valuable research resources.

Relevance of Vegetative Stage Screening to the Reproductive Stage

The question of screening for drought tolerance at the vegetative stage of growth and its relevance to tolerance at the reproductive stage was considered doubtful on the basis of available data and information at this stage. The subject was not pursued in discussions.

Statistical Designs and Handling of Data

A statistician is generally involved in agricultural experiments, including those on drought, only after an experiment has been conducted, during the

analysis of data. This sometimes proves to be very costly because of use of unsound experimental design, rendering useless the value of data collected, as very limited or no proper inferences can be drawn. It is thus necessary to involve a statistician as a member of team at the stage of conceptualization and planning of the experiment to improve the precision of experiments.

A good discussion took place on the role of statistics in planned drought research. Robust statistical designs that would enable handling of large populations, either germplasm, double haploid lines (DHLs), or recombinant inbred lines (RILs) that would minimize the error variance component in experiments were discussed and references were made to published work. Statistical methods of analysis of data to better account for error component of variance and thereby increasing the variance component due to treatments, in particular in the identification of QTL, were discussed.

Mention was also made of a common tendency to reject experiments based upon high value of coefficient of variance (CV%). An example was quoted in pearl millet experiments in which, despite a very high CV% (ranging from 150% to 2000%), very useful information was extracted on germplasm enhanced for downy mildew (a fungal disease) resistance, culminating in the release of a high-yielding, downy mildew resistant variety.

Strategies for Development of Drought-Tolerant Varieties in a Specific Time Frame

It is common knowledge that drought-tolerant varieties, in general, have low yield potential. Therefore, selection for very high levels of drought tolerance in varieties would impose a penalty in the years when drought is moderate or mild because these varieties would then be unable to take advantage of favorable soil moisture conditions.

Therefore, the degree of drought tolerance incorporated needs to match with the magnitude of drought in the target environment, determined on the basis of probability analysis of long-term climate data in the target environment. This would ensure resilience in varieties that would respond to intermittent favorable soil moisture conditions and would combine high yield with stability of yield under drought. The need in research is to focus on the physiological basis of resilience traits (plasticity in response to variable soil moisture regime) so that systematic efforts could be made to incorporate the character in new varieties.

It was felt that good field phenotyping facilities are now in place for creating managed drought environments in India, Thailand, and perhaps in some other countries. Screening the breeders' populations in those managed stress environments to document the progress made should now be a priority.

Information presented at the workshop showed that large numbers of traits related to drought-tolerant germplasm have been identified in many crops, including rice. A suggestion was made that multiple crosses be made at one location involving all the germplasm identified with traits of drought tolerance in all possible combinations. Early generation segregating material (F3s) could then be shared with all partners in the drought program for evaluation in their target drought environments and deriving drought-tolerant varieties that are specifically adapted to their drought conditions, based on the combined criteria of traits and yield. This approach would expedite the process of development of many drought-tolerant varieties in a short period of time.

Another approach suggested was to improve the yield levels in the landraces that have high adaptation to specific drought-prone areas and tolerance to many biotic and abiotic stress factors that prevail there. Varieties developed using this approach would not need intervention with tools for QTL mapping, or advanced statistics, because high yield can be easily measured with simple methods. It would thus be possible to retain the maximum drought tolerance genotype with yield built upon it.

Plant Stands Under Drought

Poor plant stand establishment in receding moisture conditions was considered to be an important factor affecting grain yield. Examples were quoted from wheat, maize, and sorghum of germinating seed becoming completely dry with transient drought and yet being able to resume growth and development when soil moisture conditions become favorable. The mechanism of this unique response in these crops *is* not well enough understood at present to think of initiating a breeding program. The best option seems to be that of agronomic management, e.g., seed priming, if a large proportion of the crop has failed to reseed the crop.

Conclusions

- With the knowledge now gained in drought research it should be possible to predict the gains from drought research in terms of products (drought-tolerant varieties) in a definite time frame.
- It is imperative that a time frame is now fixed for delivering final or intermediate products of drought research for validation.
- Emphasis should be on scaling up of the evaluation capacity of new genetic technologies, drought-tolerant varieties, and movement to on-farm farmer participatory evaluation to confirm the effectiveness of genetic management technologies in water limiting conditions.
- A high priority should be on translating new knowledge gained in drought research into developing usable technologies
- Investment in *re-search* (looking at what has already been discovered and verified many times) needs to be stopped.
- Thrust areas of research that have been adequately explored need to be concluded and new approaches defined and pursued.
- All the available information and practices need to be collated as a standard protocol on screening for drought tolerance in a manual and made available to researchers in national and international programs.

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About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research that can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is cosponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.

About the Rockefeller Foundation

The Rockefeller Foundation is a knowledge-based, global foundation with a commitment to enrich and sustain the lives and livelihoods of poor and excluded people throughout the world. In order to maximize its resources and leverage the Foundation's strengths, grant making is organized around four thematic lines of work: Creativity and Culture, Food Security, Health Equity, and Working Communities. A cross-theme of Global Inclusion supports, promotes, and supplements the work of these themes. In addition, the Foundation supports a number of programs that are developing or in transition, among them the Africa Regional Program, Communication for Social Change, Public/Private Partnerships, and Global Philanthropy.



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