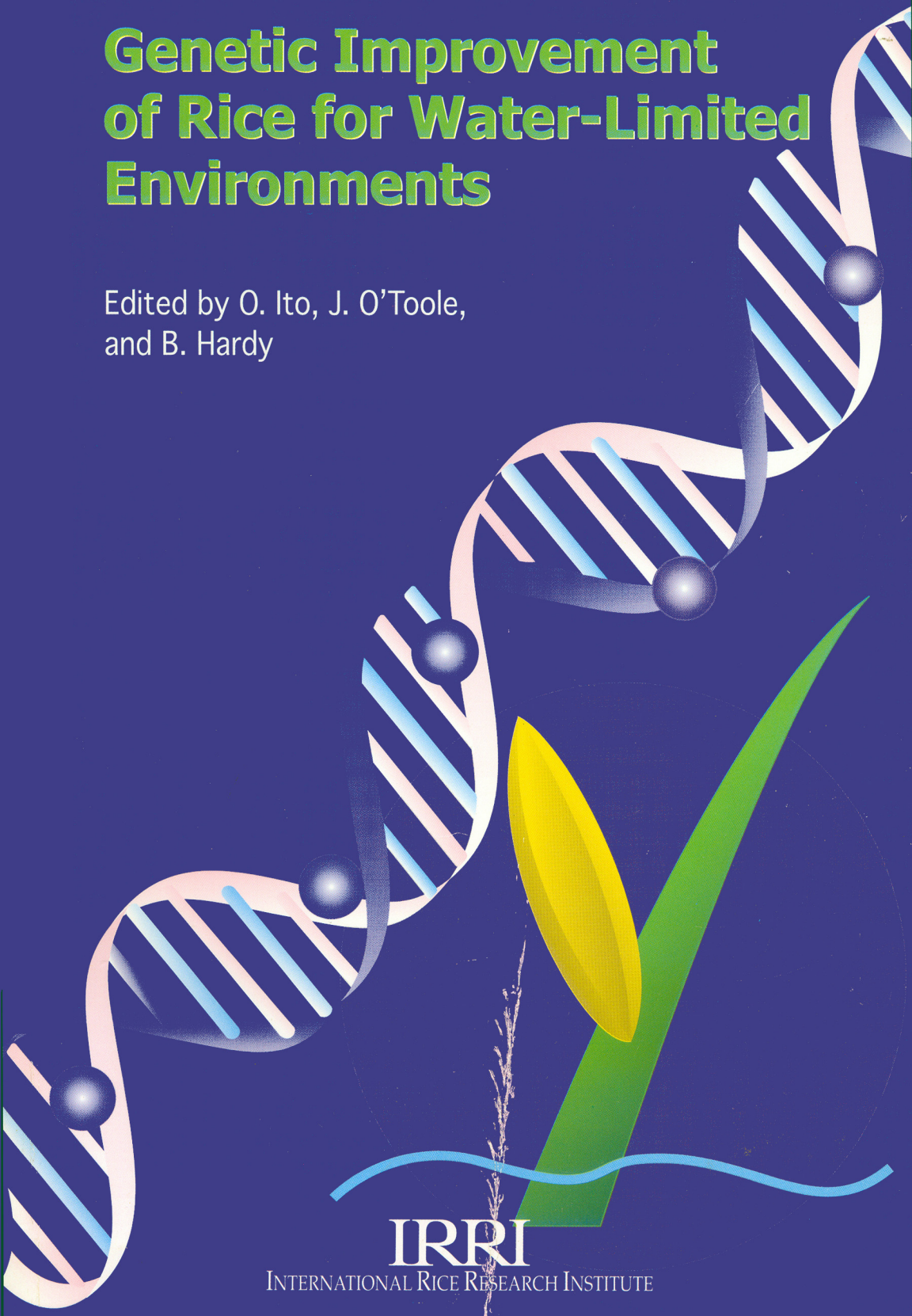
Genetic Improvement of Rice for Water-Limited Environments

Edited by O. Ito, J. O'Toole, and B. Hardy



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Contents

Foreword

Rice, unlike other cereals, has remarkable adaptability to a wide range of hydrological conditions. Although it can achieve its highest yield in waterlogged conditions under irrigation, it is widely grown in rainfed lowlands and uplands where soils are kept aerobic for a certain period or the entire period of crop growth. Aerobic exposure drastically changes the micro-environment for rice growth and increases environmental constraints that seriously affect crop production.

About 45% of total rice area is currently planted without irrigation and about 25% of total rice production depends on that area. The major environmental constraint for rice production in that area is water deficit, to which rice is more susceptible than other cereals. The gap in productivity between irrigated and nonirrigated areas is still huge. Demand for rice because of population increases expected in the near future can no longer be satisfied only by more yield from irrigated areas. Much more effort should be made to narrow the gap through genetic improvement of rice for water-limited environments, including poorly or inadequately irrigated fields. Today, we are frequently reminded of the looming water crisis for agriculture in Asia as competition with urban and industrial users requires rice production to continue with less than ideal water resources.

The issue of tolerance for water deficit in rice is somewhat classical and has been considered intractable. Many rice scientists, however, now believe that genetic improvement for this trait is possible given the recent advances in rice molecular biology and genetics and opportunities for interdisciplinary collaboration. The International Rice Research Institute was thus pleased to cosponsor, with The Rockefeller Foundation, the Workshop on Genetic Improvement of Rice for Water-Limited Environments, 1-3 December 1998, at its headquarters in Los Baños.

The objectives of the workshop were to (1) review the current status of research on marker-aided selection in rice and other crops, (2) examine collaborative research at the interfaces of breeding, physiology, and molecular genetics, (3) identify opportunities to exploit emerging knowledge and techniques, and (4) develop strategies to use research results for the production and testing of improved rice varieties. This publication contains the papers presented at the workshop and the recommendations developed from the group discussions. We hope that this book will guide future work on improving the adaptability of the rice plant to water-limited environments.

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Critical characteristics of rainfed rice environments and implications for rice improvement

L.J. Wade

This chapter begins by outlining the area and productivity of rainfed rice in upland, rainfed lowland, and flood-prone ecosystems of South and Southeast Asia. Critical characteristics of these rainfed systems are then discussed, particularly in relation to soil conditions and the implications for root growth, water extraction, and other traits likely to contribute to drought tolerance. Implications for effective phenotyping of breeding populations and for selection of lines with improved adaptation to different types of water stress are discussed. The chapter concludes that further field data are needed for identifying quantitative trait loci associated with drought tolerance, and that the benefits from incorporating particular traits must be demonstrated.

Rainfed rice is grown on approximately 59 million hectares worldwide, representing 45% of the total area planted to rice (IRRI 1993a). Water stress is the most severe limitation to the productivity of rice in rainfed ecosystems (Widawsky and O'Toole 1990). Thus, drought stress has a significant impact on rice production under rainfed conditions, where yields average 1.1, 2.3, and 1.5 t ha^{-1} under upland, rainfed lowland, and flood-prone conditions, respectively (IRRI 1993a). Table 1 presents the distribution of rice area and yield by ecosystem for the major rainfed rice-producing countries in South and Southeast Asia.

Ecosystems for rice are associated with toposequence position and are defined by the water regime encountered (Fig. 1). In the uplands, soils remain aerobic throughout the season, whereas in rainfed lowland and flood-prone ecosystems ponding and submergence can occur in the bunded fields (Garrity et a1 1986). These changing soil conditions have enormous consequences for nutrient availability, weed competition, and the adaptive strategies that the plant may need for successful performance (Wade et a1 1998). Spatial heterogeneity and seasonal variability in the timing, duration, and intensity of water stress and its interactions with other factors further complicate the development of strategies that will likely lead to the development of higher yielding varieties on average, with less probability of crop failure. This is especially important in subsistence agriculture, where survival of the farmer and family largely depends on the season's harvest.

Table 1. Distribution of rice area and yield by ecosystem for the major rainfed rice countries of South and Southeast Asia.

Country	Distribution of area (000 ha)			Yield by ecosystem (t ha^{-1})		
	RL[a]	FP	UR	RL	FP	UR
Bangladesh	5,166	2,492	875	2.5	1.6	0.8
Cambodia	864	756	36	1.5	0.9	0.9
India	13,926	2,954	6,330	2.4	1.5	0.8
Indonesia	713	1,018	1,121	3.0	1.7	1.6
Lao PDR	393	0	234	2.6	0.0	1.4
Myanmar	2,512	1,159	290	3.0	1.5	1.0
Nepal	942	118	43	2.2	0.8	1.0
Philippines	1,198	69	68	2.0	1.3	1.0
Sri Lanka	454	25	60	2.5	1.0	1.0
Thailand	8,568	660	52	1.8	2.0	1.5
Vietnam	1,763	692	504	2.0	1.5	1.0

[a] RL = rainfed lowlands, FP = flood-prone areas, UR = upland rice.

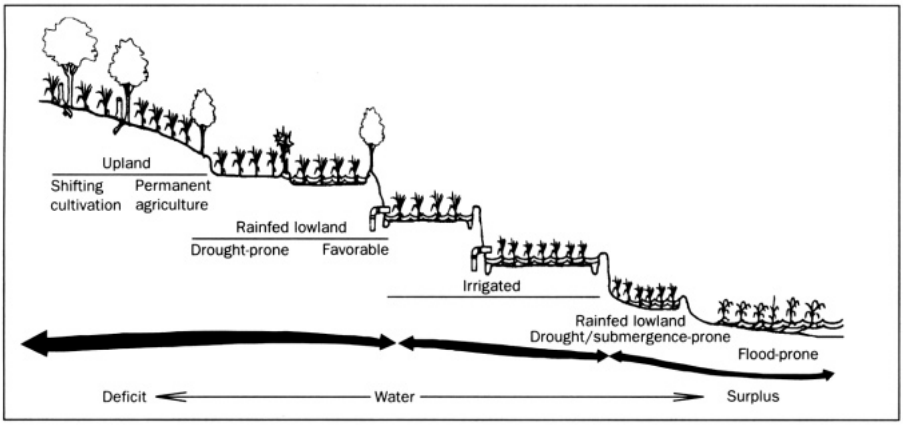

Fig. 1. Characteristics of rice ecosystems.

Rice ecosystems are characterized by the natural resources of water and land, and by the adaptation of the rice plant to them. Irrigated rice may be found at any point in a toposequence if water delivery is available.

Upland
Level to steeply sloping fields; rarely flooded, aerobic soil; rice direct seeded on plowed dry soil or dibbled in wet, nonpuddled soil.

Rainfed lowland
Level to slightly sloping, bunded fields; noncontinuous flooding of variable depth and duration; submergence not exceeding 50 cm for more than 10 consecutive days; rice transplanted in puddled soil or direct seeded on puddled or plowed dry soil; alternating aerobic to anaerobic soil of variable frequency and duration.

Irrigated
Leveled, bunded fields with water control; rice transplanted or direct seeded in puddled soil: shallow flooded with anaerobic soil during crop growth.

Flood-prone
Level to slightly sloping or depressed fields: more than 10 consecutive days of medium to very deep flooding (50 to more than 300 cm) during crop growth; rice transplanted in puddled soil or direct seeded on plowed dry soil; aerobic to anaerobic soil: soil salinity or toxicity in tidal areas.

This chapter discusses the critical characteristics of rainfed rice environments, especially in relation to soil conditions before, during, and after drought, and their implications for root growth, water extraction, and other traits likely to confer some level of drought tolerance. Implications for effective phenotyping of breeding populations for different target subecosystems and for selection of lines with improved adaptation to different types of water stress are discussed. The chapter concludes by emphasizing the need to collect further field phenotyping data for identifying quantitative trait loci (QTLs) for drought tolerance, and to understand and demonstrate the likely benefits from incorporating specific traits for drought tolerance for target subecosystems.

Critical characteristics of rainfed rice systems

Upland rice is grown higher in the toposequence on aerobic soils, sometimes on sloping lands. Three predominant agroclimatic zones with different risks of drought and sets of additional constraints are recognized, which differ geographically and climatically: dry plateaus of South Asia with permanent integrated systems, hilly subhumid areas of mainland Southeast Asia with slash and burn, and equatorial humid areas with perennials in Indonesia, southern Vietnam, and southern Philippines (Courtois and Lafitte, this volume).

Likewise, in rainfed lowlands, several subecosystems predominate: late-season drought, early season drought, fast onset late-season drought, short-term submergence (up to 10 d), and combinations of these (Wade et al 1997). With long-duration, traditional cultivars (Mackill 1986), late-season drought is most severe, but, with the change to short-duration, direct-seeded rice (Fujisaka et al 1993), early season drought is increasing in importance. The flood-prone ecosystem encounters greater water depths for longer durations than rainfed lowlands and water may be stagnant or may change in depth quickly. Floodwater quality problems caused by turbidity, low oxygen supply, extreme pH, tidal influences, and salinity may also arise (Setter et al 1995).

It is interesting to examine plant available water in different systems. Puddling changes the water content-tension relationship, with more easy-to-extract water available in puddled soils (Boling et al 1999). The effectiveness of water extraction is also related to soil condition and establishment method, with transplanted rice on puddled soils extracting less water than dry-seeded rice on unpuddled soils. This response is attributed to the different rooting patterns and different water content-tension relationships in the contrasting systems (Boling et al 1999, Table 2). When plant available water is compared from 0 to 120 cm in upland conditions, and from 0 to 30 cm in puddled soil conditions, the actual amounts of extractable water can be quite similar (Jongdee 1999). Yet the ease with which plants can extract that water differs because of contrasts in soil anaerobiosis, water content-tension relationships, and the vertical distribution of roots and soil water.

In the uplands, water extraction is related to root length density, with an extensive and deep root system likely to be advantageous (O'Toole 1982, Puckridge and O'Toole 1981). In rainfed lowland and flood-prone areas, evidence is less clear. In

Table 2. Water retention of puddled and nonpuddled soil.

Soil moisture tension (kPa)	Puddled soil ($cm^3\ cm^{-3}$)	Nonpuddled soil ($cm^3\ cm^{-3}$)
0	0.70	0.51
1	0.62	0.49
10	0.47	0.39
100	0.35	0.26
1,000	0.26	0.16

Source: Boling et al (1999).

pot studies simulating rainfed lowland conditions, genotypes differed in patterns of water extraction (Kamoshita et al 1999), and these patterns were related to changes in root distribution under drought (Azhiri-Sigari et al 1999). In the field, genotypes differed in capacity to penetrate a hardpan (Samson et al 1999) and were able to extract water from deeper layers (Wade et al 1999). But it remains to be demonstrated whether the generally low root length densities at depth in rainfed lowlands in the field can extract all of the water that should be available (Samson and Wade 1998).

The contrast in soil conditions between anaerobic flooding and aerobic drought provides special challenges for root growth and water extraction in rainfed lowland and flood-prone areas (Samson and Wade 1998, Wade et al 1998). Roots tend to be shallow in rainfed lowlands, perhaps because plenty of water is often available earlier in the season when roots are developed. Oxygen supply to roots is mainly through aerenchyma in these conditions, which may restrict roots to shallower zones, as may the presence of chemical (low pH) or physical barriers (hardpans or the transition to a nonpuddled soil layer). The rapid onset of water stress in soils of poor water-holding capacity may prevent roots from exploring deeper soil layers. In drying surface soil, signals from roots may result in stomatal closure and reduced photosynthesis, thereby reducing assimilate supply to roots and discouraging further root exploration of deeper soil layers where water may still be available. Root turnover under anaerobic/aerobic transitions may influence the capacity to extract water from depth.

Table 3 summarizes the conditions encountered in different rice ecosystems for root growth and function. While uplands may encounter severe problems with hardpans, low pH subsoils, and rapid onset of drought on coarse-textured soils, rainfed lowland and flood-prone areas are also exposed to anaerobic/aerobic soil transitions and their implications for oxygen supply, root signals, and the severe contrast between saturated and unsaturated conditions. These situations must be considered in defining environments for phenotyping of breeding populations for drought tolerance and identifying appropriate QTLs. At issue is not simply the capacity of a line to develop good roots in conditions of aerobic water stress, but in the actual conditions likely to be encountered in target environments (Table 3).

Table 3. Critical characteristics of rainfed rice environments for phenotyping breeding populations for drought avoidance and drought tolerance.

Factor	Upland rice[a]	Rainfed lowland rice	Flood-prone rice	Comments
Oxygen	–	***	***	Anaerobic/aerobic soils
Physical barriers	**	**	**	Hardpans, puddled layers
Chemical barriers	***	**	**	Acid subsoils
Rate of stress onset	**	***	***	Low WHC[b], wet/dry contrast
Nutrient distribution	**	**	**	Poor subsoils
Root signals	*	***	**	Root tips in dry/wet soils

[a] *** = believed to be extremely important, ** = believed to be moderately important, * = believed to have minor importance, - = no limit. [b] WHC = water-holding capacity.

Adaptive strategies for rainfed rice

Alternative strategies may be employed to reduce the impact of abiotic stresses in order to ensure consistency of crop performance (Ludlow and Muchow 1990). Breeders have been most successful in using the "escape" strategy, whereby the crop is exposed to low levels of abiotic stress, either by reducing crop duration or by altering the phenological pattern so that sensitive growth stages do not coincide with periods likely to encounter severe abiotic stress. Examples are short-duration rice to escape late-season drought, growing of boro rice in winter to escape severe flooding in the wet season, or the use of direct seeding of photoperiod-insensitive lines to minimize exposure to terminal drought. If complete escape is not possible, or reduced duration restricts yield potential too seriously, some level of abiotic stress "avoidance" or "tolerance'' may also be useful.

An avoidance strategy implies that the genotype minimizes its exposure to the problem by gaining access to additional resources or by reducing losses of the critical resource. For prolonged submergence, an avoidance strategy would involve rapid stem elongation so that some of the canopy could remain above the floodwater, permitting conductance of oxygen to stem bases and roots. This is also advantageous for photosynthesis if floodwater is turbid. For drought, an avoidance strategy would involve a genotype gaining access to additional reserves of soil water, some from soil layers already explored or more from deeper soil layers not previously explored (O'Toole 1982). Drought avoidance therefore implies better root systems, although a smaller canopy and greater leaf rolling may reduce evaporative loss of the finite water resource. In their review, Fukai and Cooper (1995) believed that root traits for gaining access to additional reserves were more important than shoot traits for reducing losses.

A tolerance strategy implies that the genotype is able to bear the consequences of some level of exposure to the abiotic stress, but still continue essential functions. For short-term submergence (up to 10 d), a tolerant genotype would not elongate, but

would survive using anaerobic respiration until the stress was relieved (Setter et al 1997). Reserves of nonstructural carbohydrates, higher levels of alcohol dehydrogenase and pyruvate decarboxylase, and free-radical scavenging mechanisms are implicated as beneficial here. Likewise for drought, the ability of a genotype to tolerate partial tissue dessication, adjust osmotic potential, and maintain cell turgor, and, again, free-radical scavenging mechanisms are believed to be beneficial.

Tolerance strategies may buy the crop time, but avoidance strategies should have a potential for greater impact, if additional reserves can be exploited. This may depend on whether the genotype can express a capacity to develop better and more effective roots, given constraints to their expression in different target subecosystems (Table 4; Samson and Wade 1998, Wade et al 1998). For drought, it appears that genotypes developed to date possess either better avoidance (greater maximum rooting depth, greater hardpan penetration capacity) or better tolerance (better osmotic adjustment capacity). A special research challenge to follow from identifying QTLs for avoidance and tolerance traits would be to combine these strategies in one genotype, ideally with appropriate phenology, so as to use the escape strategy as well.

This review necessarily focuses on abiotic stress tolerance, especially for water stress. Recently, other reviews have considered adaptive strategies for nutrient stresses (Lafitte 1998) and for weed competition (Fofana et al 1995), which are also important considerations in rainfed systems.

Critical issues for phenotyping of rice for water-stress tolerance

In evaluating traits for improved adaptation to drought, both constitutive and adaptive (inducible) traits may be important. For example, a genotype that is able to develop a better root system in well-watered conditions before the onset of drought (constitutive traits) may have a head start for extracting water from deeper layers as the drought proceeds. In contrast, a genotype that is able to modify its root system to better extract water from deeper layers as water stress intensifies relies on adaptive traits expressed only under water stress.

It is important to recognize the difference between constitutive and adaptive traits, and to develop phenotyping procedures capable of identifying genotypes possessing advantages in one or both types for the target ecosystem. To do so effectively, the phenotyping conditions must reflect situations representative of those that occur in the target subecosystems (Table 4). These conditions are quite different for upland rice than for rainfed lowland and flood-prone rice. In upland rice, constitutive traits

Table 4. Critical issues for phenotyping of rice for water stress.

Trait	Upland rice	Rainfed lowland rice
Constitutive	Aerobic, well-watered	Anaerobic, well-watered
Inducible	Aerobic, appropriate drought	Aerobic, following anaerobic

are expressed in well-watered conditions in which soils are aerobic and, as water stress proceeds, inducible traits are expressed in aerobic conditions as well. In contrast, for rainfed lowland and flood-prone rice, constitutive traits are expressed in anaerobic soils with ponded water, whereas inducible traits are expressed as water stress intensifies in aerobic soil conditions. These contrasts have implications not only for root traits but also for maintenance of leaf water potential, osmotic adjustment, and dehydration tolerance (Nguyen et al 1997), and for the capacity of the plant to maintain leaf water potential with variation in internal resistance to water transport (Jongdee 1999).

Last but not least is the challenge of providing a defined and repeatable drought condition for consistent phenotyping (Blum 1988). Field conditions provide more realistic conditions than pots, where restricted soil volumes and disturbed soils alter the expression of drought tolerance. But untimely rainfall in the field may preclude effective screening. Dry-season screening is sometimes employed to avoid periods of higher rainfall expectation. But evaporative demand may be considerably higher than that to which plants would normally be exposed in dry periods in the wet season, thus altering the expression of traits that should be beneficial under the targeted drought condition. For this reason, rainout shelters are sometimes used to permit us to exclude untimely rainfall during the correct season. But rainout shelters are expensive and difficult to maintain. The net result is extreme difficulty in reliably phenotyping breeding poulations for drought tolerance in appropriate field conditions closely related to those of the target environment. We are likely to conclude that the greatest limitation to further progress in identifying QTLs and developing markers for effective drought tolerance is the lack of suitable field data on phenotyping for drought tolerance.

Issues for interpreting avoidance and tolerance traits

Traits conferring drought avoidance and tolerance are quantitative (with large genotype by environment interactions), responsive to environment, influenced by timing and severity of stress imposition relative to crop growth stage, and modified by plant height and growth habit. Further, most of these traits are difficult to measure and require considerable effort and skill for interpretable data to result. Yet there is no alternative at present but to continue to take measurements, especially to phenotype breeding populations for drought avoidance and tolerance, because current field data sets for QTL identification are inadequate. For rainfed lowlands, for example, almost all data are from screening in controlled conditions in the greenhouse and the few field data that exist were collected in the dry season, in the absence of drought stress, or in upland conditions. Clearly, this deficiency must be addressed as a matter of urgency. The ideal of moving solely to marker-aided selection is only a dream until suitable QTLs can be identified and their contribution understood, and this will never happen until field phenotyping is adequate.

A second concern is the number of QTLs identified for each trait in the analysis of phenotypic data. Commonly, many QTLs are identified, and those identified often

change from experiment to experiment. But how many directly control expression of the trait of interest, and how many modify its expression via an influence on phenology, plant height, canopy development, and other factors that modify the rate and severity of stress onset? We need a much clearer understanding of the component traits to be combined for improved adaptation to water stress in different target subecosystems, and must demonstrate the likely benefit from incorporating the component traits. Interestingly, efforts to develop populations for phenotyping are consistent with the development of near-isogenic lines, which are robust materials for such physiological studies.

A clear understanding is only possible if related physiology studies continue along with phenotyping, with support from related disciplines such as soil physics, agronomy, population genetics, molecular biology, and plant breeding. Only such systematic studies are likely to lead to the identified goal of releasing more drought-tolerant genotypes capable of having an impact in farmers' fields. Because the intent of this workshop is to achieve this within five years, a concerted interdisciplinary effort is the only one likely to be successful. Work should proceed at various levels, from traditional breeding to molecular approaches, but must be supported by studies seeking understanding and synthesis as well. Only in combination are we likely to achieve success against this most difficult of abiotic stresses.

References

Azhiri-Sigari T, Yamauchi A, Kamoshita A, Wade LJ. 1999. Genotypic variation in response of rainfed lowland rice to drought and rewatering. 2. Root growth. Plant Prod. Sci. (In review.)

Blum A. 1988. Plant breeding for stress environments. Boca Raton, Fla. (USA): CRC Press. 223 p.

Boling A, Tuong TP, Singh AK, Wopereis MCS. 1999. Comparative root growth and soil water extraction of dry-seeded, wet-seeded and transplanted rice. Philipp. J. Crop Sci. (In press.)

Fofana B, Koupeur T, Jones MP, Johnson DE. 1995. Development of rice varieties competitive with weeds. Proceedings of Brighton Crop Protection Conference. Vol. 1. p 187-192.

Fujisaka JS, Moody K, Ingram KT. 1993. A descriptive study of farming practices for dry-seeded rainfed lowland rice in India, Indonesia and Myanmar. Agric. Ecosys. Environ. 45:115-128.

Fukai S, Cooper M. 1995. Review: development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

Garrity DP, Oldeman LR, Morris RA. 1986. Rainfed lowland rice ecosystems: characterization and distribution. Progress in rainfed lowland rice. Manila (Philippines): International Rice Research Institute. p 3-23.

IRRI (International Rice Research Institute). 1993a. 1993-1995 IRRI rice almanac. Manila (Philippines): International Rice Research Institute.

IRRI (International Rice Research Institute). 1993b. Rice research in a time of change. IRRI's medium-term plan for 1994-1998. Los Baños (Philippines): International Rice Research Institute.

Jongdee B. 1999. The importance of leaf water potential and osmotic adjustment on growth and grain yield of rice (*Oryza sativa* L.) genotypes under water deficit conditions. PhD thesis. University of Queensland, Brisbane, Australia.

Kamoshita A, Wade LJ, Yamauchi A. 1999. Genotypic variation in response of rainfed lowland rice to drought and rewatering. 3. Water extraction during the drought period. Plant Prod. Sci. (In review.)

Lafitte HR. 1998. Research opportunities to improve nutrient-use efficiency in rice cropping systems. Field Crops Res. 56:223-236.

Ludlow MM, Muchow RC. 1990. A critical evaluation of the traits for improving crop yields in water limited environments. Adv. Agron. 43:107-153.

Mackill DJ. 1986. Varietal improvement for rainfed lowland rice in South and Southeast Asia: results of a survey. Progress in rainfed lowland rice. Manila (Philippines): International Rice Research Institute. p 115-144.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetic considerations. Crop Sci. 37: 1426-1434.

O'Toole JC. 1982. Adaptation of rice to drought-prone environments. In: Drought resistance in crops with emphasis on rice. Manila (Philippines): International Rice Research Institute. p 195-213.

Puckridge DW, O'Toole JC. 1981. Dry matter and grain production of rice, using a line source sprinkler in drought studies. Field Crops Res. 3:303-319.

Samson BK, Wade LJ. 1998. Soil physical constraints affecting root growth, water extraction, and nutrient uptake in rainfed lowland rice. In: Ladha JK, Wade LJ, Dobermann A, Reichardt W, Kirk G, Piggin C, editors. Rainfed lowland rice: advances in nutrient management research. Manila (Philippines): International Rice Research Institute. p 231-244.

Samson BK, Wade LJ, Hasan M. 1999. Variation in root traits with respect to soil strength in drought-prone rainfed lowland rice. Field Crops Res. (In review.)

Setter TL, Ramakhrisnayya G, Rammaurya PC, Singh BB. 1995. Environmental characteristics of floodwater in eastern India: relevance to flooding tolerance of rice. Indian J. Plant Physiol. 38:34-40.

Setter TL, Ellis M, Laureles EV, Ella ES, Senadhira S, Mishra SB, Sarkarung S, Datta S. 1997. Physiology and genetics of submergence tolerance in rice. Ann. Bot. 79:67-77.

Wade LJ, McLaren CG, Criseno L, Amarante ST, Sarawgi AK, Kumar R, Bhambri MC, Singh ON, Ahmed HU, Rajatasereekul S, Porn-uraisanit P, Boonwite C, Harnpichitvitaya D, Sarkarung S. 1997. Genotype by environment interactions—RLRRC experience. In: Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research. p 115-125.

Wade LJ, George T, Ladha JK, Singh U, Bhuiyan SI, Pandey S. 1998. Opportunities to manipulate nutrient-by-water interactions in rainfed lowland rice systems. Field Crops Res. 56:93-112.

Wade LJ, Fukai S, Samson BK, Ali A, Mazid MA. 1999. Overview—problems and prospects for crop improvement in rainfed lowland rice. Field Crops Res. (In review.)

Widawsky DA, O'Toole JC. 1990. Prioritizing the rice biotechnology research agenda for eastem India. New York, NY (USA): The Rockefeller Foundation.

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Perspectives on plant breeding

Combining information from multi-environment trials and molecular markers to select adaptive traits for yield improvement of rice in water-limited environments

M. Cooper, D.W. Podlich, and S. Fukai

The principles used to design multi-environment trials (METs) are discussed. Results from METs provide much of the information used to make selection decisions in plant breeding programs. The resources available for conducting them must be used to give the most accurate and precise information possible. This requires attention to (1) the adequacy of the sampling of the target population of environments, (2) experimental design, analysis, and interpretation of results, (3) data and information management, (4) understanding the causes of genotype-by-environment (G x E) interactions, and (5) the merits of alternative selection strategies compared with those currently used. Most breeding programs do not optimize the use of METs to achieve breeding objectives. Much can be done to improve on common practice. Our work on rainfed lowland rice in northeast Thailand and Lao PDR has revealed that large G x E interactions are common for grain yield. A major component of these interactions results from genetic variation for flowering time and environmental variation in the timing of water deficits. Genetic variation for yield, after adjusting for the effects of flowering time, is related to the capacity of lines to maintain a favorable leaf and panicle water status. The proposed breeding strategy involves a greater use of METs in combination with targeted screening of traits contributing to drought tolerance. The value of putative drought-tolerance traits must be established prior to their use as indirect selection criteria. The efficiency of indirect selection for broad and specific adaptations by trait-based or marker-assisted selection (MAS) needs to be evaluated in comparison to the potential for direct selection for yield from optimized METs. Quantitative genetics theory in combination with computer simulation provides a powerful framework for evaluating and optimizing direct and indirect selection strategies. The relative merit of direct selection and MAS strategies is influenced by the completeness of the molecular marker description of the traits and the adequacy of the MET in providing accurate and precise information on the adaptation of breeding lines for the target population of environments.

The incidence of genotype-by-environment (G × E) interactions within the target genotype-environment system of a breeding program necessitates the use of multi-environment trials (METs) to evaluate genotype adaptation. The design of an effective multi-environment testing strategy for yield improvement, like all aspects of a breeding program, requires both a clear definition of breeding objectives and an understanding of the target genotype-environment system. Clearly, the complexity of the system will influence the nature and sophistication required in the conduct of METs and interpretation of their results. Our work on yield improvement of rainfed lowland rice in Thailand and Lao PDR has indicated a high level of complexity in this water-limited rice production system (Fukai et al 1997). Therefore, considerable attention needs to be given to the design of a MET strategy that gives accurate and precise information on the relative performance of genotypes in the target population of environments (TPE) to enable progress from selection. Recent publications by Cooper and Hammer (1996) and Kempton and Fox (1997) consider a range of issues relevant to the conduct of METs and their role in plant breeding.

Breeding programs operate over cycles of selection with the aim of developing improved genotypes for a TPE. Figure 1 provides a schematic representation of the conduct of METs within the context of a breeding program. The aspects emphasized

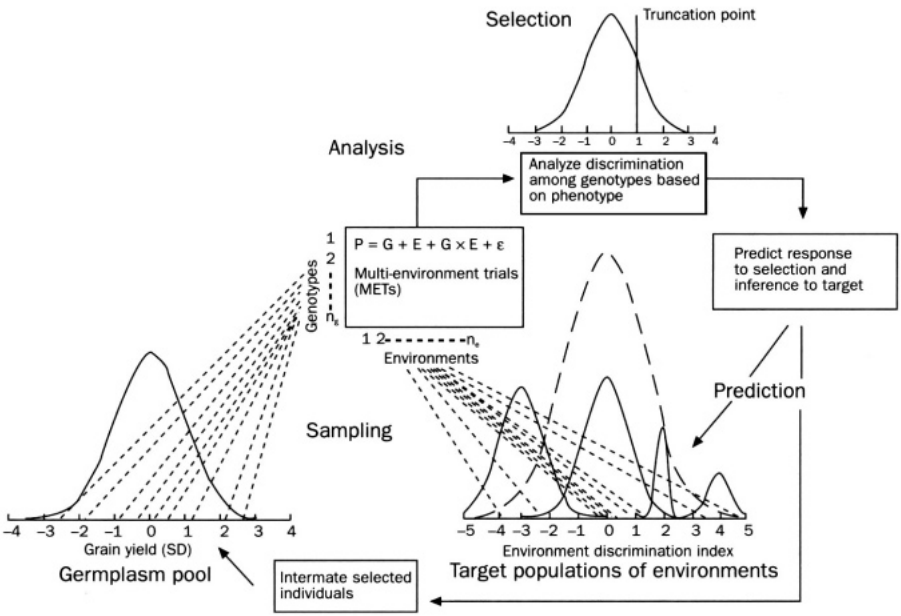

Fig. 1. Schematic structure of the role of multi-environment trials (METs) within the operation of a plant breeding program. SD = standard deviation. (Modified from Basford and Cooper 1998.)

are (1) *sampling,* the process of sampling the available germplasm and the range of environments in the TPE; (2) *analysis,* the conduct of the METs, the analysis of the results from the METs, and their interpretation, which involves an assessment of the accuracy and precision of the results; (3) *selection,* the use of the information obtained from the METs to make selection decisions; and (4) *prediction,* prediction of the performance of selected genotypes for the TPE and for further intermating and selection within the breeding program. Successful conduct of METs within a breeding program requires attention to all of these issues. Although their traditional function is to provide the breeder with data for selection, METs can also be used to identify and select for adaptive traits. Specific patterns of genetic variation observed in METs can be examined in more detail to determine their significance for improving plant adaptation. This may identify and introduce opportunities for genetic improvement by indirect selection *via* adaptive traits to complement traditional direct selection based on yield and quality results from METs. The availability of molecular markers and the scope for marker-assisted selection (MAS) have created renewed interest in the role of indirect selection strategies within plant breeding programs. But comparisons of the efficiency of marker-based selection strategies relative to conventional direct selection, using results from METs, have received limited attention to date.

The issue of selection efficiency within breeding programs can be examined in three general ways: theoretically, by simulation, and experimentally. Each approach has its own strengths and weaknesses. These methods can be used together in a complementary way to give a more comprehensive assessment of the requirements for an efficient plant breeding strategy aimed at improving the adaptation of rice to water-limited environments. The objective of this paper is to consider issues related to the efficient use of METs within a breeding program, where options for direct and indirect selection are available. The implications of these issues will be discussed in relation to current efforts to improve the adaptation of rainfed lowland rice in water-limited environments.

Theoretical issues

Quantitative genetics provides a framework for evaluating the relative efficiency of direct and indirect selection strategies within a plant breeding program. Prediction equations can be defined and used as a basis for comparisons between alternative strategies and optimization of resource allocations within a strategy. Optimization of the MET component of a breeding strategy requires joint consideration of the accuracy of discrimination among genotypes in the MET relative to that expected within the TPE and the precision by which differences in the relative performance of the genotypes are measured in the MET.

For direct selection, the heritability of the traits to be subjected to selection is fundamental. Where METs are conducted across sites for more than one year, the results can be analyzed taking into consideration the cross-classification structure of

years and sites. From the analysis of variance, components of variance can be estimated and line mean heritability *(h^2)* can be estimated as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_{gs}^2}{s} + \frac{\sigma_{gsy}^2}{ys} + \frac{\sigma_\varepsilon^2}{ysr}}$$

where σ_g^2 is the genetic component of variance, σ_{gy}^2, σ_{gs}^2, and σ_{gsy}^2 are the G × E interaction components of variance involving years, sites, and year-site combinations, respectively, σ_ε^2 is the pooled within-environment error component of variance, and y, *s,* and *r* are the numbers of years, sites, and replicates within a year-site combination, respectively. With this expression for heritability, it can be seen that the components of G × E interaction act to reduce heritability. It can also be seen that increasing replication across sites and years and within year-site combinations can increase heritability. The impact of these forms of replication will depend on the magnitude of the interaction and error components of variance. The equation also shows that heritability is increased by the use of experimental techniques and methodologies that contribute to a reduction in experimental error. This expression for heritability, and appropriate modifications of it, can be applied to prediction of response to selection for broad adaptation across the types of environments for a TPE or to selection for specific adaptation to repeatable types of environments or regions encountered within the TPE. The review article by Nyquist (1991) summarizes many of the issues associated with the estimation and use of heritability in predicting response to selection for broad adaptation to a TPE. For a treatment of selection for both broad and specific adaptation, see Cooper and Hammer (1996).

Where G × E interactions exist, a condition for any estimate of heritability to be a reliable indicator of the scope for making progress from selection is that the results must be based on a sample of environments that matches the expected mixture of "types" of environments in the TPE. Here a type of environment is defined as a set of biophysical conditions that result in a specific pattern of discrimination among genotypes. It is commonly assumed that the environments sampled in METs are a random sample from the TPE. If this were the case, the larger any such random sample were, the more likely that the MET would match the TPE. In reality, however, METs are extremely small samples from the TPE and the environments are unlikely to be a random sample. Many situations can result in METs that are mismatched with the TPE. This is a key issue in the long-standing debate on whether METs can be conducted on research stations or whether they need to be conducted on-farm.

In theory, the match between genotype discrimination in a MET and that expected in the TPE can be measured by either the genetic covariance or the genetic correlation between the discrimination among genotypes in the MET and the TPE. In practice, the performance of genotypes in the TPE is unknown. But the implications of mismatches between a MET and the TPE can be examined using the theoretical framework given by Cooper et al (1996). Consider two examples based on perfor-

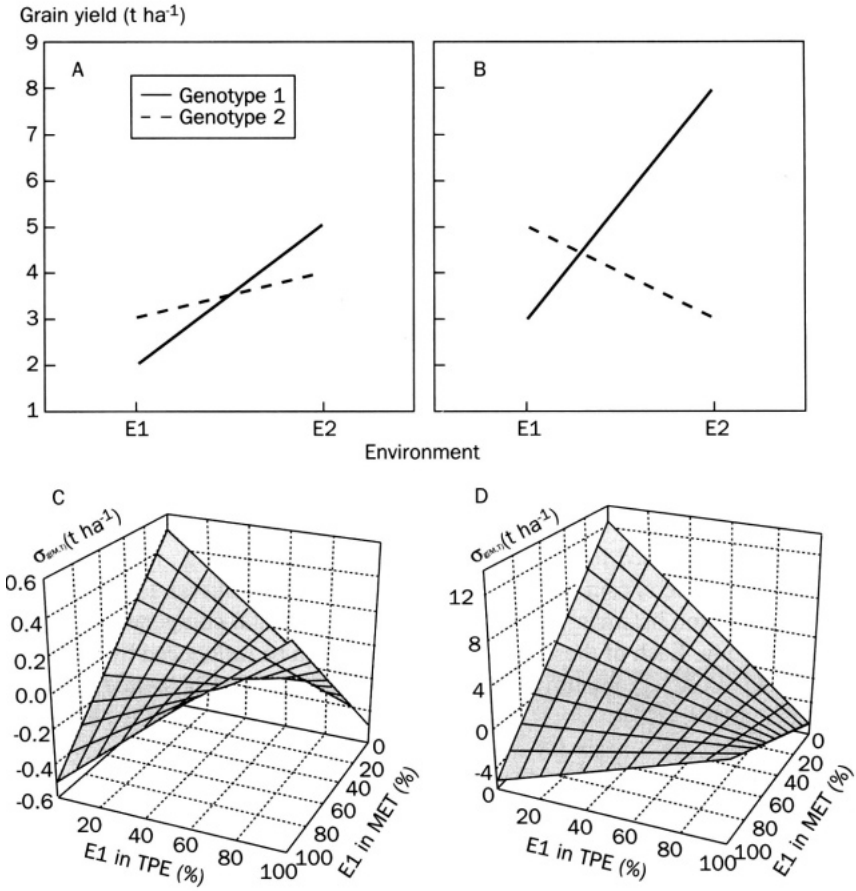

Fig. 2. Graphical representation of the influence of genotype-by-environment interactions on the genetic covariance ($s_{g(M,T)}$) between the relative performance of two genotypes in a multi-environment trial (MET) and the target population of environments (TPE) as the frequencies of the two types of environments (E1 and E2) change in the MET and the TPE. Two examples are considered: (1) where only crossover interaction exists (A, C), and (2) where a combination of crossover and heterogeneity of variance interactions exists (B, D).

mance profiles of two genotypes across two types of environments, referred to as E1 and E2 (Fig. 2). In the first example, there is only crossover interaction (Fig. 2A) and in the second there is a combination of crossover interaction and interaction because of heterogeneity of genetic variance (Fig. 2B). The relative performance of the genotypes can be computed for MET and TPE situations where different frequencies of both types of environments occur. The genetic covariance based on genotype performance can then be computed for different combinations of MET and TPE as the frequency of the environment types changes (Fig. 2C, D). A match between the MET

and TPE can be considered as a combination where the frequencies of environment types in the MET and TPE are similar and a mismatch where the frequencies diverge. For both examples, where there is sufficient divergence between the environmental composition of the MET and the TPE, there is a negative genetic covariance between genotype performance in the MET and the TPE. The graphical presentation of the results for the two genotype profiles (Fig. 2) shows that, when there is a mismatch between the MET and the TPE in the presence of crossover G × E interactions, response to selection in the TPE can be negative. This can occur even when estimates of heritability from the MET suggest that a positive response to selection is expected. A further point to note from these examples is that, even if the correct environment types are sampled in the MET, if they are in proportions different from those in the TPE, it is still possible to have a negative response to selection in the TPE. Therefore, it is not sufficient to define an environment type as relevant. It is also necessary to have an estimate of the contribution of the environment type to the TPE to determine the optimal contribution of information from the environments sampled in METs to selection decisions.

For indirect selection, the heritability of the target trait (e.g., yield) and the heritability of the trait used to implement the indirect selection (e.g., maturity, drought score, root characters, osmotic adjustment, or molecular markers), together with the genetic correlation between both traits, are the fundamental components of the prediction equation. Each of these genetic parameters can be influenced by G × E interactions. Therefore, to obtain reliable estimates, these parameters should be determined from the results of METs that sample the range of conditions expected in the TPE. This provides a basis for evaluating the contribution of any adaptive trait to yield. Where molecular markers have been identified to be polymorphic within a cross, and are associated with a trait that is to be manipulated by selection, their role in selection can be considered within an indirect selection framework. The heritability of the marker variation should be close to 1.0 when a reliable marker technology is used. The genetic correlation will be a function of the closeness of the linkage and degree of linkage disequilibrium between the marker and loci contributing to variation for the target quantitative trait, the so-called quantitative trait loci (QTLs). For yield improvement of rice in water-limited environments, these QTLs may be associated with the control of flowering time, rooting depth, osmotic adjustment, and resistance to water flow in xylem vessels.

Lande (1992) constructed a prediction equation to evaluate the relative efficiency of MAS for a quantitative trait within the context of a pedigree breeding program. For this treatment of MAS, a single target environment was considered and a selection index combining both phenotypic and marker information was used to identify situations where response from MAS was greater than that from direct selection based on phenotypic data alone. Using the equation given by Lande (1992), the expected selection response from MAS for different numbers of lines and plants within lines and different proportions of total genetic variance explained by the markers (p) can be examined. Although this framework does not explicitly deal with the effects of G × E

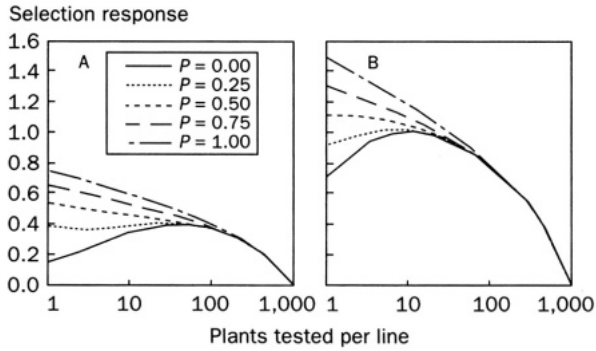

Fig. 3. Theoretical selection response per generation for selection among inbred lines in a pedigree breeding program as a function of the number of plants tested per line and the proportion of total genetic variance (p) explained by molecular markers. The selection scenario was based on a total of 50,000 plants measured and the top 50 lines selected (Lande 1992). Selection response is scaled in units of individual plant phenotypic standard deviations for the population as a whole. (A) $h^2 = 0.05$ and (B) $h^2 = 0.20$ on a single-plant basis.

interactions, some indication of their effects can be obtained by considering that they contribute to a reduction in heritability. Figure 3 shows two examples. In the first situation, the heritability on a single-plant basis is 0.05 (Fig. 3A), and the second heritability on a single-plant basis is 0.20. The first case represents the situation with the greatest amount of G × E interaction. In both cases, it is assumed that a total of 50,000 plants can be measured and that the best 50 lines are selected. Therefore, the maximum number of lines that can be examined is 50,000, with 1 plant line''. At the other extreme, 50 lines could be evaluated, with 1,000 plants line''. If 50 lines are evaluated and selected, however, no selection pressure is applied and no response is achieved. Hence, the selection response decreases to zero as the number of plants per line approaches 1,000.

We can observe several points from the examples. First, using the selection index of Lande (1992), there is an increase in response to selection from improvement of heritability from 0.05 (Fig. 3A) to 0.20 (Fig. 3B). This increase in heritability could be achieved by improved experimental technique to control environmental variation, experimental designs and analyses that adjust for the effects of environmental variation, or increased replication within and across environments in METs. Second, response to selection changes as a function of the number of plants evaluated per line. Third, response to selection improves as the proportion of genetic variance accounted for by the molecular markers increases for both levels of heritability. Fourth, response to selection can be improved in the absence of any information from molecular markers by increasing the number of plants evaluated within a line from one to some optimum for both levels of heritability. Fifth, there are situations where selection on phenotypic information alone (i.e., $P = 0$) can achieve a greater selection response than the combined phenotype-marker index (i.e., P >O). For example, with a herita-

bility of 0.20, a greater response to selection is achieved for $P = 0.0$ when phenotypic evaluation is based on 10 plants line^{-1} compared with $P = 0.25$ when 1 plant line^{-1} is evaluated (Fig. 3B). It is clear from this theoretical treatment that the design of MAS strategies cannot be considered in isolation from the design of METs. These issues are examined further by computer simulation below.

There is a long history of attempts to use physiological principles as a basis for guiding the genetic improvement of complex traits such as grain yield. Adaptation to water-limited environments has been of particular interest. Jackson et al (1996) evaluated the role of physiological understanding of adaptation in plant breeding within an indirect selection framework. They identified three main areas where physiological understanding can contribute to breeding programs: (1) identification of types of target environments for effective selection in core breeding programs, (2) identification of traits that could be used as indirect selection criteria in core breeding programs, and (3) identification of traits as selection criteria for introgression programs. Physiological approaches to breeding have generally concentrated on the second and third of these areas, with only limited emphasis on the first. It is in the area of defining target environments and designing appropriate METs, however, that there is likely to be the greatest scope for improving the efficiency of breeding rice for water-limited environments.

Computer simulation

Investigations into the efficiencies of using MAS in breeding programs should be considered in combination with the design of METs. A theoretical argument for this was given above. But many issues relevant to breeding strategy are difficult to investigate within this theoretical framework. In such situations, investigation by computer simulation can be a powerful tool to extend the theoretical treatment of the problem (Podlich and Cooper 1998). In this section, we use a computer simulation methodology to consider two issues: (1) the power of experiments to detect QTLs contributing to the traits that are to be manipulated in a breeding program and (2) the impact of G × E interactions and the size of METs on response from MAS.

Beavis (1994) examined the influence of heritability and the size of plant population on the power of experiments to detect QTLs contributing to a quantitative trait (Fig. 4). The power of the experiments was measured as the frequency with which QTLs were correctly identified from 200 simulations. Using simulation methodology, Beavis considered a quantitative trait controlled by either 10 or 40 QTLs. Segregation for a diploid genome within an F_2 population was simulated for three levels of heritability on a single-plant basis (0.30, 0.63, 0.95) and three population sizes (100, 500, and 1,000 plants). Beavis (1994) found a strong interaction between heritability and population size for the power of the experiments. With low heritability and small population sizes (100 to 500 individuals), the experiments had limited power to detect QTLs. Even when the heritability was increased to 0.95, with a population size of 100, the experiment was still weak in detecting QTLs. For the genetic model based on 10 QTLs, the combination of a population size of 500 individuals and heritability of

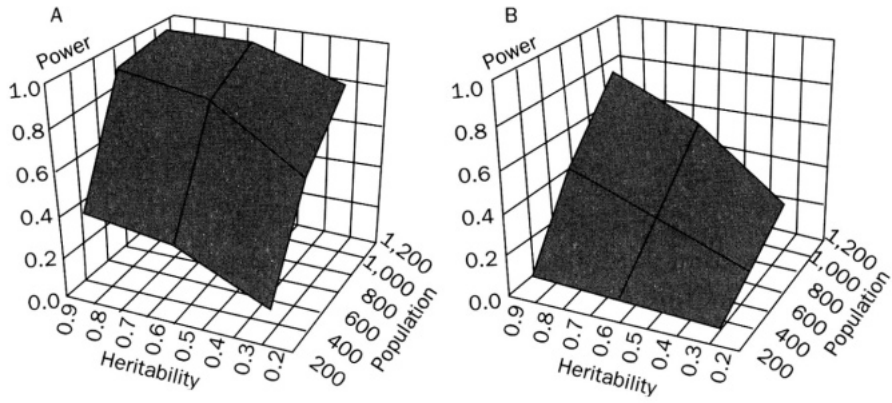

Fig. 4. The results of Beavis (1994) on the simulated power of a QTL experiment to correctly identify known QTLs in an F_2 population as a function of individual plant heritability and population size for quantitative trait models based on (A) 10 QTLs and (B) 40 QTLs.

0.63 was powerful enough to reliably detect 86% of the QTLs. But this combination was weak for the genetic model based on 40 QTLs, detecting only 29% of them. For the more complex genetic model, the combination of a large population (1,000 individuals) and a high heritability was required to detect 77% of the QTLs. Experimental evaluation of these findings by Pioneer Hi-Bred International, in an experiment based on 976 F_5 maize testcrosses evaluated across 19 environments, confirms the results of the simulation experiments conducted by Beavis (Dr. S. Openshaw, Pioneer Hi-Bred International, 1998, personal communication). A significant observation was that small METs based on 100 to 250 lines tested in a limited number of environments were unreliable in detecting QTLs and that the effects estimated for the QTLs were generally biased up.

These results have strong implications for the conduct of METs that generate phenotypic data to be used in linkage studies as the basis for detecting QTLs for MAS in breeding. For quantitative traits, even those controlled by a modest number of genes (5 to 10 QTLs), large METs with substantial numbers of lines and replications across environments will be required to sample the genetic variation and achieve the level of heritability necessary to reliably characterize the architecture of these traits. Beavis and Keim (1996) considered several of the issues associated with the analysis of METs to take into account QTL × E interactions.

The QU-GENE software (Podlich and Cooper 1998) was used to conduct a simulation experiment that examined the joint impact of G × E interactions and size of MET on response to selection from three selection strategies: (1) selection on phenotypic information alone (PS), (2) selection on molecular marker information alone (MS), and (3) an optimized index combining phenotypic and marker information (i.e., MAS). The breeding scenario considered was analogous to the pedigree breed-

ing strategy considered theoretically by Lande (1992) and discussed above. A quantitative trait controlled by 50 independent QTLs of equal additive effect was simulated. The extent to which the molecular markers were associated with the QTLs was varied in two ways: (1) by manipulating the number of QTLs identified by flanking molecular markers, either 25 or all 50 QTLs identified, and (2) by manipulating the tightness of the linkage between the molecular markers and the QTLs, measured as recombination frequencies of 0.0 (perfect association), 0.05, 0.20, and 0.40 (weak association). Two G × E interaction scenarios were considered: (1) no systematic G × E interaction in the TPE, and (2) systematic G × E interaction in the TPE, such that the crossover G × E interaction component of variance was approximately 2.5 times that of the genotypic component of variance.

In our rainfed lowland rice METs, we have observed the interaction component of variance for grain yield to be up to four to six times the genetic component of variance (Cooper and Somrith 1997, Fukai and Cooper 1999). Heritability on a single-plant basis was assumed to be 0.20 in the situation with no G × E interaction. The within-experiment environmental component of variance was assumed to be N (0, σ^2). An estimate of σ^2 was obtained from the scenario with no G × E interaction and used throughout the simulation experiment. Two sizes of MET were considered: (1) ope environment sampled at random from the TPE, and (2) 10 environments sampled at random from the TPE. The pedigree breeding strategy commenced by crossing two homozygous parents with the favorable alleles for the 50 QTLs dispersed equally between the parents. The parents were used to generate an F_1 that was self-pollinated to produce an F_2 population. Selection was conducted among F_2-derived lines in the F_3 generation. Following Lande (1992), it was assumed that 50,000 plants could be measured phenotypically and by markers. The top SO F_2-derived lines were then selected. Therefore, some comparisons could be made between the results of the simulation experiment and the theoretical treatment given by Lande (Fig. 3).

For the MET based on a single environment, the number of F2-derived lines ranged between 50,000 (1 plant line^{-1}) and 50 (1,000 plants line^{-1}). For the MET based on 10 environments, the number of F_2-derived lines ranged from 5,000 (10 plants line^{-1} and 1 plant environment^{-1}) to 50 (1,000 plants line^{-1} and 100 plants environment^{-1}). For each combination of treatments, 250 simulations were conducted and the mean result examined. The response to selection was examined for the three forms of selection (PS, MS, and MAS) as a function of the number of plants sampled per line (single-environment MET) or number of plants per environment (10-environment MET). To assist comparisons between genetic models based on different levels of G × E interaction, response to selection was scaled as a percentage of the performance of the best possible genotype, possessing all favorable QTLs (target genotype) that could be produced by recombining the genes from the two parents.

For the case with no systematic G × E interactions, the response to selection varied in relation to the number of plants tested per line (Fig. 5A) in a manner comparable to the theoretical predictions (Fig. 3). Therefore, as with the theoretical treatment, in the absence of molecular marker information, increasing the replication from

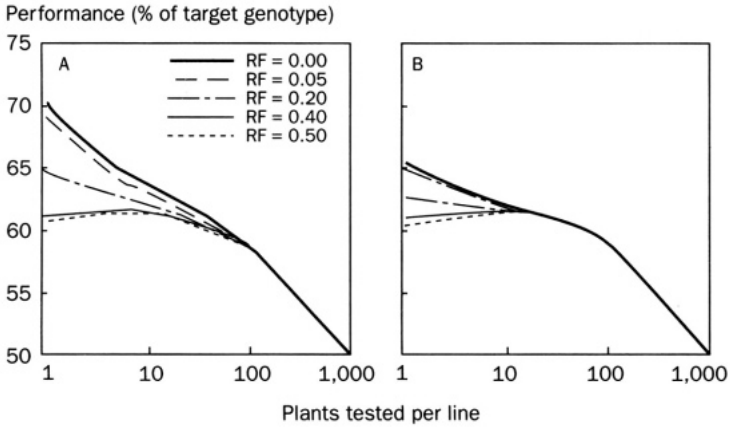

Fig. 5. Simulated selection response per generation for a quantitative trait controlled by 50 QTLs, plotted as a function of the number of plants tested per line, the recombination frequency (RF) between flanking molecular markers and the QTLs, and the number of QTLs identified by molecular markers: (A) all 50 QTLs, (B) 25 QTLs. Selection was among F_2-derived inbred lines in the F_3 generation of a pedigree breeding program. The selection scenario was based on a total of 50,000 plants measured and the top 50 lines selected (Lande 1992). Selection response is scaled as a percentage of the best possible response that can be achieved from recombining the 50 QTLs; 50% represents no response and 100% the maximum response.

one plant to an optimal number contributed to an increase in the response to selection. Maximum response for selection based on phenotype alone resulted from replication based on 10 plants line^{-1} (Fig. 5A). As the linkage between the markers and the QTLs strengthened, MAS provided an opportunity to increase the response to selection over phenotypic selection when low levels of replication were used to evaluate the lines. When only 25 of the QTLs were identified (Fig. 5B), the improvement in response over phenotypic selection by MAS was less than when all QTLs were identified. Evaluation of MAS where the quantitative traits are only partially explained by markers is a more realistic scenario given our current understanding of the inheritance of quantitative traits.

For the quantitative trait model incorporating G × E interactions, two situations were considered: (1) where all QTLs were associated with markers (Fig. 6), and (2) where half of the QTLs were identified by markers (Fig. 7). Response to selection was maximized by MS and MAS selection when all QTLs were associated with tightly linked markers and the 50,000 lines were evaluated in a single environment (Fig. 6A). Evaluation of the 50,000 lines in a single environment was also the situation where phenotypic selection was the least effective. Therefore, the difference between MAS and phenotypic selection was the greatest for this situation. As expected, conducting a MET based on 10 environments increased the response achieved by phenotypic selection (Fig. 6B). But this necessitated sampling fewer lines and the effectiveness of both the MS and MAS strategies decreased. Clearly, with such a strong char-

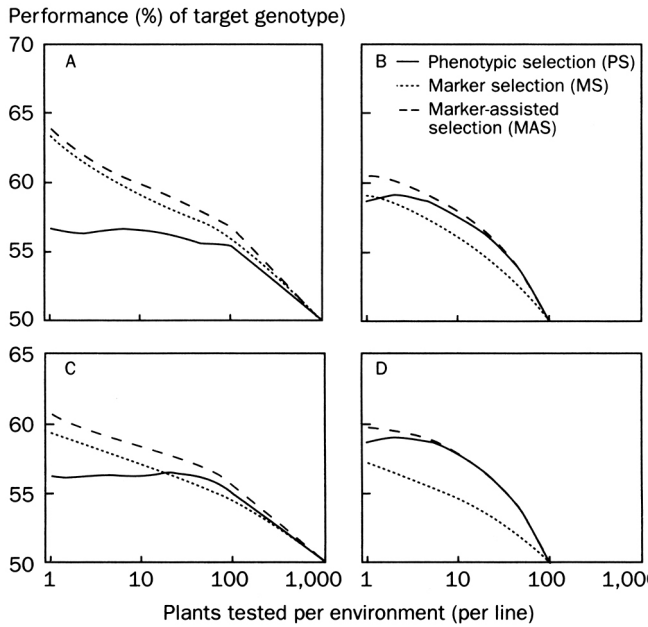

Fig. 6. Simulated response per generation for selection on phenotype alone (PS), molecular markers alone (MS), and marker-assisted selection (MAS) for a quantitative trait controlled by 50 QTLs influenced by genotype-byenvironment interactions, plotted as a function of the number of plants tested per line. All 50 QTLs were associated with molecular markers and the recombination frequency (RF) between the flanking molecular markers and the QTLs was either 0.05 (A, B) or 0.20 (C, D). The multienvironment trial (MET) was based on 1 (A, C) or 10 (B, D) environments. Selection response is scaled as a percentage of the best possible response that can be achieved from recombining the 50 QTLs; 50% represents no response and 100% the maximum response.

acterization of the trait by markers, there was no advantage to either MS or MAS from conducting a more extensive MET, whereas there was the expected advantage for phenotypic selection. Such a comprehensive description of the QTLs contributing to a quantitative trait may be considered as an ideal to aim for if MAS is to be at its most effective. For most situations in plant breeding, however, we are nowhere near this ideal. Therefore, it is instructive to examine the relative effectiveness of the three selection strategies for a MET based on 1 or 10 environments as we reduce the adequacy of the molecular marker description of the trait.

When the strength of linkage between the markers and the QTLs was decreased by increasing recombination frequency from 0.05 (Fig. 6A, B) to 0.20 (Fig. 6C, D), there was a reduction in the response to selection from both the MS and MAS strategies. As expected, response from phenotypic selection was unaffected. For evaluation of lines in a single environment, however, both the MS and MAS strategies were superior to phenotypic selection for all levels of replication, with a slight advantage

of MAS over MS (Fig. 6C). The conduct of a MET based on 10 environments contributed to a further reduction in the effectiveness of the MS and MAS strategies (Fig. 6D). This, combined with the increase in response from phenotypic selection, resulted in phenotypic selection being comparable to MAS and superior to MS selection, except at the lowest levels of replication where MS and MAS still had an advantage over phenotypic selection.

When tight linkage was achieved for half of the QTLs contributing to the trait, response to selection was maximized with MAS in combination with low levels of replication in a single environment (Fig. 7A). There was a slight advantage of MAS over the MS strategy. Evaluating lines in the 10-environment MET improved phenotypic selection over the MS strategy and made it comparable with MAS, except at the lowest levels of replication (Fig. 7B). The relative merit of the three selection strategies changed when the linkage between the molecular markers and QTLs was reduced (Fig. 7C, D). There was a slight advantage for MAS based on the 10-environ-

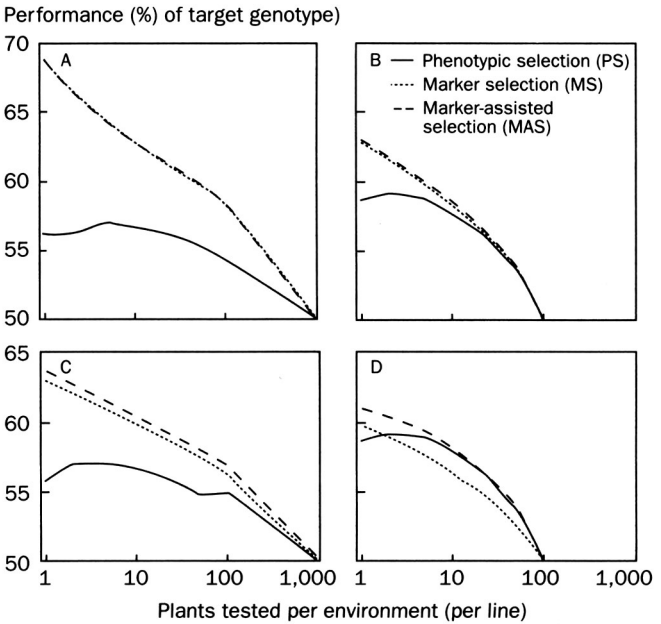

Fig. 7. Simulated response per generation for selection on phenotype alone (PS), molecular markers alone (MS), and marker-assisted selection (MAS) for a quantitative trait controlled by 50 QTLs influenced by genotype-by-environment interactions, plotted as a function of the number of plants tested per line. Twenty-five of the 50 QTLs were associated with molecular markers and the recombination frequency (RF) between the flanking molecular markers and the QTLs was either 0.05 (A, B) or 0.20 (C, D). The multi-environment trial (MET) was based on 1 (A, C) or 10 (B, D) environments. Selection response is scaled as a percentage of the best possible response that can be achieved from recombining the 50 QTLs; 50% represents no response and 100% the maximum response.

ment MET over the single-environment MET. This was apparent with replication levels of 1 to 10 plants environment^{-1} for the 10-environment MET (10 to 100 plants total) (Fig. 7D) when compared with the same levels of replication in the single-environment MET (10 to 100 plants) (Fig. 7C). Both phenotypic selection and MAS were superior to the MS strategy when a 10-environment MET was conducted (Fig. 7D). This latter situation, where half of the QTLs were associated with markers and intermediate linkage levels were achieved, is probably still better than most of the situations that exist for the quantitative traits we are working with in breeding programs. These results emphasize the clear need for the design of MAS strategies that jointly consider the quality of the molecular marker description of the traits and the design of METs. Simulation analyses, such as those conducted for the example considered here, can serve as a basis for identifying target levels of molecular marker characterization of quantitative traits that will be necessary if benefits from MAS are to be realized.

Experimental results

Fukai and Cooper (1995) used an indirect selection framework to define target environments for rainfed lowland rice and evaluate putative traits for improving drought tolerance. In general, matching phenology with the water availability of an environment has a major impact on yield variation and G × E interactions for yield of rice in water-limited environments. Yield variation among genotypes with similar phenology, however, indicates genetic variation for adaptation to water-limited environments that could be exploited to improve the drought tolerance of rainfed lowland rice (Cooper and Somrith 1997, Fukai and Cooper, 1999). Breeding programs need to exploit this latter source of genetic variation. We have found that genetic variation for ability to maintain a high leaf and panicle water potential is associated with significant variation in adaptation to water-limited environments (Jongdee et al 1997, Fukai and Cooper 1999).

The strong influence on grain yield of both genetic variation for flowering time and environmental variation in timing of drought development necessitates particular attention to characterizing the water balance of environments sampled in rainfed lowland rice METs. For example, in 1997 at the Chum Phae drought-screening facilities in northeast Thailand, a severe terminal water deficit developed (Fig. 8). The 450 experimental lines, sampled at random from 7 breeding populations, and the check cultivars flowered between 80 and 135 days after sowing (DAS). There was no standing water in the paddy during the majority of this period. A rainfall event reduced the water deficit in the paddy between 80 and 90 DAS (Fig. 8A). Grain yield was strongly influenced by the flowering time of the lines (Fig. 8B). The earliest flowering lines (80 to 85 DAS) and those flowering after 95 DAS generally had a lower yield than those flowering between 85 and 95 DAS. Those lines flowering in this period would have obtained some relief from the water deficit because of the coincidence of flowering with the significant rainfall event. Selection for grain yield alone would emphasize early flowering lines in this trial.

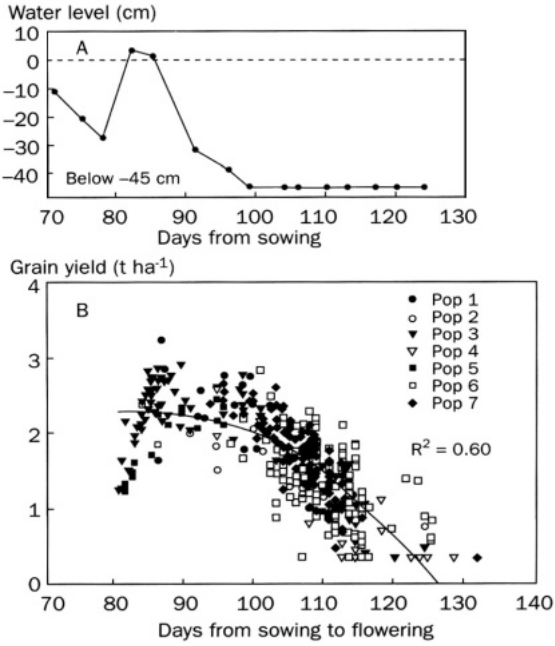

Fig. 8. Grain yield variation among rainfed lowland rice breeding lines from seven populations at the Chum Phae drought screening facilities in 1997: (A) the water level in the paddy relative to the soil surface as a function of days from sowing; (B) grain yield of individual lines as a function of days from sowing to flowering.

We have consistently observed this dominating influence of flowering time on yield of rainfed lowland rice in breeding METs (Henderson et al 1996, Cooper and Somrith 1997, Fukai and Cooper 1999). Although the influence of flowering time on yield was strong, there was still significant variation for grain yield among lines flowering at a similar time. Unless the analysis of yield variation in trials such as these takes into account the nonlinear effects of flowering time and timing of water deficit, it will be difficult to make genetic progress for grain yield from direct selection. Appropriate adjustments can be made by either separating breeding lines into separate flowering time groups or by statistically adjusting for the effects on yield of variation in flowering time prior to selection. We would recommend a combination of both practices. Clearly, it is critical that selection for yield should take into account the effects of variation in plant developmental patterns and in particular genetic control of variation in flowering time. At the same time, a practical strategy is required. Table 1 summarizes the process that we would recommend for the analysis and interpretation of the results from METs. Table 2 summarizes the selection targets for the drought-screening nursery at Chum Phae.

Table 1. Considerations for the analysis and interpretation of genotype-by-evironment (G × E) interactions in terms of their application to selection in plant breeding. The three levels of activity under the three applications categories—analysis methods, objectives of analysis, and selection strategy—should be interpreted in a cumulative manner as the forms of G × E interaction within the genotype-environment system are understood with increasing levels of detail and the likelihood of the patterns of G × E interaction being repeatable is quantified. (Modified after DeLacy et al 1996.)

Form of G × E interaction	Application in plant breeding		
	Analysis methods	Objectives of analysis	Selection strategy
Nonrepeatable	Analysis of variance by residual maximum likelihood (REML). Best linear biased predictors (BLUPs) of line performance.	1. Estimate components of variance to determine the relative sizes of sources of variation and estimate heritability. 2. Characterize the form of G × E interactions by examining them for: (a) Heterogeneity and lack of correlation partition. (b) Rank change and no rank change partition. (c) The impact of rank change on the composition of the select group of genotypes.	Selection for broad adaptation. Decisions on MET size; i.e., how many test environments (years, sites, specific screens), replicates, and genotypes to use. Appropriate experimental designs.
Mixture of repeatable and nonrepeatable	Indirect selection relationships among types of environments. Pattern analysis (combined use of cluster analysis and ordination methods).	3. Relationships among environments measured in terms of genetic correlations and indirect response to selection. 4. Grouping, ordination, and partitioning of G × E interaction for groups of genotypes and environments. 5. Investigation of the causes of differences in patterns of adaptation. 6. Environmental characterization to identify types of environments sampled in the MET and their relevance to the TPE.	Selection for broad adaptation and specific adaptation to types of environments.
Repeatable	Biophysical models	7. Interpretation of the biophysical basis of G x E interactions.	Selection for specific adaptation in combination with broad adaptation.

Table 2. Selection targets for improvement of grain yield of rainfed lowland rice, within a flowering time (maturity) group, against late-season drought using the Chum Phae drought-screening facilities in northeast Thailand.

Water regime	Target traits
Irrigated conditions	High potential yield with • High harvest index • Intermediate height Small dry matter at anthesis
Rainfed conditions with drained water prior to anthesis	Minimal delay in flowering Maintenance of favorable plant water status • Visual scoring for green leaf retention (drought score) • Reduced spikelet sterility

Source: Fukai and Cooper (1999).

An important consideration in progressing from basic to more sophisticated levels of analysis and interpretation of results from METs is whether any of the observed G × E interactions are likely to be repeatable (Table 1). If any are considered repeatable, then there is an expectation that specific types of environments observed in METs will be encountered at other sites or in future years and that the specific patterns of genetic variation associated with the types of environments are relevant to achieving breeding objectives. Where interactions are considered to be nonrepeatable, then selection for broad adaptation would be appropriate. Where interactions are considered to be a mixture of repeatable and nonrepeatable events, we need to consider the nature of the interactions and selection for a combination of broad and specific adaptation. Here, the relationships among the alternative analytical methodologies need to be appreciated (Cooper and DeLacy 1994, Cooper et al 1996). For situations where the patterns of adaptation are considered to be highly repeatable, detailed analysis by a combination of pattern analysis and biophysical models of plant adaptation would be warranted.

Our experience with rainfed lowland rice METs in northeast Thailand and Lao PDR suggests that the observed G × E interactions for yield in water-limited environments are the result of a complex mixture of repeatable and nonrepeatable events. The influence of flowering time can be accommodated by stratifying the breeding lines on maturity and appropriate analysis of results. Specific adaptation associated with repeatable G × E interactions has been associated with the ability of lines to maintain high leaf and panicle water potential under water deficit. Lines able to maintain high panicle water potential suffered less delay in flowering and had a higher proportion of fertile panicles and spikelets, more filled grains, and a higher grain yield. Detailed experiments on a more limited set of lines indicated that genotypic variation for maintenance of leaf water potential was related to plant size and resistance to water flow within the plants. Other traits we have examined that were not related to a genotype's ability to produce higher yield under water-limited conditions

are osmotic adjustment and the root characters root mass density, maximum root depth, and root-pulling resistance. To date, we have only examined these traits in a limited set of water-deficit conditions and for a specific reference genotype population. If there are any specific adaptation advantages associated with these traits, they may be associated with G × E interactions we have yet to encounter.

A common problem in breeding METs is the large component of experimental error. These errors act to reduce heritability and therefore response to selection. Much work has been done on developing experimental designs and analytical methodologies to adjust for systematic environmental variation within experiments. Basford et al (1996) gave an overview of these techniques and discussed their application in the analysis of METs. The impact of adjusting for the spatial environmental variation encountered in rainfed lowland rice experiments is demonstrated for four environments sampled as part of a MET conducted in northeast Thailand in 1997 (Fig. 9). Each environment had a change in the ranking of the lines following adjustment of the best linear unbiased predictors (BLUPs) for spatial variation within the paddy.

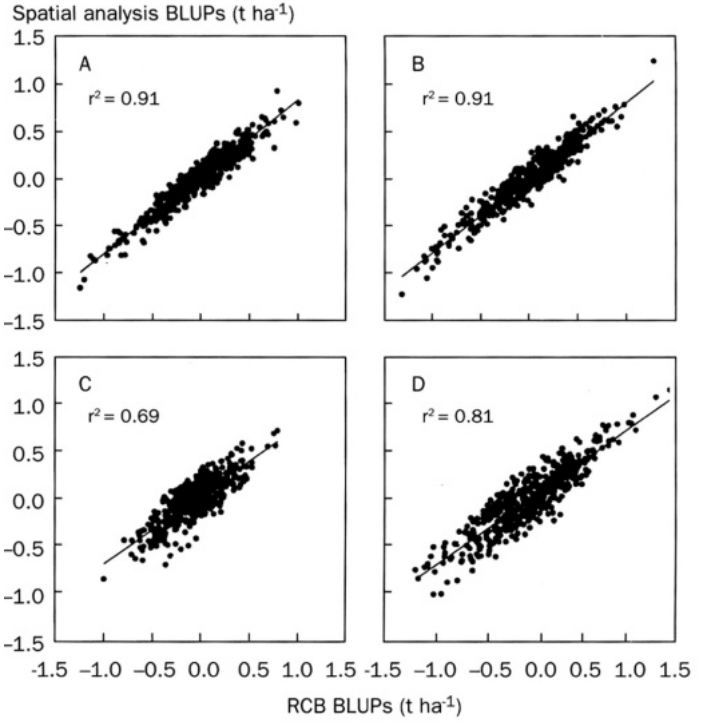

Fig. 9. Association between the best linear unbiased predictors (BLUPs) computed on a randomized complete block model (RCB) and following spatial analysis for four sites in northeast Thailand in 1997: (A) Khon Kaen, (B) Phimai, (C) Sakon Nakhon, and (D) Ubon Ratchathani.

The adjustment was more pronounced at the Sakon Nakhon (Fig. 9C) and Ubon Ratchathani sites than at the Khon Kaen (Fig. 9A) and Phimai (Fig. 9B) sites. This example demonstrates that the spatial variation within the paddies used to conduct rainfed lowland rice experiments is sufficient to have significant effects on the selections taken from an experiment.

Conclusions

The design of METs for a breeding program is based on the need to make accurate and precise predictions about the adaptation of breeding lines in a TPE. With the improvements that have been made in experimental design and analysis, together with our increasing understanding of the causes of G × E interactions, we can do much to improve selection efficiency based on the phenotypic information generated from METs. The central role of METs as a source of information on genotype adaptation in the TPE is emphasized in Figure 1. With the objective of prediction underpinning the design of METs, the optimization of their design requires attention to the effects of G × E interactions across all facets of the breeding program. The strategy we are recommending for rainfed lowland rice breeding in Thailand and Lao PDR is a shift away from intense early generation selection at a single site (intrastation trials) to early generation METs across sites for at least 2 yr (interstation testing) (Fukai and Cooper 1999). The results from these METs are combined with information from the targeted drought-resistance screening at Chum Phae. This strategy will improve the accuracy and precision of the evaluation of breeding lines over the current strategy and increase the opportunities for Thai and Lao rice breeders to identify broadly adapted lines and at the same time select for any repeatable specific adaptations observed.

The availability of molecular marker methodologies and their applications to the understanding of the inheritance of putative drought-resistance traits in rice provide opportunities to further improve the effectiveness of breeding programs. But we need to avoid certain traps. Quantitative genetics and selection theory provide a framework for both (1) identifying and quantifying the opportunities that exist with our current capabilities, and (2) defining the necessary targets to aim for if the potential of MAS is to be realized.

References

Basford KE, Cooper M. 1998. Genotype × environment interactions and some considerations of their implications for wheat breeding in Australia. Aust. J. Agric. Res. 49: 153- 174.

Basford KE, Williams ER, Cullis BR, Gilmour A. 1996. Experimental design and analysis for variety trials. In: Cooper M, Hammer GL, editors. Plant adaptation and crop improvement. Wallingford (UK): CAB International in association with IRRI and ICRISAT. p 125-138.

Beavis WD. 1994. The power and deceit of QTL experiments: lessons from comparative QTL studies. In: Proceedings of the Forty-Ninth Annual Corn and Sorghum Industry Research Conference. Washington, D.C. (USA): American Seed Trade Association. p 250-266.

Beavis WD, Keim P. 1996. Identification of quantitative trait loci that are affected by environment. In: Kang MS, Gauch HG Jr., editors. Genotype-by-environment interaction. New York (USA): CRC Press. p 123-149.

Cooper M, DeLacy IH. 1994. Relationships among analytical methods used to study genotypic variation and genotype-by-environment interaction in plant breeding multi-environment experiments. Theor. Appl. Genet. 88:561-572.

Cooper M, DeLacy IH, Basford KE. 1996. Relationships among analytical methods used to analyse genotypic adaptation in multi-environment trials. In: Cooper M, Hammer GL, editors. Plant adaptation and crop improvement. Wallingford (UK): CAB International in association with IRRI and ICRISAT. p 193-224.

Cooper M, Hammer GL. 1996. Plant adaptation and crop improvement. Wallingford (UK): CAB International in association with IRRI and ICRISAT. 636 p.

Cooper M, Somrith B. 1997. Implications of genotype-by-environment interactions for yield adaptation of rainfed lowland rice: influence of flowering date on yield variation. In: Fukai S, Cooper M, Salisbury J, editors. Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held at Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research. p 104-114.

DeLacy IH, Cooper M, Basford KE. 1996. Relationships among analytical methods used to study genotype-by-environment interactions and evaluation of their impact on response to selection. In: Kang MS, Gauch HG Jr., editors. Genotype-by-environment interaction. New York (USA): CRC Press. p 51-84.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

Fukai S, Cooper M. 1999. Plant breeding strategies for rainfed lowland rice in northeast Thailand. In: Horie T, Geng s, Amano T, editors. World food security and crop production technologies for tomorrow. (In press.)

Fukai S, Cooper M, Salisbury J, editors. 1997. Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held at Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research. 248 p.

Henderson SA, Fukai S, Jongdee B, Cooper M. 1996. Comparing simulation and experimental approaches to analysing genotype by environment interactions for yield in rainfed lowland rice. In: Cooper M, Hammer GL, editors. Plant adaptation and crop improvement. Wallingford (UK): CAB International in association with IRRI and ICRISAT. p 443-464.

Jackson P, Robertson M, Cooper M, Hammer G. 1996. The role of physiological understanding in plant breeding; from a breeding perspective. Field Crops Res. 49:11-37.

Jongdee B, Fukai S, Cooper M. 1997. Genotypic variation for water relations and growth during the vegetative stage among six rice lines contrasting in maintenance of high leaf water potential. In: Fukai S, Cooper M, Salisbury J, editors. Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held at Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research. p 180-191.

Kempton RA, Fox PN, editors. 1997. Statistical methods for plant variety evaluation. London (UK): Chapman and Hall. 191 p.

Lande R. 1992. Marker-assisted selection in relation to traditional methods of plant breeding. In: Stalker HT, Murphy JP, editors. Plant breeding in the 1990s. Wallingford (UK): CAB International. p 437-451.

Nyquist WE. 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10:235-322.

Podlich DW, Cooper M. 1998. QU-GENE: a simulation platform for quantitative analysis of genetic models. Bioinformatics 14:632-653.

Notes

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Improving rice for drought-prone upland environments

B. Courtois and R. Lafitte

Drought is the main cause of yield instability in upland rice. But this general statement has to be put into perspective. Four different production systems of upland rice (shifting cultivation, integrated rice-based, perennial-based, and cash-based) are encountered in Asia. They correspond to three different agroecological zones with different risks of drought and sets of additional specific constraints. We define different breeding priorities for the different production systems and present the available germplasm in the light of improvement of resistance to water deficit. We discuss some important points for upland rice improvement, notably the strong G × E interactions and their implications for decentralization of breeding work, and the tradeoff between productivity and stability. More specifically, we present the current breeding strategy for drought resistance, highlighting the difficulties involved in establishing relevant drought conditions and choosing relevant selection criteria. We emphasize the use of molecular markers to improve selection efficiency. We chose to focus on improving individual traits rather than on performance under stress. We are developing near-isogenic lines by introgressing QTLs for a structural root system and osmotic adjustment into elite backgrounds. This approach allows us to reconcile the need for rapid impact and the testing of physiological hypotheses on the value of the various traits.

Upland rice in Asia: target zones and breeding priorities

Upland rice is an important crop in Asia and occupies about 12 million hectares (IRRI 1997). The conditions for upland rice production in Asia are highly diverse. Our main entry point to structure and understand this diversity is through production systems. The two driving forces that determine the nature of production systems are population pressure and market integration (Pandey 1996). Increasing population pressure through natural growth and/or migration pushes farming systems to become more intensive and sedentary. Increasing market access moves the systems from a subsistence orientation toward more commercial production of nonrice crops. Based on these two parameters, we generally identify four production systems (Table 1): shifting cultivation, integrated rice-based, perennial-based, and cash-based systems.

Another way to structure the upland ecosystem is to use an agroecological characterization that determines the potential and constraints of the zones. Because physical and human factors are not independent, each agroecological zone corresponds to a dominant mode of exploitation of the environment. This association is not perfect

Table 1. Characterization of upland rice production systems and breeding priorities.

Production system	Shifting cultivation	Integrated rice-based	Extensive perennial-based	Intensive cash-based
Market integration	Low	Low	High	High
Population pressure	Low	High	Low	High
Proportion of total upland rice area and trend	14% (↘)	70% (→)	14% (↗)	2% (↗)
Location	Northeast India, north Myanmar, Lao PDR, north Vietnam, north Thailand, South China (Yunnan), Indonesia (Sumatra, Irian Jaya, Kalimantan, Sulawesi)	Eastern India, Bangladesh	Indonesia (Sumatra, Kalimantan, Sulawesi), south Vietnam (Hauts Plateaux)	North Thailand, south Philippines (Mindanao)
Major agroecologies	Hilly subhumid and equatorial	Dry	Equatorial	Hilly subhumid and equatorial
Varietal types: Traditional (T) Improved (I)	T = tropical japonica (group 6), e.g., Vieng in Lao PDR, Klemas and Arias in Indonesia; I = no improved varieties	T = aus types (group 2), e.g., Gora types in India; I = indica (group 1), Kalinga III, or intermediate aus/ indica (e.g., Vandana)	T = tropical japonica; I = indica (e.g., Danau Laut Tawar in Indonesia); japonica (e.g., Way Rarem, Jatiluhur) or intermediate indica × japonica	T = tropical japonica (e.g., Dinorado in the Philippines) or indica; I = indica (e.g., UPL Ri-5)
Breeding targets	Priority 2	Priority 1	Priority 1	Priority 2
Major breeding priorities	Yield stability Drought tolerance (intermittent stresses) Interference with weeds	Yield stability Drought tolerance (terminal + intermittent drought stresses) Interference with weeds Productivity under low inputs Grain quality for local consumption	Productivity under moderate level of inputs Disease resistance (blast, sheath blight) Shade tolerance Adaptation to nutrient-deficient acid soils Grain quality for marker needs	Productivity under moderate to high inputs Grain quality for market need (aroma, pericarp color, amylose content, etc.)

Table 2. Characteristics of the three major agroecological zones of upland rice production.

Agroecological zone	Aus	Hilly subhumid	Equatorial humid
Location	Eastern India (Assam, Bihar, Orissa, Madhya Pradesh, Uttar Pradesh, West Bengal), Bangladesh	Northeast India, north Myanmar, Lao PDR, north Vietnam, north Thailand, South China (Yunnan), Indonesia	Indonesia (Sumatra, Kalimantan), Malaysia, south Vietnam (Hauts Plateaux), south Philippines (Mindanao)
Latitude	30° to 20°N	30° to 15°N	15ºN to 5°S
Longitude	80° to 95°E	90° to 110°E	95° to 125°E
Elevation (m)	100–150	300–2,000	300–1,000
Slope	Gently rolling	Steep: up to 60%	Gently rolling
Rainfall (mm)	800–1,400	1,200–3,000	>2,500
Length of rainy season	3 mo	4–5 mo	>5 mo
Rainfall pattern	Monomodal	Trend to bimodality	Monomodal
Risk of drought	High	Moderate	Moderate to low
Type of drought	Terminal + intermittent	Intermittent	Intermittent
Drought intensity (wk)	4–5	2–3	1–2
Light intensity during cropping season	Moderate	Moderate	Low
Temperature during cropping season	Not limiting	Cold problems above 600 m	Not limiting
Soil pH	5.5–6.5	5.0–6.0	3.5–5.0, locally >6.0
Soil fertility	Very poor	Medium, locally good	Poor, locally good
Potential for high yield	Limited	Good	Limited, locally very good

because of socioeconomic differentiation among upland farmers, but it gives a fair idea of the realities. Three zones are usually recognized on the basis of climate, soil conditions, and slope (Table 2):

1. The hilly subhumid areas of mainland Southeast Asia, where shifting cultivation and slash-and-burn systems are dominant,
2. The dry plateau areas of eastern India and Bangladesh, where rice is grown under permanent integrated systems, and
3. The equatorial humid areas of Indonesia, south Vietnam, and south Philippines, where perennial-based systems are found in unfavorable areas and cash-based systems in areas with better soils.

Shifting cultivation

This traditional production system is sustainable in situations with low population pressure. It is a subsistence system with no inputs, relying on a long fallow period to

reestablish soil fertility. Under increasing population and land pressure, the system exceeds its carrying capacity and starts to have a negative impact on the environment (erosion in the watershed and deforestation). For this reason, in several Southeast Asian countries, government policies have been implemented to reduce this system's extent (e.g., Lao PDR, Keoboualapha et a1 1996). The typical shifting cultivation system is progressively evolving either toward degraded forms of slash and burn or toward more permanent and intensive agriculture (see the cash-based system described below). Its importance is therefore decreasing, though it still represents 14% of the upland rice area. The northern Thailand upland area has already undergone such a transition (Trébuil et a1 1997), associated with a strong reduction in the relative importance of upland rice. A similar shift is likely to occur in the surrounding countries.

Shifting cultivation covers some 1 million hectares in extreme northeast India (Assam, Tripura, Manipur, Nagaland, Aruchnal Pradesh), north Myanmar, north Thailand, north Vietnam, Lao PDR, and South China, which corresponds to the hilly semihumid subecosystem. But some slash and burn is also found in Indonesia (10% of the upland rice area in Sumatra, West Kalimantan, and Sulawesi according to Fagi 1996) under equatorial conditions.

In the hilly semihumid subecosystem, rainfall ranges from 1,200 to 3,000 mm. This is enough to sustain the needs of the crop, but the distribution can be erratic, with risk of moderate drought spells (2–3 wk) during the cropping season. Elevation varies from 300 to 2,000 m, with sometimes steep slopes (15–60% in Lao PDR, Keoboualapha et a1 1996) and severe problems of soil conservation. Cold can be a serious constraint common to all areas above 600 m (Keepthong et a1 1996). The main constraints are weeds and poor soil fertility. Drought is an intermittent problem. Blast can be important locally or annually but is not a general problem. Average yield varies widely: from 3.0 to 4.5 t ha^{-1} locally for a first year of slash and burn down to less than 1 t ha^{-1} for harsher conditions (Van Keer et a1 1998).

Upland rice varieties grown are generally traditional tropical japonicas (isozymic group 6 in Glaszmann's classification, 1987). The plant type is a panicle weight type with a limited number of tillers and panicles and a high number of long and broad grains. Early plant vigor is essential for competitiveness with weeds. A high proportion of the varieties are glutinous, the preference for a given grain type being related to ethnic group. In slash-and-burn systems, farmers have not adopted any improved varieties.

Integrated rice-based systems

Integrated rice-based systems are found in situations with high population pressure but limited market access. Upland rice is grown in permanent fields in rotation with a range of winter crops (wheat, early vegetables, pulses, oilseeds) or fallows, depending on the availability of residual soil moisture after harvest of upland rice. Livestock-crop integration is strong, with crop residue being used as cheap forage for livestock and livestock manure compensating for the absence of mineral fertilization. These areas usually have a continuum from upland to lowland fields along the

toposequence, with farmers often possessing fields in all hydrological conditions. In such cases, the dominant crop is the lowland one; upland rice receives a second priority.

These are the dominant systems across Asia (70% of the upland area), located mainly in South Asia—in eastern India (Assam, Bihar, Madhya Pradesh, Orissa, West Bengal, and Uttar Pradesh) and Bangladesh (Prasad et al 1996, Biswas et al 1996). These areas correspond to the "aus" subecosystem ("aus" means early). In India, upland rice is grown as a monsoon crop while in Bangladesh it is a premonsoon crop. The topography is from gently rolling to flat, with elevation varying from 100 to 500 m. The rainfall pattern is generally monomodal, with 800 to 1,400 mm per year and a peak in July-August. The subecosystem is characterized by very short monsoon or premonsoon growing periods, associated with high risk of drought. The type of drought is different in the two countries: drought during the early vegetative phase is more common for premonsoon crops, whereas terminal drought is the most common situation for monsoon crops. Even for monsoon crops, however, the risks of intermittent drought stress are not negligible, with 2-wk drought spells between mid-July and mid-September, at the intermediate stage of crop growth, being quite common in eastern India (IRRI 1993, Mishra et al 1996, Singh et al 1996).

The effect of drought is reinforced by the loose structure of the soils, which are very often sandy, with poor water-holding capacity and prone to crust formation. Soils are either old alluviums or red lateritic. They are poor and generally unbalanced in major nutrients and therefore prone to brown spot disease. Soil pH ranges from 5.5 to 6.5. Some marginal problems of salinity are observed in Bangladesh (Biswas et al 1996).

The main abiotic constraints are drought and soils of poor fertility and structure. The main biotic constraints are weeds, brown spot, termites, and other soil-borne pests. Average yield varies between 0.8 and 1.2 t ha^{-1} (Prasad et al 1996) but some improved varieties can reach 2.0 to 2.5 t ha^{-1} (P.K. Sinha, CRURRS, Hazaribagh, personal communication).

The upland rice varieties grown are either traditional aus varieties (isozymic group 2 in Glaszmann's classification, 1987) or improved indica or indica × aus varieties (isozymic group 1). The plant type is a "panicle number" type. These varieties are characterized by very good early vegetative vigor, extreme earliness (around 90 d to maturity), high amylose content (usually more than 20%), and susceptibility to lodging and blast, which are not common in such a system. Farmers use a low proportion of improved varieties in this environment (Courtois et al 1998).

Association with perennial plantations

Newly emerging perennial-based systems where rice and other annual crops are grown in association with permanent plantations are developing in Indonesia, notably in transmigration areas, in large estates as well as smallholder plantations (Fagi 1996), and in Vietnam (Hong et al 1996). These represent roughly 14% of the Asian upland rice area. They are favored by government policies. In these cropping systems, up-

land rice is intercropped in young tree plantations during the first 34 yr of tree growth but disappears when the shade from the tree canopy becomes too pronounced. Rice benefits from the inputs given to the trees while the plantation benefits from the weed control used on rice. Trees can be rubber trees, oil palms, coconut trees, teaks (25 yr for turnover), or faster-growing species such as acacias or cashew nuts (7–8 yr). This system is seen as a way to rehabilitate degraded soils in areas invaded by alang-alang grass (*Imperata cylindrica*). Such areas are often considered unsuitable for growing annual food crops under traditional systems.

This system is found in the equatorial subecosystem in latitudes from 15°N to 5°S. Elevation varies from 300 to 1,000 m. Rainfall always surpasses 2,500 mm, with a very long rainy season (more than 5 mo). The risks of drought are limited, though never completely absent. The high rainfall is associated with extremely poor soils with high acidity (pH varying from 3.5 to 5.0), low organic matter content and cation exchange capacity, low phosphorus availability, and high aluminum toxicity. Fertilizer application to overcome soil constraints increases the incidence of humidity-favored diseases (blast and sheath diseases). Minimum tillage is practiced and herbicides are used to control weeds. Yields can reach 2.5 to 3 t ha^{-1} when diseases are controlled. Traditional japonica varieties are not adapted to this system. Although they possess the necessary adaptation to acid soil and resistance to diseases, their plant type does not respond well to inputs. Current improved varieties are mostly indicas, but the durability of their resistance to blast is limited. Some improved japonicas are also used. Varieties with intermediate to high amylose content are preferred.

Intensive cash-based system

The intensive cash-based system does not yet represent much area (estimated at 2%) but its importance will increase with the "demise of subsistence agriculture" predicted by Pingali (1997). Existing examples (e.g., north Thailand, Trébuil et al 1997) show that this evolution leads to a decrease in upland rice area to the benefit of more income-generating crops, a concentration of upland rice in more favorable areas, and an increase in rice productivity through the use of inputs. Whether or not upland rice maintains a significant role in a purely market-driven system depends on the price of rice relative to that of other upland crops. Some specific features such as uncommon grain quality or the perception of upland rice as an organic farming crop (R. Hondrade, USM, Philippines, personal communication), however, might give some substantial advantage to upland rice locally in comparison with lowland rice.

This system is present either in the equatorial area with relatively favorable soils of neutral pH (e.g., Mindanao in the Philippines or Java in Indonesia) or in regions with favorable weather conditions where permanent cultivation is replacing the slash-and-bum system (e.g., north Thailand). Yield in farmers' fields can then reach 3 t ha^{-1}.

The varieties grown in these conditions are often chosen for their productivity (improved varieties are all indicas) or for their comparative advantage on the market because of their specific grain quality (e.g., traditional varieties such as Dinorado, an

aromatic variety in Mindanao, or Lubang Red, with red pericarp, in the Visayas). They have an intermediate amylose content.

In addition to the four systems described, it is worthwhile to consider a system that may develop in Asia in the future: intensive rice cultivation in irrigated aerobic soils on the model of what already exists in Japan on a small scale and in Brazil on a wider scale. If appropriate high-yielding varieties are available, this system can be expected to replace paddy rice in lighter soils as the availability of water declines in the future. The varieties needed for such a system must have a considerably higher yield potential than most current upland varieties, but this is not an unachievable goal, as shown by the performance of Maravilha, a recent upland variety released in Brazil (EMBRAPA-CNPAF 1997).

Table 1 summarizes the different systems with their main characteristics and derived breeding priorities for each. Our two main targets, the integrated rice-based system and rice associated with perennial plantations, were chosen on the basis of area covered, trend in area evolution, and degree of local government support for the production system. Drought tolerance, weed interference, and blast resistance, combined with the proper yield level and grain quality needed in each system, are therefore our breeding priorities.

Germplasm resources for improved tolerance for water deficit

We have seen that the diversity of the upland environment translates into a diversity of varieties, the upland germplasm of Asia belonging to three different varietal groups. The strong structure of *Oryza sativa,* with two main and four small varietal groups (Glaszmann 1987), plays an important role in rice breeding. These varietal groups differ not only for genetic markers but also for morphological and physiological features, traits that were initially used to classify rice varieties before the availability of molecular markers (Kato et al 1928, Matsuo 1952, Oka 1958). Though there is some within-group variability, the groups have some strong common features, including certain traits related to drought tolerance.

Sets of upland rice varieties were evaluated for root system development (Courtois et al 1996a). Tables 3 and 4 present some of these results. Lilley and Ludlow (1996) evaluated osmotic adjustment of a broad sample of varieties. For these two drought-tolerance parameters, the overall pattern is as follows: the japonica types generally have a deep, thick root system and good root penetration but poor dehydration tolerance and low osmotic adjustment. The indica types have shallow, thin root systems and show better dehydration tolerance and osmotic adjustment. The situation of the aus varieties, which were evaluated in limited numbers, is less clear: they seem to perform like indica varieties for osmotic adjustment while their root system can be deep but with relatively thin roots. The favored drought-tolerance strategy of the aus group is via escape with very short duration.

The same varieties were also evaluated at IRRI for field performance under drought, but the within-group variation tends to be as great as the between-group variation. Performance under drought integrates the action of many different mecha-

Table 3. Variance decomposition within and between varietal groups for a sample of 104 upland rice varieties for different root traits.

Source	df[a]	MRL	THK	TRW	DRW	Ratio	R/S	DR/S
Variety[b]	103	**	**	**	**	**	**	**
Between groups	5	**	**	**	**	**	**	**
Within groups:								
0	3	*	ns	*	*	*	**	**
1	26	**	**	**	**	**	ns	**
2	8	ns	ns	*	ns	*	ns	ns
Int. 1/2	6	**	**	ns	*	**	ns	**
6 Trop.	50	**	**	**	**	**	**	**
6 Temp.	5	**	ns	**	ns	ns	ns	ns
Error	293							

[a] df = degrees of freedom, MRL = maximum root length, THK = thickness, TRW = total root weight, DRW = deep root weight, ratio = deep root weight/total root weight, R/S = root-to-shoot ratio, DR/S = deep root-to-shoot ratio. ns = nonsignificant; * and ** = significant at 5% and 1% level, respectively. [b] 1 = Indica, 2 = aus, 6 = japonica, 0 = intermediate.
Source: Courtois et al 1996a,b.

Table 4. Average of root parameters per varietal groups.

Varietal group[a]	Nb[b]	MRL (cm)	THK (mm)	TRW (g)	DRW (g)	Ratio (%)	R/S (%)	DR/S (%)
0	3	89.3 cd	1.10 ab	0.73 c	0.173 c	20.23 cd	24.31 b	5.06 bc
1	26	87.3 d	1.10 ab	1.01 ab	0.178 c	15.96 e	25.69 ab	3.96 c
2	8	106.1 a	1.09 ab	1.11 a	0.307 a	26.00 a	27.72 ab	6.88 a
Int. 1/2	6	93.2 cd	1.10 ab	1.13 a	0.262 ab	21.35 bc	26.93 ab	5.62 ab
6 Trop.	50	101.1 ab	1.14 a	0.88 b	0.232 bc	25.00 ab	28.36 a	6.80 a
6 Temp.	5	95.2 bc	1.11 ab	0.92 b	0.173 c	17.35 de	27.49 ab	4.51 bc
Mean		96.5	1.11	0.95	0.220	21.8	27.31	5.79
cv (%)		10.8	6.8	24.5	43.4	24.9	19.3	30.8

[a] 1 = indica, 2 = aus, 6 =japonica, 0 = intermediate. [b] Nb = number of varieties sampled, MRL = maximum root length, THK = thickness, TRW = total root weight, DRW = deep root weight, ratio = deep root weight/total root weight, R/S = root-to-shoot ratio, DR/S = deep root-to-shoot ratio. Means in columns followed by the same letter are not significantly different at the 5% level.
Source: Courtois et al (1996a,b).

nisms that are diversely represented in different groups, which blurs the patterns observed for individual traits. As true intermediates are seldomly found because of reproductive barriers between the subspecies (Second and Ghesquiere 1995), our dominant strategy is to remain close to the plant type and the local genetic base used in a given production system, introgressing missing traits from other varietal groups. In our situation, this means introgressing indica varieties from South Asia with alleles for deep and/or thick roots, and introgressing japonica varieties from Southeast Asia with osmotic adjustment.

We are so far relying on the genetic variability existing within *O. sativa.* It is not clear whether this variability is broad enough for significant improvement of drought

tolerance in upland rice. For traits such as root depth (O'Toole and Bland 1987), adequate variability is reported to exist within the *O. sativa* species, but variation for traits such as cuticular transpiration is more limited. African scientists have started to use the other cultivated species, *O. glaberrima,* as a source of variability for drought tolerance (Jones et al 1996), but the variability of this species is known to be limited based on neutral marker data as well as agronomic data (Pham 1992). From the point of view of evolution, rice is an aquatic crop and most of its physiological mechanisms are those of an aquatic crop. To be able to adapt to an upland environment, upland rice should be transformed into a truly aerobic crop. Among rice wild relatives, only *O. granulata* and *O. meyeriana* (both perennial, diploid, and carrying the G genome; they are probably the same species, according to B. Lu, IRRI, personal communication) are truly aerobic. But their characteristics in terms of drought resistance are not very well known and material under evaluation has limited introgression from the wild parent. Further exploitation of this material through breeding would need more powerful tools than those presently available to increase the amount of introgression. The range of possibilities should not be seen as restricted to rice genomes. For mechanisms controlled by one or a few genes, the existing transformation systems allow us to introduce drought-related genes coming from another genus, as shown by Xu et a1 (1996), or kingdom, as in Pilon-Smits et a1 (1995).

Preferred breeding strategy

IRRI's breeding strategy for the uplands takes into account the diversity of the production systems and agroecologies presented above. Four additional features of upland rice influence this breeding strategy.

The first important point is that selection should be performed under conditions relevant for the target production systems in terms of inputs. We do not breed for situations with no inputs because we anticipate little improvement beyond traditional varieties. We also eliminate conditions with very high levels of inputs, which are not relevant for Asia. The second element is the necessary decentralization of the breeding work in the target zone. A three-year study was conducted to quantify G × E interactions for yield between breeding sites (Courtois et al 1996b, IRRI 1998). Table 5 summarizes the results. Environment was the dominant source of variation but G × E interactions were very important, accounting for more than five times as much of the observed variation as did the effect of variety. The interaction biplot for the AMMI model confirmed that the best breeding strategy was to breed in the target zones, the best performances for a given variety being recorded in the environment for which it was selected. The similarities between the dendrograms coming from pattern analysis of the interactions and from isozymic data showed that adaptation was clearly genetically determined and well related to the subdivision into varietal groups. This strengthened the recent IRRI decision to devolve varietal development to NARS, which have an obvious comparative advantage in selection for specific adaptation. IRRI now focuses more on developing and distributing the elements necessary for NARS to conduct their breeding programs.

Table 5. Analysis of variance using an AMMI model based on three years of yield data for 16 upland rice varieties in 11 sites of 6 countries (1994-96).

Source	df[a]	Sum of squares	Mean square	% of total sum of squares
Genotype	15	16.82	1.121	4.7
Environment	30	244.46	8.149	68.9
G × E	378	93.33	0.247	26.3
IPCA1	44	26.15	0.594	28.0
IPCA2	42	19.50	0.464	20.9
IPCA3	40	11.50	0.287	12.3
IPCA4	38	10.58	0.279	11.3
Residuals	214	25.60		27.5
Total	423	354.61		100.0

[a] df = degrees of freedom, IPCA = interaction principal component axis.

Even with decentralization of the breeding work at stations representative of the target zones, the rate of adoption of improved upland rice varieties is low (Courtois et al 1998). We are now trying to determine whether decentralization of the selection process in farmers' fields and/or including farmer participation in the selection process would improve the rate of adoption and, if so, under what conditions. Specific adaptation should not be pursued to the point where it is detrimental to yield stability, however, as variability among years is not negligible.

The third element is the tradeoff between productivity and yield stability. The optimal balance varies according to the production system: in systems with limited inputs, priority is given to yield stability, primarily through drought tolerance, whereas in high-input systems, high productivity is necessary. Whether or not it is possible to incorporate both features in the same variety is still under debate though the problem now seems to be more on methodological issues of how to achieve this merging, rather than questions on the theoretical possibility itself. A classical genetic study on *Drosophila* showed that stability and productivity were unrelated and that it was possible to manipulate them independently (Caligari and Mather 1975). More recent results using marker-aided genetics indicated that quantitative trait loci (QTLs) controlling specific adaptation and QTLs controlling mean performance were partly mapping in different genomic areas (Romagosa et al 1996). This was confirmed for results involving tolerance for abiotic stresses (Ribaut et al 1997, Monforte et al 1997). The results of our G × E interaction study show that japonica types have good yield stability but at a low yield level, whereas indica types are relatively unstable but have a good yield potential. So, improvements in both backgrounds might be possible, as shown, in the case of the japonicas, by the high productivity of Maravilha (EMBRAPA-CNPAF 1997).

The last point to consider is that most of the traits that are important for upland rice breeding are quantitative. This requires the use of breeding methods that allow the manipulation of multiple alleles and increase the frequency of the favorable ones

(recurrent selection for a random improvement; molecular markers for a more directed one).

Screening for drought tolerance

Crop management and germplasm improvement are two nonexclusive options for tackling drought problems. A nonquantified margin of progress is possible through manipulation of land preparation and fertilization practices (Kondo 1996). The risks of upland rice production with current varieties tend to discourage farmers from adopting such measures in most areas. Germplasm improvement is one important alternative that limits the problem of technology transfer to farmers and, at the same time, can encourage the intensification of crop management practices.

Two main types of drought occurrence are found in Asia: terminal drought stress and intermittent drought stress. Terminal drought stress is specific to South Asia. The problem has been tackled successfully through phenology manipulation: most of the varieties are less than 90 d in duration. Possible improvement could come from developing a technique to screen for better translocation of assimilates from stems to grain after flowering, for years when the rains end early.

Intermittent stress is more widespread. Though its timing, duration, and severity vary, the probability of occurrence is never insignificant. It is considered in Brazil that, in the sensitive period, every day beyond 7 d without rain reduces yield by 10% (B. Pinheiro, EMBRAPA-CNPAF, personal communication), so, even in favorable environments like the equatorial one, risks are not negligible. Moreover, the risk factor discourages farmers from applying inputs, and this limits yield.

In both cases, we assume that the crop is ecologically suited to the target areas, and, in a normal agricultural situation, we aim at developing varieties with the least yield reduction under stress. Survival mechanisms are of less interest.

To improve a given trait, breeders need three major elements: a simple and efficient screening technique, genetic variability, and an understanding of the genetic control of the trait. The constraint to improving drought tolerance is generally the screening technique, either because it is too complicated to be used at a mass level, or because the establishment of proper drought conditions is unreliable, too complex, or too costly. In addition, the adaptive value of many putative drought-tolerance traits remains unclear.

Drought conditions

Both classical data (Acevedo and Cecarelli 1989) and molecular data (Ribaut et al 1997) have shown that the best environment for screening for drought tolerance is under stress conditions. Major issues are how to create these conditions, how to control them, and what kind of stress to impose. Greenhouse screening, though well controlled, is generally artificial (rate of stress development too rapid) and unadapted to the high volume of material coming from breeding programs. Such screening can be useful, however, for specific situations such as screening mapping populations for root system traits (Yadav et al 1997) or osmotic adjustment (Lilley et al 1996).

For screening under field situations, there are two options: to use naturally occurring drought spells during the rainy season or to create artificial conditions during the dry season.

Natural drought spells were and still are the classical way to eliminate varieties with low yield under stress. The risk that some of the material performs well because it escapes the drought, however, can never be eliminated.

Dry-season tests are an interesting option though not possible everywhere because of cold temperature during dry seasons (e.g., Central Rainfed Upland Rice Research Station, Hazaribagh) or excessive vapor pressure deficit (e.g., IRRI station during late dry season). The manipulation of sowing dates is possible to limit the effect of these problems, but this is delicate to handle for practical reasons. Other options like shelters from rain are simply impractical because of their high cost. Comparisons of different techniques of irrigation (sprinkler, furrow, and drip irrigation) showed that they had different effects on the soil moisture profile, soil impedance, and variety performance (Lafitte, unpublished results). The choice of irrigation method used to irrigate the plots before the stress period and to water the control plots was not neutral.

The second problem to handle is the phenology of the varieties. A small change in phenology can induce a major shift in yield. Garrity and O'Toole (1994) reported that each additional day of delay in flowering after 4 d of stress resulted in an 8% reduction in grain yield. This is consistent with results from Brazil and illustrates the extent to which the effect of stress depends on flowering date. If it is relatively easy to screen for stress at the vegetative stage, it is much more difficult to perform such screening at the reproductive stage with the range of duration generally present in a breeding program. Staggered planting dates are complex to handle and not possible for insufficiently characterized material. The recent development of a drip irrigation system allows management of irrigation on a plot basis and ensures that all entries are stressed at the same phenological stage. This should provide the tool necessary for screening materials that vary in maturity (Lafitte, unpublished results). Regression analysis is another option to adjust for differences in flowering dates when their range is not too broad (Garrity and O'Toole 1994).

Specific experimental designs

Field-scale micro-variations in soil properties are known to be high under upland situations (Dobermann et al 1995). This variability is even more acute under drought conditions (Blum 1993). Therefore, the use of specific designs such as alpha-lattices or augmented designs, developed to reduce the effects of variability when a large number of lines are tested, is preferred. Alpha-lattice designs have been found to provide increased precision in measuring traits that are strongly sensitive to sampling time (e.g., canopy temperature, relative water content). Moving means analysis (Townley-Smith and Hurd 1973) is another useful alternative for such traits.

Screening criteria

One of the problems with rice is the wide array of traits that are said to contribute to drought tolerance (Ludlow and Muchow 1990 for a general review, Fukai and Cooper 1995 for a review in rice). Most of them have a proven effect on the plant's water status, but the situation is less clear for their influence on yield under stress. With the exception of a deep root system (Mambani and Lal 1983), most traits have no proven effect. One reason is that the material needed to test their importance is not available because most varieties vary for many traits at the same time. This emphasizes the need to produce near-isogenic lines for the different mechanisms involved in drought tolerance (Price and Courtois 1999). This would provide physiologists with the appropriate material to test hypotheses on the importance of various traits.

Although the screening criteria to select for a specific trait are relatively well defined, the techniques and indices to evaluate the global drought tolerance of a variety are still not very satisfactory. The difference in yield between stressed and unstressed plots is generally used, but this approach is not really compatible with the evaluation of breeding material at early stages because of the volume of seeds required and the need for replication. Moreover, the heritability of the various indicators for performance under stress, notably yield, is usually very low (Blum 1993).

Our strategy is to rely on screening for individual mechanisms that can affect yield and can easily be selected, rather than rely on the whole-plant reaction. This is a risky strategy as it is not certain whether the effect of improvement for a specific trait will not be compensated for by changes in another trait. Now, however, it is probably the only possibility. We continue to attempt to relate performance in specific water-limited environments to those specific mechanisms.

Besides the work on phenology (which we consider very important but which is not limited by methodological problems as long as the appropriate duration is correctly defined), we are concentrating on the root system (deep and thick roots) and on osmotic adjustment. Under field conditions, we still rely on leaf drying as an indicator of stay-green ability, and on final yield. Our present target is to improve the genetic potential of our varieties for these different traits, but we are very conscious that an important aspect is realization of this potential under field conditions. For the root system especially, expression of the potential can be clearly limited by management techniques (e.g., soil preparation, compaction, fertility) or by biotic factors (e.g., nematodes).

Traits of high-yielding rice for water-limited environments

In addition to a relatively compact root system in indica types and limited ability for osmotic adjustment in japonica types, rice exhibits several other traits that may constrain its performance in aerobic environments. For example, rice leaves have a very low resistance to nonstomatal water loss, apparently because of the properties of the cuticle (Nguyen et al 1997). This results in profligate water loss without carbon fixation. In addition, rice leaves generally roll at only moderate levels of water deficit compared with other crops. Although this is an effective method of shedding excess

radiation and maintaining a favorable canopy energy balance, it can limit productivity in dryland environments. Modification of these two leaf characteristics might be expected to improve performance in upland environments. While genetic variation exists in rice for nonstomatal water loss, even the best rice varieties for this trait suffer large losses relative to other crops. If a single gene can increase cuticular resistance, this trait might be a candidate for transformation. Leaf-rolling response varies widely among rice varieties, and QTLs have been identified for leaf rolling (e.g., Price et a1 1997). Therefore, marker-aided selection could be used to improve this trait. One result of these changes would be an increase in leaf temperature under stress, and the impact of this on productivity must be assessed.

Roots of upland rice also exhibit some characteristics that reflect the aquatic adaptation of the species. For example, aerenchyma and a suberized hypodermis persist when the crop is grown in aerobic conditions. The formation of air spaces in the cortex may have some impact on root penetration, and can also restrict the radial transport of water through the root symplast. Cultivar differences have been reported in the response of root porosity to growth conditions (Colmer et a1 1998). A suberized hypodermis is not considered a major barrier to water uptake in moist soils, but the permeability of that layer to water is strongly affected by soil drying (Moreshet et a1 1996). We are now seeking rice cultivars or related species that do not form aerenchyma and/or do not develop a suberized hypodermis in aerobic conditions. Alternatively, we could attempt to improve the concentration and activity of water channels in the water-conducting root tissues of the exodermis and the cortex, but such channels have not yet been characterized in rice. Axial resistance to water movement may also be important in rice, but this may be partially addressed by increasing root thickness, because xylem vessel diameter tends to increase with root thickness.

Another aspect of rice that must be improved to achieve stable upland yields is its extreme sensitivity to water deficit at flowering. The basis of this sensitivity may be pollen sterility if stress coincides with meiosis, though there is controversy about whether sterility results from a root-sourced signal or a disruption of carbohydrate metabolism in developing pollen grains (Kobata et a1 1994, Sheoran and Saini 1996). Poor grain set may also be due to the abortion of fertilized ovules because of an inadequate supply of current assimilate or inability to mobilize stem reserves.

Currently, we are developing screening methods to identify genotypes with reduced water loss from leaves and panicles, that maintain root activity in dry soil, and that have reduced sensitivity of grain production to moisture deficit because of greater carbohydrate mobilization and/or an ability to maintain pollen fertility. The results do not yet allow breeding for these traits.

Role of marker-aided selection, weight given in the program, and limitations

Limited progress has been made in improving the drought tolerance of upland varieties, perhaps because of the limited efficiency of the screening methods. The use of

molecular markers is seen as a way to short-circuit the weakness of screening techniques based on phenotype only, as well as to improve the understanding of the genetic control of traits. The possibility to decompose a quantitative trait into its Mendelian components, and to manipulate them independently, is expected to improve considerably the prospects for success in breeding for drought tolerance. For drought-related traits, marker-aided selection is seen not so much as complementing phenotyping but as replacing it completely during selection because of the impossibility of phenotypic screening on individual plants and, in some cases, its destructive nature. Phenotyping, necessary for confirmation of success, would be postponed until after the marker-aided selection step.

The first generation of work between 1986 and 1996 focused on developing indica × japonica populations that are polymorphic for the traits of interest and identifying markers linked with the genes of interest. Genes controlling structural root traits (Champoux et al 1995, Yadav et al 1997, Price and Tomos 1997, Shen et al, in preparation), osmotic adjustment (Lilley et al 1996), root penetration (Ray et al 1996), and leaf rolling (Champoux et a1 1995, Courtois et a1 1996a) were tagged.

These studies showed that for some traits not all alleles with positive effects were included in the best parent, and that it was therefore possible to build lines better than the best parent. Some results with wild species (Tanksley and McCouch 1997) confirmed that hidden variability was probably even more common than imagined. The studies confirmed that the traits were highly quantitative and that the most prevalent situation was to have a few QTLs explaining between 10% and 20% of the variability and a multitude of QTLs with a smaller effect. The obvious targets for transfer through marker-aided backcrosses are of course those with a major effect.

Our knowledge of how the effects of these QTLs vary from environment to environment and of their allelic values in different genetic backgrounds is slowly improving. To evaluate adaptation to upland conditions, a number of trials were performed with the existing populations under different drought-stress conditions (Table 6). These results are under analysis. Progress has been limited, however, by statistical weaknesses in the QTL detection (Hyne et al 1995). Sophisticated methods such as composite interval mapping (Zheng 1994) are now available and these should improve QTL analysis. Errors based on phenotyping or genotyping problems are also slowing progress. There is still much to be done in this respect.

Marker-aided genetics, which is based on statistical correlations and is prone to errors, would certainly benefit from an understanding of what molecules the QTLs are actually coding for. Various attempts to use known genes directly as markers gave promising results (Causse et al 1995, Teulat et al 1998). In an attempt to validate the QTLs identified in rice populations and understand their role, we are planning to look at the co-segregation between candidate genes and QTLs in collaboration with H. Nguyen from Texas Tech University, starting with genes possibly involved in osmotic adjustment.

Table 6. Evaluation of the mapped populations under upland conditions.

Population	Size[a]	No. and type of markers	Traits studied	Locations and season
IR64 × Azucena	135 DH lines	175 RFLP + 110 microsatellites	Root system (greenhouse + field) Performance under field conditions (stress at vegetative + reproductive stages)	IRRI (1995 DS, 1996 DS, 1998 DS) CRURRS (1995 WS)
Azucena × Bala	194 SSD lines	100 RFLP	Performance under field conditions (stress at vegetative stage)	IRRI (1995 DS, 1997 DS) WARDA (1996 DS, 1997 DS) CRURRS (1998 WS)
Co 39 × Moroberekan	280 SSD lines	150 RFLP	Performance under field conditions (stress at vegetative + reproductive stages)	IRRI (1995 DS) IRRI (selected lines: 1998 WS, 1999 DS)
IAC 165 × Co 39	144 SSD lines	135 RFLP + microsatellites	Root system (greenhouse) Performance under field conditions (stress at vegetative stage)	IRRI (1998 DS, 1999 DS)
IR62266-42-6-2 × CT9993-5-10-1-M	220 DH lines	313 RFLP + AFLP	Performance under field conditions (stress at vegetative stage)	IRRI (1998 DS)

[a]DH = doubled-haploid, SSD = single seed descent, RFLP = restriction fragment length polymorphism, AFLP = amplified fragment length polymorphism, DS = dry season, WS = wet season. CRURRS = Central Rainfed Rice Research Station, Hazaribagh, Bihar, India; WARDA = West Africa Rice Development Association, Bouake, Côte d'Ivoire.

The next step is to use the QTLs. Though the body of knowledge on QTLs is still not broad enough to produce a high degree of confidence in the output, we have started work to transfer QTLs to improved varieties. This represents an attempt to validate the hypotheses and to demonstrate that the concept is workable in the framework of a breeding program.

Our preferred strategy is backcrossing the QTLs for any drought-tolerance mechanism into an elite background and, if necessary, pyramiding these QTLs. Several advantages are linked with this approach. It allows the production of improved material that is immediately useful and probably easily accepted by farmers if the genetic background chosen is already a cultivated variety. It allows the production of the near-isogenic lines we need to validate our hypotheses on the importance of various traits. It also allows us to test the pleiotropic effect of the QTLs, notably on yield, and their interactions with the genetic background.

Three projects are ongoing in the upland program. One is the production of near-isogenic lines introgressed with QTLs for root depth in an IR64 background, as a follow-up to the results of QTL analysis obtained by Yadav et al (1997). Progress on this project is reported by Shen et al (this volume). The other two projects aim at transferring QTLs to improved varieties through either simple marker-aided backcrossing (Price and Courtois 1999) or the advanced backcross-QTL analysis method when neither of the parents is well characterized for the trait to be transferred. The advanced backcross-QTL analysis developed by Tanksley and Nelson (1995) seems to reconcile our need for rapid impact and for genetic analyses. There is a risk of narrowing the genetic base if we consistently use the same elite recurrent parent, but this is unlikely for upland rice because the recurrent parent will differ from country to country. Table 7 gives details on the three approaches.

The type of markers presently available still limits the scope of the projects because co-dominant markers are needed for the backcrossing work. Restriction fragment length polymorphism markers fit this requirement but the technique is too slow to manipulate a large number of plants in the 4 mo of a breeding season. Microsatellites are the markers of choice (highly polymorphic, co-dominant, polymerase chain reaction-based), but their number is still limited in rice (McCouch et al 1997). Progress needs to be made in this area as well.

We are conscious that transformation could give the same results faster and at a lower cost for single-gene introgression and we acknowledge that, to modify in depth the physiology of the rice plant, transformation might be the only way to go. But transformation has moderate chances to produce releasable products in the immediate future because the genes to be used are not yet clearly defined and because of intrinsic limitations of the technique, intellectual property rights issues, and environmental and health concerns. We believe that, with some improvement in the type of markers and the techniques, marker-aided selection is the most reasonable approach for improving drought tolerance for upland rice in the next five years.

Table 7. Development of near-isogenic lines having introgressed drought-tolerance trait.

Recipient	Donor	Trait of interest	Goal	Method[a]	Collaborators/reference	Stage reached (December 1998)
IR64	Azucena	Root depth and thickness	Validation of hypotheses	Back-cross	L. Shen, K. McNally Z. Li, IRRI (this volume); S. McCouch, Cornell University	BC_3F_2
Kalinga III	Azucena	Root depth and thickness	Cultivar for South Asia	Backcross	K. Steele, University of Bangor, UK; A. Price, University of Aberdeen, UK	BC_3F_1
IR60080-46A	IR62266-42-6-2	Osmotic adjustment	Cultivar for Southeast Asia	ABC-QTL	H. Nguyen, Texas Tech University, USA; S. Robin, IRRI	BC_3F_1 (150 plants)
IR60080-46A	IR63919-38-1-B	Osmotic adjustment	Cultivar for Southeast Asia	ABC-QTL	H. Nguyen, Texas Tech University, USA; S. Robin, IRRI	BC_3F_1 (150 plants)
Vandana	Moroberekan	Blast resistance, root thickness	Cultivar for South Asia	ABC-QTL	H. Leung, IRRI P.K. Sinha, K. Prasad, N. Mandal, M. Variar, CRURRS, Hazaribagh, India	BC_3F_1 (80 plants) BC_2F_1 (90 plants)

[a] ABC-QTL = advanced backcross-QTL analysis method (Tanksley and Nelson 1995).

References

Acevedo E, Cecarelli S. 1989. Role of the physiologist-breeder in a breeding program for drought resistance conditions. In: Baker FWG, editor. Drought resistance in cereals. Wallingford (UK): CAB International. p 117-139.

Biswas JK, Salam MA, Khatum S. 1996. Upland rice in Bangladesh. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. Proceedings of the Upland Rice Research Consortium Workshop, 4-13 Jan. 1996, Padang, Indonesia. Manila (Philippines): International Rice Research Institute. p 39-44.

Blum A. 1993. Selection for sustainable production in water-deficit environments. In: International Crop Science I. Madison, Wis. (USA): Crop Science Society of America. p 343-347.

Caligari PDS, Mather K. 1975. Genotype x environment interaction. III. Interactions in *Drosophila melanogaster*. Proc. R. Soc. London B191:387-411.

Causse M, Rocher JP, Henry AM, Charcosset A, Prioul JL, De Vienne D. 1995. Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key-enzyme loci. Mol. Breed. 1:259-272.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Colmer TD, Gibberd MR, Wiengweera A, Tinh TK. 1998. The barrier to radial oxygen loss from roots of rice is induced by growth in stagnant solution. J. Exp. Bot. 499: 1431-1436.

Courtois B, Chaitep W, Moolsri S, Sinha PK, Trébuil G, Yadav R. 1996a. Drought resistance and germplasm improvement: on-going research in the Upland Rice Consortium. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 154-175.

Courtois B, McLaren CG, Ynalvez M, Hernandez J, Endaya V, Du M-J, Du A, Hondrade E, Chatel M, Guimaraes E, Jones M, Semon M, Darwis S, Syarif AA, Chaitep W, Moolsri S, Prasad K, Sinha PK. 1996b. Old and new tools to analyze genotype x environment interactions: impact on upland rice breeding. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 177-195.

Courtois B, Singh RK, Pandey S, Piggin C, Paris T, Sarkarung S, Singh VP, McLaren G, Baghel SS, Sahu RK, Sahu VN, Sharma SK, Singh S, Singh HN, Singh A, Singh ON, Sisodia BVS, Misra CH, Roy JK, Chaudhary D, Prasad K, Singh RK, Sinha PK, Mandal NP. 1998. Breeding better rainfed rice varieties through farmer participation: some early lessons from Eastern India. In: Proceedings of 2nd International Seminar of CGIAR SWP on Participatory Research and Gender Analysis, Quito, Ecuador, 6-9 September 1998.

Dobermann A, Goovaerts P, George T. 1995. Sources of soil variation in an acid Ultisol of the Philippines. Geoderma 68:173-191.

EMBRAPA-CNPAF (Centro Nacional de Pesquisa de Arroz e Feijão). 1997. Manejo de cultivar Maravilha. Infome Técnico 1. Goiânia, Brazil. 38 p.

Fagi AM. 1996. Status and prospects of upland rice in Indonesia. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 14-27.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-87.

Garrity DP, O'Toole JC. 1994. Screening for drought resistance at the reproductive phase. Field Crops Res. 39:99-110.

Glaszmann J-C. 1987. Isozyme classification of Asian rice varieties. Theor. Appl. Genet. 74:21-30.

Hong HH, Quoc NG, Xuan VT. 1996. Upland rice production in Vietnam: present situation and prospect for development. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 61-70.

Hyne V, Kearsey MJ, Pike DJ, Snape JW. 1995. QTL analysis: unreliability and bias in estimation procedures. Mol. Breed. 1:273-282.

IRRI (International Rice Research Institute). 1993. Program report for 1992. Manila (Philippines): IRRI. 316 p.

IRRI (International Rice Research Institute). 1997. Rice almanac. Manila (Philippines): IRRI, WARDA, CIAT. 181 p.

IRRI (International Rice Research Institute). 1998. Program report for 1997. Manila (Philippines): IRRI. p 58-61.

Jones M, Dingkhun M, Aluko GK, Semon M. 1996. New breeding approaches for upland rice improvement: the use of *O. sativa* × *O. glaberrima* crosses. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 274-283.

Kato S, Kosaka H, Hara S. 1928. On the affinity of rice varieties as shown by the fertility of rice plants. Centr. Agric. Inst. Kyushu Imp. Univ. 2:241-276.

Keepthong V, Petpisit V, Witayasari W, Pintanon P. 1996. Varietal screening and selection of upland rice for cold tolerance. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 216-221.

Keoboualapha B, Schiller JM, Manivong V. 1996. Prospects and priorities for upland rice in the Lao PDR. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 45-55.

Kobata T, Tanaka S, Utumi M, Hara S, Imaki T. 1994. Sterility in rice subject to drought during the booting stage occurs not because of lack of assimilate or of water deficit in the shoot but because of dehydration of the root zone. Jpn. J. Crop Sci. 63:510-517.

Kondo M. 1996. Interaction of nutrient and water in upland rice with emphasis on root ecophysiology. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 141-153.

Lilley JM, Ludlow MM. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. Field Crops Res. 48:185-197.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Ludlow MM, Muchow RC. 1990. A critical evaluation of traits for improving crop yield in water-limited environments. Adv. Agron. 43: 107-153.

Mambani B, Lal R. 1983. Response of upland rice cultivars to drought stress. III. Screening varieties by means of variable moisture along a toposequence. Plant Soil 73:73-94.

Matsuo T. 1952. Genecological studies on cultivated rice. Bull. Nat. Inst. Agric. Sci. Jpn. D3: 1-111.

McCouch S, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T, Blair M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol. Biol. 35:89-99.

Mishra GN, Sinha PK, Shukla VD, Prasad K. 1996. Rainfall analysis for planning upland rice crop in Bihar plateau. Indian J. Agric. Sci. 66(7):392-396.

Monforte AJ, Asins MJ, Carbonell EA. 1997. Salt tolerance in *Lycopersicon* species. IV. Genotype by salinity interaction in quantitative trait loci detection: constitutive and response QTLs. Theor. Appl. Genet. 95:706-713.

Moreshet S, Huang B, Huck MG. 1996. Water permeability of roots. In: Waisel Y, Amram E, Katkafi U, editors. Plant roots: the hidden half. 2nd Edition. New York (USA): Marcel Dekker. p 659-678.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop. Sci. 37: 1426-1434.

Oka HI. 1958. Intervarietal variation and classification of cultivated rice. Indian J. Genet. Plant Breed. 18:78-89.

O'Toole JC, Bland WL. 1987. Genotypic variation in crop plant root systems. Adv. Agron. 41:91-145.

Pandey S. 1996. Socioeconomic context and priorities for strategic research on Asian upland rice ecosystems. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 103-124.

Pham J-L. 1992. Evaluation des ressource génétiques des riz cultives en Afrique par hybridation intra- et intérspecifique. PhD thesis. University of Orsay, France. 132 p.

Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek P, Smeekens SCM. 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol. 107:125-130.

Pingali P. 1997. From subsistence to commercial production systems: the transformation of Asian agriculture. Am. J. Agric. Econ. 79:628-634.

Prasad K, Singh CV, Singh RK, Mathur KC. 1996. Prospects and priorities of upland rice in India. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series No. 16. Manila (Philippines): International Rice Research Institute. p 1-13.

Price A, Courtois B. 1999. Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. Plant Growth Reg. (In press.)

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice. II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95: 143-152.

Price AH, Young EM, Tomos AD. 1997. Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice. New Phytol. 137:83-91.

Ray JD, Yu LX, McCouch SK, Campoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice. Theor. Appl. Genet. 92:627-636.

Ribaut JM, Jiang C, González-de-León, Edmeades GO, Hoisington DA. 1997. Identification of QTL under drought situation in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor. Appl. Genet. 94:887-896.

Romagosa I, Ullrich SE, Han F, Hayes PM. 1996. Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley. Theor. Appl. Genet. 93:30-37.

Second G, Ghesquiére A. 1995. Cartographie des introgressions réciproques entre les sous-espéces indica et japonica de riz cultivé. In: Techniques et utilisation des marqueurs moléculaires. INRA, Paris, France, Les Colloques 72:83-93.

Sheoran IS, Saini HS. 1996. Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sex. Plant Reprod. 9:161-169.

Singh VP, Sastri ASRAS, Baghel SS, Chaudhary JL. 1996. Agro-climatic atlas of Eastern India. Volume 1. IGAU and IRRI. 96 p.

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063-1066.

Tanksley SD, Nelson JC. 1995. Advanced back-cross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92: 191-203.

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A. 1998. Several QTLs involved in osmotic adjustment trait variation in barley. Theor. Appl. Genet. 96:688-698.

Townley-Smith TF, Hurdt EA. 1973. Use of moving means in wheat yield trials. Can. J. Plant Sci. 53:447-450.

Trébuil G, Kam SP, Turkelbom F, Shinawatra B. 1997. System diagnoses at field, farm and watershed levels in diversifying upland agro-ecosystems: toward comprehensive solutions to farmers' problems. In: Teng PS et al, editors. Applications of systems approaches at the farm and regional level. Dordrecht (Netherlands): Kluwer Academic Publishers. p 99-114.

Van Keer K, Trébuil G, Courtois B, Vejpas C. 1998. On-farm characterization of upland rice varieties in North Thailand. Int. Rice Res. Notes 23(3):21.

Xu D, Duan X, Wang B, Hong B, David Ho T-H, Wu R. 1996. Expression of a late embryogenesis abundant protein gene, *HVA*1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol. 110:249-257.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Zheng ZB. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

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Improving rice for drought-prone rainfed lowland environments

S. Sarkarung and G. Pantuwan

More than 50% of the 40 million ha of rainfed lowland rice area in South and Southeast Asia is affected by drought annually, which has contributed to significant yield losses. A breeding program to improve rice cultivars for drought-prone environments began by establishing shuttle breeding programs in different target environments. Evaluation and selection of early generation material are carried out at the key site in Ubon, northeast Thailand, and at satellite stations across the region. Breeding objectives for drought-prone rice environments were prioritized and ideotypes described. Breeding strategies to tackle drought have been formulated as follows: (1) identify component traits that are related to drought tolerance, (2) generate diversified sets of germplasm adapted to drought-prone conditions, (3) develop field techniques for mass screening of rice cultivars for drought tolerance, (4) develop mapping populations for the identification of molecular markers that are closely related to genes that control drought tolerance, and (5) begin a marker-aided selection program.

Field techniques for mass screening of rice cultivars for drought tolerance were established and routinely used to evaluate breeding lines for drought tolerance. Doubled-haploid and recombinant inbred populations were developed for the mapping of genes that control root-pulling tolerance, root penetration ability, and osmotic adjustment. Phenotypic evaluation of these DH lines for root-pulling tolerance, drought score, and important agronomic traits was accomplished. A database of varietal responses to drought has been established and is available for researchers.

Many advanced breeding lines were identified to possess a high level of drought tolerance and recovery ability at the vegetative stage when subjected to different moisture stresses in the dry season. Experiments to investigate the mechanisms responsible for drought tolerance in those breeding lines are under way.

Water deficit commonly occurs during the growing season of the rice crop in rainfed lowlands; the intensity depends on duration and frequency. In South and Southeast Asia, more than 50% of a total of 40 million ha is affected annually. These areas are found mainly in northeast Thailand, eastern India, Bangladesh, and Indonesia. Yield losses from drought are significantly high. In northeast Thailand alone, yield reduction is estimated to be between 13% and 35% (Jongdee et a1 1997). Aprolonged dry spell can take place in both the vegetative and reproductive stages. Drought at the reproductive stage is believed to be more detrimental to grain yield, but severe stress during the early vegetative stage can also cause significant crop damage. Germplasm development for drought conditions is one of the priorities in IRRI's rainfed lowland breeding program.

Decentralizing breeding activities

Because of the heterogeneity of the rainfed lowland ecosystem, major breeding activities were transferred from IRRI headquarters to key sites representative of the major rainfed lowland rice environments. This was accomplished by establishing shuttle breeding programs in collaboration with the major rice-growing countries in this region (Sarkarung 1995).

Our strategies are to expose genetic materials, mainly F_2s, to growing conditions that include environmental and biotic factors that influence optimal development and high productivity of the rice crop, such as the interactive effects of rainfall pattern, temperature, soil conditions, maturity period, and seventy of diseases and insects. Two main breeding stations were chosen: the Ubon Rice Research Center (URRC), in Ubon Ratchathani, northeast Thailand, to represent the drought-prone environment, and the Central Rice Research Institute (CRRI), in Cuttack, eastern India, for the submergence-prone environment. Both of these stations are supported by satellite stations (Sarkarung et al 1995). The key sites for the drought-prone environment are Ubon, Raipur (India), and Rajshahi (Bangladesh).

Generating diverse sets of germplasm

Traditional rice cultivars known to adapt well under water-deficit conditions in South and Southeast Asia were characterized for their main attributes related to drought tolerance and subsequently used as donors in the breeding program. Table 1 demonstrates the main characteristics of selected cultivars possessing different attributes. These cultivars provide high stability under critical environmental changes but have shortcomings such as disease and insect susceptibilities. African-based CIAT and Asian-based IRRI germplasm has been an important source of resistance for major biotic and abiotic stresses, as well as improved grain quality (long, slender grains and good cooking quality). Crosses were made to cover major drought-prone rice areas such as eastern India, northeast Thailand, and Bangladesh. The spillover from selections made in Ubon has been useful to more favorable rainfed lowland areas in Myanmar, Lao PDR, and the Philippines, where mild drought occurs frequently.

Table 1. Main characteristics of selected traditional cultivars commonly grown under rainfed lowland conditions of South and Southeast Asia.

Cultivar	Origin	Main attributes
KDML105	Thailand	Adapted to low fertility, high recovery under drought
NSG19	Thailand	High osmotic adjustment, adapted to low soil fertility
Safri 17	India	Drought tolerance at reproductive stage
Rajshree	India	Drought tolerance at seedling stage
MGL 2	India	Deep roots
FR13A	India	Drought tolerance at seedling stage, submergence tolerance

Evaluating and selecting material adapted to drought conditions

Tolerance for drought stress is a prerequisite to rice cultivars targeted for water-limiting environments, mainly to ensure that new cultivars have a high probability of success during the critical stress period. Fukai and Cooper (1995) stated that the most important mechanism for drought escape is an appropriate phenology that matches crop growth and development with rainfall patterns. They further suggested the need to look for the other mechanisms for drought tolerance such as root depth, dehydration tolerance, and drought recovery.

Besides drought tolerance, other important traits such as grain yield, eating quality, and resistance to blast and bacterial leaf blight disease are important. Our task for breeding is therefore to incorporate the traits required into rice cultivars targeted for drought-prone environments.

For the screening and evaluation of breeding materials for adaptation to drought conditions, key sites that complement each other were selected based on the nature of drought occurence. For example, because of erratic rainfall in June and July in northeast Thailand, a water deficit during the vegetative stage is common. On the other hand, rapid cessation of rains induces a water shortage in the postflowering period (Raipur). A different drought situation occurs in Rajshahi, where subsoil hardpan impedes root penetration of shallow-rooted cultivars and prevents roots from taking up available water in a deeper soil layer (Wade et al 1997). Our strategy is to select materials that perform well at these sites and later intercross them to combine those traits. Our goal is therefore to identify superior genotypes that adapt to different types of water stress.

Segregating populations (mainly F_2 are initially exposed to different target environments of the rainfed lowlands, with emphasis on key sites (e.g., Ubon, Raipur, and Rajshahi; Fig. 1). This approach was mentioned in the survey conducted by Jackson et al (1996) on the impact of physiological research on plant breeding. It demonstrated potential pathways for breeding programs to have an impact in drought-prone environments via three important factors: (1) choice of environments in which to conduct selection trials, (2) identification of selection criteria and traits for the introgression program, and (3) identification of traits for indirect selection criteria.

In northeast Thailand, where more than 4 million ha of rainfed lowland rice area are located, early and late-season drought are common. Growing environments are quite diverse, ranging from extremely poor soils to medium-high fertility conditions. Early maturing and weakly photoperiod-sensitive varieties are more suitable for the low-yielding environment, whereas medium-duration varieties perform better under higher inputs.

At the URRC research station, F_2 and F_4 generations are evaluated under well-drained, light-textured soils and F_3 are transplanted at the Nakorn Si Thammarat Rice Research Station for generation advance (Fig. 1). Alternating the planting of materials between the two distant sites not only shortens breeding time but also facilitates the identification of photoperiod responses to daylength. Under the growing condi-

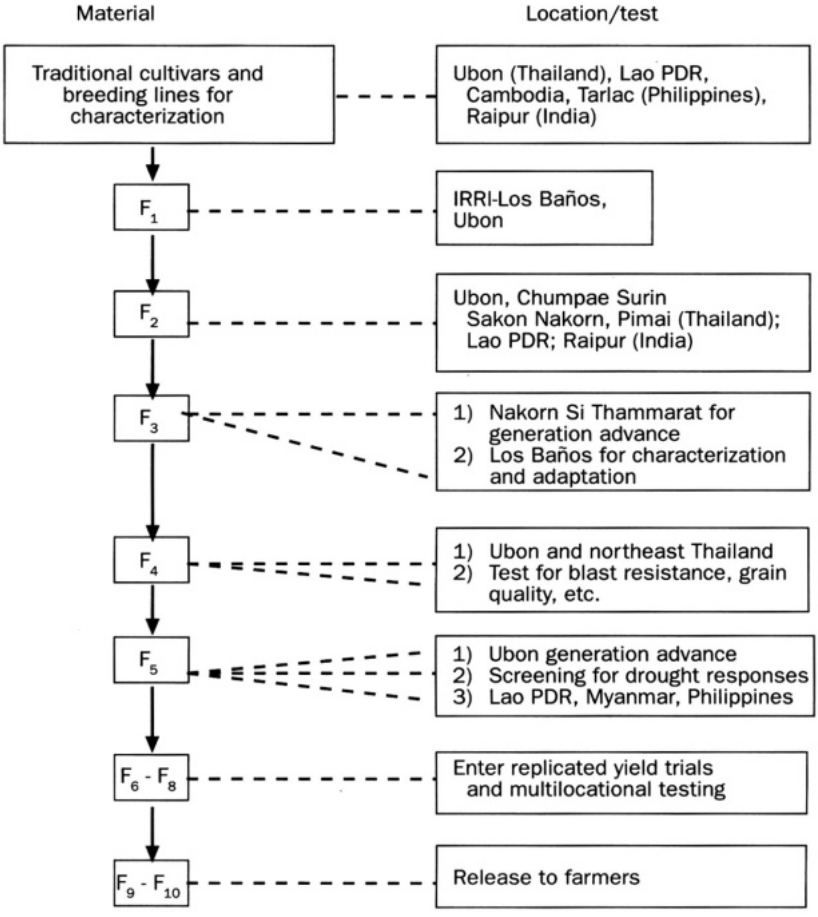

Fig. 1. Flow of breeding materials for drought-prone environments.

tions in Ubon, materials are normally subject to water shortage in the early vegetative stage, mainly because of irregular rainfall from mid-July to mid-August. The responses of each line are recorded based on their symptoms; highly sensitive entries are usually rejected. Entries that showed some level of tolerance were normally uprooted by hand to examine root strength (which refers to the amount of force applied). F_4 families are again exposed to conditions similar to those applied to F_2s. This undertaking is time-consuming and error-prone. Based on our previous experience, not all deep-rooted cultivars/lines were tolerant of drought, indicating that other traits/mechanisms also operate in the water-deficit conditions of the rainfed lowlands.

General breeding objectives are established as follows:

1. Tolerance for water deficit at the vegetative and reproductive stages.
2. Resistance to major diseases and insects (e.g., blast, bacterial blight, brown planthopper, green leafhopper, gall midge, etc.).
3. Good seedling vigor.
4. Early to medium duration (115–135 d).
5. Intermediate height (110–130 cm).
6. Good grain quality and diverse grain types.
7. Yield stability (average farm yield of 3–4 t ha^{-1}).

Because of unpredictable rainfall in most rainfed lowland rice-growing areas, farmers are unable to apply fertilizers at the appropriate times. Under this management, our approach is to develop rice cultivars that have high stability in these adverse environments. There is thus a tradeoff between high yield and yield stability.

Identifying component traits associated with drought tolerance

In rainfed lowland rice fields where soil hydrology often varies from anaerobic to aerobic conditions and vice versa, root depth and root thickness may not provide as adequate a level of drought tolerance as they do in well-drained upland fields. The deep and thick roots may play a minor role under these circumstances. Unfortunately, little research has been carried out in rainfed lowlands to determine the mechanisms/traits responsible for drought tolerance. Under this complexity, simple references could be drawn from NSG19 and KDML105, the leading varieties for the drought-prone environment of northeast Thailand. NSG19 has a high osmotic adjustment (OA) and intermediate root depth; it shows tolerance for drought in both the vegetative and after-flowering periods. On the other hand, KDML105 possesses a different mechanism under water shortage; it displays a high recovery ability when moisture availability is adequate in the soil. This variety has an intermediate level of OA and a superficial root system.

These varieties were included along with other major rainfed lowland varieties such as MGL2 and Mahsuri in studies conducted by Lilley and Ludlow (1996) on the magnitude of genetic variation in OA and dehydration tolerance in diverse rice cultivars. At a conference on rice biotechnology held in Bali, Indonesia, the working group on drought research recommended that, in the rainfed lowlands, besides the major root traits (e.g., root depth, root thickness, root length density, etc.), other component traits associated with drought tolerance be investigated as well, such as OA, root penetration ability, and dehydration tolerance. Our breeding program for drought-prone environments had incorporated those key traits as selection criteria. Using the majority of cultivars/lines developed for the rainfed lowlands, methodologies for measuring root penetration ability were developed at Texas Tech University (TTU) using wax-petrolatum layers as a substitute for the subsoil hardpan (Yu et al 1995). Field experiments to measure root penetration of the subsoil hardpan were then carried out at Rajshahi (Samson et al 1995).

Developing doubled-haploid populations for molecular research

To provide an understanding of the molecular and physiological aspects of drought research, doubled-haploid (DH) populations were developed from the parents involving deep roots, high root penetration, and low OA (CT9993) and shallow roots, low root penetration, and high OA (IR62266). Our main objectives were to develop homozygous lines that possess different component traits. Linkage maps were developed at TTU using the 220 DH lines; these DH populations segregate for root penetration ability, OA, drought score, and root-pulling resistance.

Phenotyping of the DH lines for root-pulling resistance under field conditions was carried out at URRC in the 1996 wet season using a modified root-pulling scale previously employed by O'Toole and Soemartono (1981). Under the dry-seeded conditions at Ubon, the results showed high genetic variability among DH lines in root-pulling resistance, drought score, plant height, maturity period, tiller number, and yield and yield components (Sarkarung et al 1997). Root pulling refers to the force (in kg) required to uproot a rice plant. It collectively includes root depth, diameter and size, volume, and mass. Table 2 shows root-pulling resistance, root mass density at 0-15-, 15–30-, and 30–45-cm soil depths, and total root mass of the 220 DHs and their parents. There was transgressive segregation in these DH populations for root-pulling resistance, root mass density, and total root mass (Table 2). Genotypic differences in root-pulling resistance were also recorded between the parents. The deep-rooted parent, CT9993, also had a higher root mass density than the shallow-rooted one in the topsoil profiles (0–15 cm and 15–30 cm). But its roots beyond 30 cm were almost negligible.

The heritability of root-pulling resistance was 80%, indicating that this trait is highly heritable and under simple genetic control. Selection for root-pulling resistance in the early generation is effective (unpublished data), as opposed to findings by Ekanayake et al (1985) under transplanting conditions at IRRI, Los Baños.

These DH populations were also phenotyped for drought score and canopy temperature at Ubon in the 1996 dry season. Genotypic variation existed in expression of tolerance. It has not been determined in these studies, however, what traits were responsible for the variation. Some 220 DH lines and their parents were evaluated under field conditions at Ubon for drought score and canopy temperature (Table 3). Drought score was recorded at 1-wk intervals as stress developed from mild to severe. Drought scores were significantly different from mild to severe water deficits (stress 1 to 4). In this environment, genotypes also showed differences in canopy temperature at early and late stress (Table 3). It was interesting to note that the deep-rooted parent, CT9993, displayed a higher degree of susceptibility to drought than the shallow-rooted parent, IR62266. The canopy temperatures of the two parents were similar under both stress conditions (Table 3).

In Israel, the 100 selected DH lines based on field drought score and other morphological characters at Ubon were evaluated for drought responses under uniform soil and no rain. The results were reported by Blum et al (this volume). Studies on root traits and water extraction at different soil depths were done under controlled

Table 2. Minimum, maximum, and mean root-pulling resistance, root mass density, and total root mass of 220 doubled-haploid lines (DHLs) and parents in the 1996 wet season at Ubon, Thailand.

Root traits	DHLs		CT9993	IR62266	Mean	Significance
	Min.	Max.				
Root-pulling resistance (kg)	27.9	101.9	75.0	45.3	56.9	**
Root mass density (mg cm^{-3})						
0–15 cm	0.382	1.993	1.162	0.731	0.863	**
15–30 cm	0.010	0.185	0.047	0.022	0.054	**
30–45 cm	0.001	0.069	0.008	0.025	0.021	**
Total root mass (g m^{-2})	66	311	183	117	141	**

**Significant at 0.01 level.

Table 3. Minimum, maximum, and mean drought score and canopy temperature of 220 doubled-haploid lines (DHLs) and parents at different levels of drought stress, 1995-96 dry season at Ubon,Thailand.

Factors	DHLs		CT9993	IR62266	Mean	Significance
	Min.	Max.				
Drought score[a]						
Stress 1	0.00	3.51	1.67	1.58	1.30	**
Stress 2	1.18	6.15	4.25	3.58	3.47	**
Stress 3	1.83	8.09	5.42	4.17	4.38	**
Stress 4	4.94	8.90	7.58	6.50	6.87	**
Canopy temperature (°C)						
Early stress	25.6	29.7	27.2	27.8	27.5	**
Late stress	30.0	36.4	33.3	32.8	33.1	**

**Significant at 0.01 level. The Standard Evaluation System for Rice (SES) was employed: 0 = no symptoms, 9 = all plants apparently dead. Stress 1 = mild stress, stress 4 = severe stress.

conditions at IRRI (Kamoshita et al, this volume). Different methods for measuring OA using rainfed lowland rice cultivars were compared under screenhouse conditions at TTU (Babu et al 1999). The thermocouple psychrometry method was developed for measuring OA of DH lines and their parents (Babu et al, this volume). Data obtained from field experiments in Ubon and Bet Dagan (Israel) and from controlled conditions at IRRI and TTU were used in the mapping populations for genes controlling root-pulling resistance, root penetration, and OA at TTU (Zhang et al, this volume, "Molecular dissection of drought resistance in rice: from physio-morphological traits to field Performance").

It is imperative to investigate the performance of these DH lines under different drought-prone conditions to determine which combination has a comparative advantage under water-limiting environments. In complex rainfed lowland environments, however, a combination of traits would probably be required for drought tolerance.

In 1997 and 1998, several DH lines possessed different combinations of component traits:

- deep roots, high root penetration, high OA
- low root penetration, low OA, shallow roots
- shallow roots, high OA, deep roots

A group of DH lines that consists of high and low root-pulling resistance was assembled for multilocational testing; these lines have a similar canopy arrangement and maturity period. In 1997 and 1998, they were evaluated under controlled (fully irrigated) and water-stress conditions (drought imposed in the postflowering stage) at three locations—Ubon, Chumpae, and Surin in northeast Thailand. The results of these experiments are being analyzed.

Developing ideotypes for drought-prone environments of the rainfed lowlands

Because of highly diverse drought-prone rice environments, ideal plant types may vary from one targeted area to another. Different types of plant architecture have been designed based on amount of rainfall, soil type, and hydrological conditions. Besides the ability of a rice plant to adapt to a water deficit, a basic understanding of the mechanisms for drought tolerance is needed, particularly for those related to drought during the reproductive stage. Ideotypes of rice cultivars suited to drought should have the following traits:

1. One or a combination of the major component traits (e.g., root depth, OA, recovery ability, etc.)
2. Fast initial growth for good ground cover
3. Erect upper canopy structure
4. Intermediate height (110–130 cm)
5. Stiff stem
6. Resistance to lodging
7. Medium tillering (6–8 productive tillers)
8. Large and dense panicles with high grain weight
9. Early to medium duration (105–135 d); phenology to fit the rainfall pattern
10. Harvest index of 0.5 or higher

These traits serve as selection criteria for breeders.

In drought-prone rice areas of northeast Thailand and eastern Madhya Pradesh, India, where water deficit can be severe, intermediate height with a large panicle size would provide better stability than semidwarf types. On the other hand, in an environment with moderate water stress (e.g., favorable rainfed lowland), semidwarf genotypes with medium to high tillering would be a better choice for maximizing yield potential. Certain morphological traits can re-enforce the ability of the rice plant to be more efficient in dry soils by conserving soil moisture (early seedling vigor, high leaf area index), economizing water use (medium tiller number), and having crop plasticity (intermediate plant height).

Screening rice cultivars for drought tolerance at the vegetative stage

It is important to evaluate breeding material for drought tolerance at the seedling stage because under drought-prone conditions the rice crop normally experiences a prolonged period without rain in the early monsoon season. Starting from the F_5 generation or when the breeding lines become more uniform, screening for drought response is employed; mass screening is normally carried out in the dry season using irrigation water. Breeding lines displaying a high level of drought tolerance and desirable agronomic traits are further tested under drought conditions in both the vegetative and reproductive stages.

At the URRC farm, breeding lines were dry-seeded in hills. The experimental plots were flooded after germination and were maintained as under rainfed lowland conditions. Irrigation water was suspended when the seedlings were about 50 d old. Drought score, recovery ability, and other parameters were recorded (Sarkarung et a1 1997). Our main objectives were to characterize the genotypes that respond to different levels of water stress and to identify those displaying a high level of drought tolerance. The methodologies for mass evaluation of drought tolerance at the reproductive stage are being refined to facilitate the screening of breeding lines at this stage.

In the 1998 dry season, more than 200 breeding lines were evaluated for drought response and recovery ability. The 50-d-old seedlings were subjected to varying levels of drought stress when irrigation water was cut off. Drought score was taken at 1-wk intervals for 4 wk and the recovery score was taken 4 d after rewatering. Table 4 presents the drought score and recovery score of the selected breeding lines at different stages of stress development and their ability to recover from drought in comparison with check varieties NSG19 (drought-tolerant) and IR20 (drought-susceptible). Under a limited water supply, these breeding lines demonstrated the ability to survive and regenerate new green parts after the stress was relieved. Figure 2 shows the responses of a few selected breeding lines to water deficit at different scoring times and recovery scores; IR68796-36-2-B-1-1-B had the lowest drought score and the fastest recovery from stress to resume growth. In contrast, IR20, a drought-susceptible check, was slow to recover from drought. It was important, however, to note that NSG19, the resistant check, was unable to recover quickly due to its photoperiod-sensitive nature. This stage is, of course, the most sensitive to water deficit.

Using molecular markers to select for “difficult traits”

Plant breeders have high expectations from molecular research to facilitate the selection and identification of superior genotypes at the molecular level. This new technology can complement a conventional breeding program and make it more efficient. Marker-aided selection (MAS) is one of the top priorities in our germplasm improvement program. Using the doubled-haploid populations of CT9993/IR62266, many important traits such as root-pulling resistance, green leaf retention (drought score),

Table 4. Drought score and recovery score of selected breeding lines evaluated at Ubon during the dry season, 1997.

Cultivar	Combination	Drought score[a]					Recovery score[a]
		20 Feb	27 Feb	6 Mar	13 Mar	20 Mar	23 Mar
IR70828-18-1-B-B	IR54081-CPA-3-B-1-3/ SABITA//SLK 31-2-2	1.6	3.0	4.0	5.3	5.3	1.7
IR70849-43-B-B	CT 6241-17-1-5-1/ KDML86G2 4-4//IR46329-SKN	1.3	2.7	5.0	5.3	5.3	1.7
IR70849-29-B-B-B	CT 6241-17-1-5-1/ KDML86G2 4-4//IR46329-SKN	2.7	3.3	4.7	5.7	5.7	3.7
IR68821-70-1-8-5-8-B	CT9899-32-1/ IR55829B-B//IR45@SKN-516	2.3	3.3	4.7	5.7	5.7	1.7
IR68823-9-5-B-1-2-B	CT9981-23-5-M-2/ IR55776-16-2//IR54977-UBN	2.0	2.7	4.3	5.3	5.3	2.3
IR69502-28-SKN-1-UBN-1-B-1	IR57514-PMC5-B-1-2/ IR57515-PMI-8-1-SKN// IR43524-55-1-3-2	1.7	3.0	4.7	5.7	5.3	2.3
IR69513-1-SKN-2-UBN-1-3-2	IR57514-SKN-299-3-2/ IR57515-PMI-8-1-SKN// IR43524-55-1-3-2	2.3	3.3	4.7	5.7	5.7	3.0
IR69514-51-KKN-3-UBN-1-4-3	IR57514-SKN-299-3-2/ IR57515-PMC8-1-SRN// IR54119-4-B-1-1	2.3	2.7	5.0	5.7	5.7	2.3
IR69514-51-KKN-3-UBN-1-5-1	IR57514-SKN-299-3-2/ IR57515-PMI-8-1-SRN// IR54119-4-B-1-1	2.0	3.0	5.3	6.0	5.7	3.0
IR69515-9-KKN-4-UBN-2-1-1	IR57514-SKN-299-3-2/ IR57515-PMI-8-1-SRN// IR54119-4-B-1-1	2.0	3.0	4.7	5.7	5.7	3.0
IR69515-27-KKN-1-UBN-1-1-1	IR57514-SKN-299-3-2/ IR57515-PMI-8-1-SRN// IR54119-4-B-1-1	2.0	3.3	4.7	5.7	6.3	1.7

Table continued

Table 4 continued.

Cultivar	Combination	Drought score[a]					Recovery score[a]
		20 Feb	27 Feb	6 Mar	13 Mar	20 Mar	23 Mar
IR68815-51-PMI-2-UBN-7-1-2	CT9993-5-10-1-M/ IR41431/68-1-2-3// IR57514-PMI-5-51-2	1.7	2.3	4.7	5.7	5.7	2.3
IR68815-69-PMI-6-UBN-8-2-1	CT9993510-1-M/ IR41431/681-2-3// IR57514-PMI-5-B-1-2	2.3	3.3	4.3	5.7	5.7	1.7
IR68796-27-3-B-5-1-B	CT9992-22-2-4/ IR56592-21-1-3-1//KDML105	2.3	3.3	5.7	6.7	5.7	3.7
IR68796-27-B-B-6-1-B	CT9992-22-4/ IR56592-21-1-3-I//KDML105	1.0	2.3	3.7	5.7	5.7	1.7
IR68796-36-2-B-1-1-1	CT9992-22-2-4/ IR56592-21-1-3-1//KDML105	1.0	2.0	4.7	5.0	5.0	1.0
Checks							
KDML105		2.3	3.3	5.7	6.4	7.0	4.3
IR20		3.3	4.0	6.0	6.7	7.0	5.0
NSG19		3.0	4.0	6.5	7.3	7.5	4.7

[a] Average of 4 replications. recovery score was taken 4 d after rewatering. The Standard Evaluation System for Rice (SES) was used, with a scale of 1 to 9. where 1 = 90-100% plants recovered and 9 = 0–19% plants recovered.

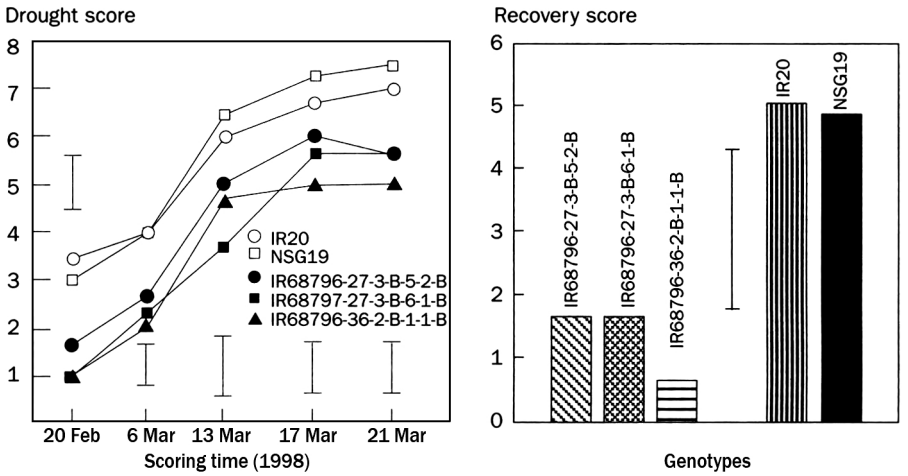

Fig. 2. Drought score and recovery ability of selected breeding lines evaluated in dry season 1997-98 at Ubon. The 50-d-old seedlings were subjected to varying levels of drought stress by stopping irrigation. The recovery score was taken 4 d after rewatering. Bars indicate significant differences at 5%.

root penetration ability, and osmotic adjustment have been mapped at Texas Tech University, and important quantitative trait loci (QTLs) have been identified. These traits are highly influenced by climatic changes and soil heterogeneity, making MAS more compelling.

Our approach for molecular selection has emphasized the genes that control drought traits, using appropriate molecular markers. Unfortunately, the traits that govern drought tolerance in rainfed lowland conditions have not been clearly understood or identified. An alternative approach is to use field phenotypic data in the search for QTL markers that are closely associated with drought tolerance, without referring to specific traits. These QTLs can be moved around in chromosomes to increase dosages (DNA), which in turn improves the level of drought tolerance.

Conclusions

Significant progress has been made on breeding rice cultivars for adaptation to water-limiting environments. This has been accomplished by exposing early generation materials to the stress conditions of the rainfed lowlands that are representative of drought-prone rice areas and by evaluating advanced breeding lines in water-deficit conditions in the dry season. Many advanced breeding lines (CT9992-22-2-2LIR56592-21-1-3-1//KDML105) adapted to drought stress in the vegetative stage were identified. These promising lines, which combine drought tolerance with good grain quality and have resistance to major diseases and insects, are being evaluated for root-

pulling resistance and yield potential in the 1999 dry season. These lines will be included in multilocational testing in the 1999 wet season.

The doubled-haploid populations developed for the molecular mapping of genes that control component traits associated with drought tolerance have been phenotyped for drought response under field conditions in northeast Thailand and at Bet Dagan, Israel. The same set of lines was also characterized under controlled conditions for OA and root penetration ability at TTU, and major root traits and water extraction at IRRI. QTL markers for root-pulling resistance, OA, and root penetration ability were identified.

References

Bahu RC, Pathan MS, Blum A, Nguyen HT. 1999. Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Sci. 39:150-158.

Ekanayake IJ, Garrity DP, Masajo TM, O'Toole JC. 1985. Root pulling resistance in rice: inheritance and association with drought resistance. Euphytica 34:905-913.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

Jackson P, Robertson M, Cooper M, Hammer G. 1996. The role of physiological understanding in plant breeding, from a breeding perspective. Field Crops Res. 49:11-37.

Jongdee S, Mitchell JH, Fukai S. 1997. Modelling approach for estimation of rice yield reduction due to drought in Thailand. In: Fukai S, Cooper M, Salisbury J, editors. Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held in Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings 77. p 65-73.

Lilley JM, Ludlow MM. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. Field Crops Res. 48:185-197.

O'Toole JC, Soemartono.1981. Evaluation of a simple technique for characterizing rice root system in relation to drought resistance. Euphytica 30:283-290.

Samson BK, Wade LJ, Sarkarung S, Hasan M, Amin R, Harnpichitvitaya D, Pantuwan G, Rodriquez R, Sigari T, Calendacion A. 1995. Examining genotypic variation in root traits for drought resistance. In: Fragile lives in fragile ecosystems. Proceedings of the International Rice Research Conference, 12-17 Feb. 1995, International Rice Research Institute, Los Baños, Philippines. Manila (Philippines): IRRI. p 521-534.

Sarkarung S. 1995. Shuttle breeding for rainfed lowland rice improvement. Rainfed lowland rice: agricultural research for high-risk environments. Manila (Philippines): International Rice Research Institute.

Sarkarung S, Singh ON, Roy JK, Vanavichit A, Pekasut P. 1995. Breeding strategies for rainfed lowland ecosystem. In: Fragile lives in fragile ecosystems. Proceedings of the International Rice Research Conference, 12-17 Feb. 1995, International Rice Research Institute, Los Baños, Philippines. Manila (Philippines): IRRI. p 709-720.

Sarkarung S, Pantuwan G, Pushpavesa S, Tanupan P. 1997. Germplasm development for rainfed lowland ecosystems: breeding strategies for rice in drought-prone environments. In: Breeding strategies for rainfed lowland rice in drought-prone environments. ACIAR Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research.

Wade LJ, McLaren CG, Criseno L, Amarante ST, Sarawgi AK, Kumar R, Bhamri MC, Singh ON, Amed HU, Rajatasereekul S, Porn-uraisanit P, Boonwite C, Harnpichitvitaya D, Sarkarung S. 1997.Genotype-by-environment interaction: RLRRC experience. In: Breeding strategies for rainfed lowland rice in drought-prone environments. ACIAR Proceedings No. 77. Canberra (Australia): Australian Centre for International Agricultural Research.

Yu LX, Ray JD, O'Toole J, Nguyen H. 1995. Use of wax-petrolatum layers for screening rice root penetration. Crop Sci. 35:684-687.

Notes

Authors' address: International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Philippines.

Citation: Ito O, O'Toole J, Hardy B, editors. 1999. Genetic improvement of rice for water-limited environments. Proceedings of the Workshop on Genetic Improvement of Rice for Water-Limited Environments, 1-3 December 1998, Los Baños, Philippines. Manila (Philippines): International Rice Research Institute. 353 p.

Perspectives on plant physiology

Water uptake by plant roots: modes of regulation

E. Steudle, H.M. Zimmermann, and T. Henzler

A composite transport model explains variability in the ability of roots to take up water. The model is based on measurements of root hydraulics (hydraulic conductivity) at the level of both excised roots and root cells (membrane level) using pressure probes and other techniques. The composite transport model integrates apoplastic and cellular components of radial water flow across the root cylinder. It explains why the hydraulic conductivity of roots changes in response to the nature (osmotic vs. hydraulic) and intensity of water flow. The model provides for an adaptation of plants to conditions of drought and other stresses by allowing for a coarse regulation of water uptake according to soil water conditions and demands for water from the shoot. The coarse regulation is physical in nature but depends on root anatomy, e.g., on the state of suberization and the existence of apoplastic barriers in the exo- and endodermis. A fine regulation results from the activity of water channels (aquaporins) in root cell membranes, which is assumed to be under metabolic control.

We have detailed knowledge of the regulation of water flow for the shoot. The cuticle prevents water losses to the dry atmosphere and stomatal width is regulated according to plant needs. There are mechanisms that either sense the water status (water potential) of the shoot or the water status of the root's surroundings is signaled to stomata over long distances. But relatively little is known about the processes that limit or even regulate root water uptake, although the water supplied to the plant by roots contributes to the overall water balance of the shoot. The reason for this is that until now we have, for technical reasons, much less information about the hydraulic architecture of the root than about the shoot. In the soil-plant-atmosphere continuum, stomata will usually dominate the hydraulic resistance. But the hydraulic resistance of the root will nevertheless contribute substantially to the water status of the plant and will be crucial for shoot growth and biomass production. So, regulation of water flow across roots is of major importance for agriculture, especially under conditions of water shortage.

This is one reason the old problem of the variability of root water uptake and its regulation is now much discussed. Discussion of the issue follows two lines. One focuses on the role of the apoplastic path across the root cylinder and how suberization of the cell surface and the formation of Casparian bands in the endo- and exodermis would result in a control of water movement across the root (Peterson 1988, Steudle and Peterson 1998). The other focuses on the role of aquaporins or water channels in root cell membranes, which have been shown to be responsible for most

of the water permeability (hydraulic conductivity) of membranes (Wayne and Tazawa 1990, Chrispeels and Maurel l994, Steudle and Henzler 1995, Maurel 1997, Schaffner 1998, Steudle and Peterson 1998). The question is how both the apoplastic and membrane-bound water flow would contribute to overall water uptake by plant roots.

It is well known that adverse factors strongly affect the suberization of plant roots and that this has consequences for the water flow (North and Nobel 1991, Stavosky and Peterson 1993, Azaizeh and Steudle 1991, Azaizeh et al 1992). Undoubtedly, anatomical conditions affect root water transport. On the other hand, evidence suggests that water channel activity could be either directly "triggered" or "gated" by external factors (drought, salinity, anoxia, nutrition, heavy metals) or that channels are under metabolic control (Steudle and Henzler 1995, Johansson et al 1996). Certainly, both components are important and perhaps they are compensatory. In the following section, we present evidence of their role, which is based on measurements of overall root water permeability (root hydraulic conductivity, Lp_r) and on measurements at the level of individual cells (membrane level, root cell Lp) using modern techniques. We discuss a model that integrates the different components.

Variability of water uptake corresponds to complex root structure

Hydraulic properties of roots are related to their anatomy, and the interpretation of root transport data requires a detailed knowledge of root structure. Changes in root suberization are most relevant. Root suberization increases with age and during stress (drought, high salinity, nutrient deprivation, anoxia, etc.). Young roots suberize when they pass through different stages of development of the endo- and exodermis. During stage I, Casparian bands are forming in the radial cell walls of the endodermis. During stage II, a suberin lamella is laid down in both the radial and tangential walls. Cell walls are eventually thickened during stage III, which results in the well-known u-shaped cross section of endodermal cells. It has been shown that the exodermis also develops Casparian bands and that this structure passes through states similar to those of the endodermis, which includes the formation of passage cells (Peterson 1988, Peterson and Enstone 1996). In young maize roots, the formation of a Casparian band in the endodermis did not affect hydraulic conductivity during stage I, which led to the conclusion that the hydraulic resistance was distributed more evenly across the root cylinder (Peterson et al 1993, Steudle et al 1993). But this may change during later stages. For the exodermis, experiments indicated a strong effect on radial hydraulic conductivity (root Lp_r; Zimmermann and Steudle 1998).

In recent years, considerable information has been collected on how changes in root structure caused by drought, high salinity, nutrient deprivation, and anoxia are translated into changes in root hydraulic properties (North and Nobel 1991, Azaizeh et al 1992, Cruz et al 1992, Birner and Steudle 1993, Stavosky and Peterson 1993, Carvajal et al 1996, Steudle and Heydt 1997, Peyrano et al 1997). Relatively little is known about the contribution of older thickened roots to overall water uptake. These

roots are covered with several layers of suberized cells, such as in woody species. Usually, it is thought that, because of suberization, these arrays do not contribute much to overall water uptake. But this has been questioned (see discussion in Kramer and Boyer 1995, p 130, and references there). For technical reasons, it is still difficult to quantify the uptake by different parts of the roots or even root zones. Unlike the situation in the shoot, much less is known about the hydraulic architecture of roots.

Besides the uncertainties related to the root apoplast, there are questions about water channels and how they contribute to the overall water permeability of roots. For technical reasons, there are to date only a few experimental approaches to measure hydraulic conductivity at the root cell level and to relate this to the overall hydraulic conductivity of the entire organ during different stages of development (Steudle and Jeschke 1983, Steudle et al 1987, Zhu and Steudle 1991, Azaizeh et al 1992, Frensch et al 1996).

Composite transport in roots

Because there is no active water transport (i.e., water transport directly coupled to chemical reactions in root membranes), water uptake by roots has been often regarded as either a simple hydrostatic (hydraulic) or osmotic process governed by root hydraulic conductivity (Kramer and Boyer 1995). But results collected over the past decade show that this is not the case. Some changes in the transport mechanism(s) for water cause changes in root hydraulic conductivity (Lp_r). These changes are related to the intensity of water flow and to the nature of forces applied to drive water across the root. Depending on the species used, there are large differences between root Lp_r observed during either osmotic water flow (such as during conventional exudation of an excised root) or hydraulic water flow (such as in transpiring plants). The problem is how water uses the different pathways that are, according to the developmental state of the root, potentially available in the root cylinder. This means that results obtained with one experimental system can be quite different from those obtained with another. The reason for the variability is related to the question, Which pathways within the root cylinder are used? In principle, there are three different pathways (Fig. 1). The first is the apoplastic path around protoplasts. The second is the symplastic path, which transports water and solutes across plasmodesmata and within the cytoplasmic continuum. Last but not least is the transcellular or vacuolar pathway, which is across membranes. Because of the high permeability of membranes to water, this latter route is special for water. For ions and other solutes present in plant cells, this component's contribution will usually be negligible. Experimentally, symplastic and transcellular components of water flow cannot yet be separated. Therefore, they are summarized as a cell-to-cell or protoplastic component (Fig. 1).

In the usual picture of water transport in roots that textbooks describe, the flow of water across the root cortex is largely apoplastic. This changes at the endodermis because of the Casparian band, which interrupts the apoplastic path. There is a transcellular transport step at the endodermis, which is usually thought to rate-limit

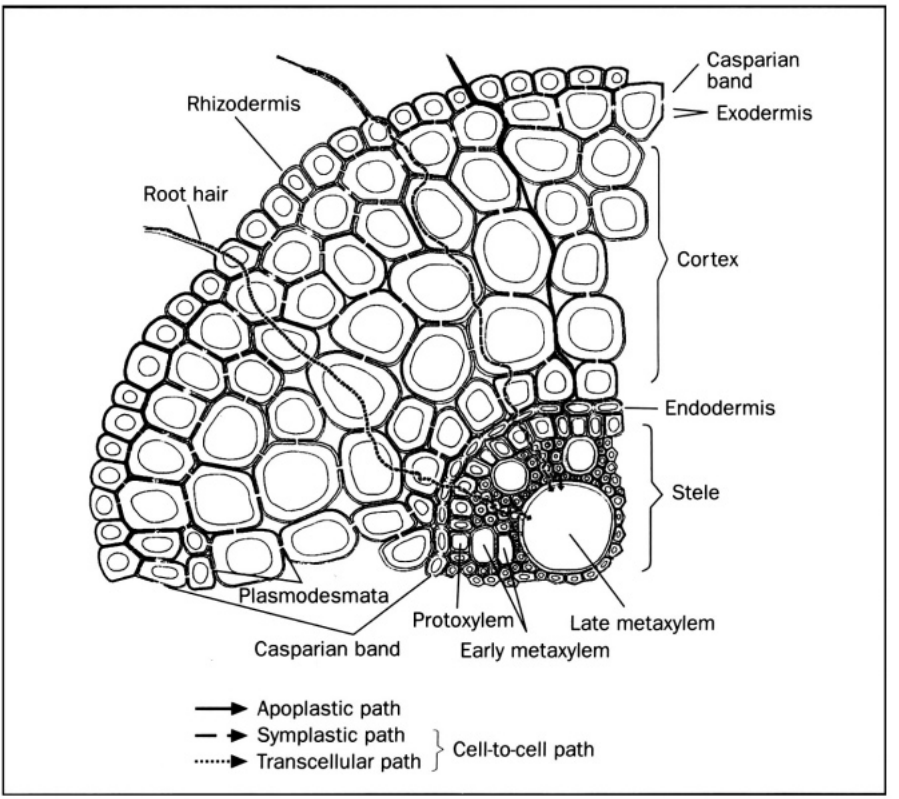

Fig. 1. Pathways for the uptake of water and solutes into the root. The apoplastic path (⟶) refers to movement within cell walls and intercellular spaces, which may be interrupted by Casparian bands in the endo- and exodermis. The symplastic path (— ▸) is through plasmodesmata and the cytosol of cells. Along the transcellular path (·····▸), water and solutes have to cross many cell membranes. This path is of minor importance for solute transport but represents an important path for water. Experimentally, the symplastic and transcellular components of water transport cannot yet be separated. The cell-to-cell component, however, is measurable (cell pressure probe). The cell-to-cell path is the sum of the symplastic and transcellular pathways.

water transport across young roots. In the stele, the situation is similar to that in the cortex.

Detailed measurements of root hydraulics have shown that this simple picture has to be modified. Depending on the conditions, the relative contribution of pathways to overall uptake or hydraulic conductivity may change substantially. A composite transport model of the root has been established, which is based in part on irreversible thermodynamics (Fig. 2; Steudle 1989, 1992, 1994a,b, 1997, Steudle and Frensch 1996, Steudle and Peterson 1998). The model shows that the different pathways may be used with different intensity. This should then result in the observed plasticity of water uptake. Besides the intensity of water flow, the physical nature of

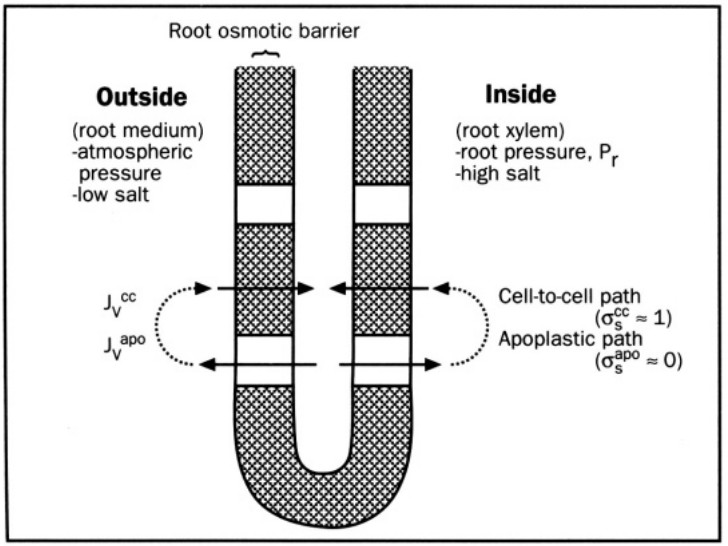

Fig 2. Composite transport model of root (schematical). The root osmotic barrier comprises cells (protoplasts) and the apoplast. The apoplastic path may be interrupted by Casparian bands in the endo- and exodermis (not shown for sake of simplicity). Water and solutes move along the two parallel pathways (cell-to-cell and apoplastic route). The cell-tocell path has a high selectivity (reflection coefficient, ($\sigma_s^{cc} \approx 1$) and the apoplastic path a very low selectivity ($\sigma_s^{apo} \approx 0$) for solutes. At low rates of transpiration, this results in a circulation flow of water in the root and in a low overall root σ_{sr} (as found experimentally): J_V^{cc} =water flow across the cell-to-cell path, J_V^{cc} = water flow across the apoplast. The model explains variable root hydraulic conductivity, which depends on the nature of the driving force and other factors. For further explanation, see text.

driving forces is crucial. In the presence of (hydrostatic) pressure gradients, the flow is largely around protoplasts (apoplastic) because this path represents a low hydraulic resistance. The data indicate that, to some extent, there should also be some apoplastic flow across the endodermis, i.e., Casparian bands appear to be somewhat permeable to water (but not to ions) during stage I or even later. This results in a high overall hydraulic conductivity of the root (root Lp_r), which is sometimes even larger than that of the plasma membrane of individual root cells. On the other hand, water flow in the presence of osmotic gradients is low, as would be the case in the absence of transpiration and during phenomena such as root exudation. Osmotic driving forces cause water movement only in the presence of membranes. So, an osmotic water flow across the root has to pass many membranes, which results in an overall root Lp_r that is then much less than that found during hydraulic water flow.

The composite transport model is based on detailed measurements of hydraulic conductivity and other transport properties (salt permeability and reflection coefficients) that have been obtained at the level of both individual cells and entire roots (see reviews: Steudle 1992, 1994a,b, 1997, Steudle and Frensch 1996, Steudle and

Peterson 1998). An important consequence of the model is that the hydraulic resistance of roots of transpiring plants will be low. Moreover, the supply from the root is adjusted according to shoot demands. In the absence of demand, there will be only osmotic gradients present due to the active uptake of solutes by the root. These, however, will cause a much smaller root Lp_r and water flow. Thus, in the absence of demand, root hydraulic resistance is high. As a consequence, the plant will not experience excessive losses of water to a dry or saline soil under these conditions. Composite transport provides some kind of coarse regulation of water flow across roots, which is just a consequence of the composite root structure. The composite transport model readily explains the variability of root hydraulic properties in terms of changes in forces that cause a switching between the pathways used. A dependence of the hydraulic conductivity along the apoplast has been discussed as well to account for some of the changes in the overall root Lp_r (Steudle and Frensch 1996). These changes in the hydraulic properties of the apoplast may be brought about by changes in the water content of cell walls and other factors. Most interestingly, root Lp_r is not as variable in some species as in others. For example, in *Phaseolus coccineus* and barley, the hydraulic and osmotic Lp_r were similar (Table 1). This has been explained by a high membrane Lp (in *P. coccineus*) or a rather tight Casparian band (in barley).

Interactions between water and solute flow

In the root, water and solutes (nutrients) interact with each other. This is most obvious under conditions of low or zero water flow (e.g., during the night when transpiration is switched off). Under these conditions, we observe phenomena such as guttation and root pressure. As solutes move across the root cylinder, osmotic concentrations within the apoplastic and protoplastic compartments change. This in turn causes a redistribution of water between pathways and compartments. The composite transport model accounts for osmotic interactions. It explains the finding that the overall reflection coefficient will be substantially smaller than unity. Solute diffusion along the apoplast is taken into account as well. Along the cell-to-cell path, the diffusive transport of solutes will usually be negligible because of the low membrane permeability of solutes (nutrients). Rates of active transport may be incorporated in the model if we have detailed information on the uptake by cells in different layers (Steudle 1992). In general, osmotic effects are important only under conditions of low transpiration. When substantial water flow is set up in a transpiring plant, osmotic concentrations within the root apoplast and the xylem are usually low and the flow is driven across the root cylinder by purely hydrostatic pressure gradients. Large changes in hydraulic conductivity usually occur when the mode of transport changes. The composite transport model accounts for these changes.

Another important point relates to water-solute interactions. Water uptake into the root xylem will reduce the osmotic concentration there and the overall force driving water into the root. This may be interpreted as some kind of feed-forward mechanism that modifies water uptake. It should be important only at low rates of transpiration. As water uptake increases with increasing transpiration, the concentration of

Table 1. Root hydraulic conductivity (Lp_r), solute permeability (P_{sr}), and reflection coefficients (σ_{sr}) of roots of herbaceous and woody species as determined with the root pressure probe and other techniques. Where available, hydraulic conductivities of root cell membranes (cortical cell Lp) are given for comparison. There are to date no data on cell Lp for tree roots. Because of the high cell Lp, there are no differences between osmotic and hydraulic water flow (Lpr) in barley and *Phaseolus coccineus.* For maize and *Phaseolus vulgaris,* there are large differences. Differences between osmotic and hydraulic water flow are much larger for roots of trees than for those of herbs. Although the data overlap somewhat, hydraulic tree root Lp, is on the average smaller by an order of magnitude than that of herbaceous plants. The difference between osmotic and hydraulic root Lp, is dramatic in trees. Differences also occur in roots of herbs. For both herbs and trees, values of root σ_{sr} are significantly lower than unity for solutes for which cell membranes exhibit a σ_s of virtual unity. For tree roots, σ_{sr} values are smaller by a factor of two than for the roots of herbs. The findings are explained by the means of the composite transport model of the root (see text).

Species	Root Lpr × 10^8 ($m\ s^{-1}\ MPa^{-1}$)		Root permeability, $P_{sr} \times 10^9$ ($m\ s^{-1}$)	Root reflection coefficient, σ_{sr} (1)	Techniques	References
	Hydraulic	Osmotic				
Herbaceous plants						
Hordeum distichon, primary root	0.3–4.3 Cell Lp: 12	0.3–4.3	–	Mannitol: ≈ 0.5	Cell and root pressure probe	[a]
Zea mays, primary root	1–46 Cell Lp: 24	0.1–5	Sucrose: 3.0 NaCl: 6–14	Mannitol: 0.4–0.7 Sucrose: 0.54 NaCl, KCl: 0.5–0.6	Cell and root pressure probe	[b] to [e]
Zea mays, root system	21	2.2	–	Nutrients: 0.85	Stop-flow technique and osmotic flow	[f] and [g]
Allium cepa, primary root	14	0.02–2	$NaNO_3$: 0.7	KCl, mannitol, $NaNO_3$, and NH_4NO_3: 0.35–0.88	Root pressure probe	[h] to [j]
Phaseolus coccineus, primary root	2–8 Cell Lp: 30–470	3–7	Mannitol: 0.15 NaCl: 0.21 KCl: 0.7–0.9	Mannitol: 0.68 NaCl: 0.59 KCl: 0.43–0.54	Root pressure probe	[h] to [j]

Table continued

Table continued.

Species	Root Lpr x 10^8 Root ($m\ s^{-1} MPa^{-1}$)		permeability, P_{sr} x 10^9 ($m\ s^{-1}$)	Root reflection coefficient, s_{sr} (1)	Techniques	References
	Hydraulic	Osmotic				
Phaseolus vulgaris, root system	30	0.56	Nutrients: 1.3	Nutrients: 0.98	Pressure chamber and osmotic flow	[f] and [k]
Woody plants						
Picea abies, root system	6.4	0.017	Not measured	Na_2SO_4 $K2SO_4$, $Ca(NO_3)_2$: 0.18-0.28	Root pressure probe	[h] to [j]
Quercus robur, root system	0.5-4.8	0.003–0.062	Not measured	Mannitol: 0.19–0.43 NaCl, KCl: 0.12–0.35	Root pressure probe	[h] to [j]
Fagus sylvatica, root system	0.35–1.6	0.022–0.11	Not measured	Mannitol: 0.29–0.82 KCl: 0.22–0.55 NaCl: 0.32–0.64	Root pressure probe	[h] to [j]

[a]Steudle and Jeschke (1983). [b]*Steudle* et al (1987). [c]Steudle and Frensch (1989). [d]Zhu and Steudle (1991). [e]Peterson et al (1993). [f]Newman (1973). [g]Miller (1985). [h]Melchior and Steudle (1993). [i]Melchior and Steudle (1995). [j]Steudle and Brinckmann (1989). [k]Fiscus (1986). [l]Rudinger et al (1994). [m]Steudle and Meshcheryakov (1996). [n]Steudle and Heydt (1997).

xylem sap is reduced. At large enough rates of uptake, the osmotic driving force will vanish. Fiscus (1975) analyzed this dilution effect quantitatively and found that it accounted for some of the apparent variability in root Lp_r reported in the older literature. This may be true, but the dilution effect cannot completely explain the variability in root hydraulics. Recent studies with excised root systems of maize have shown that most of the differences between osmotic and hydraulic Lp_r remain even when the dilution effect is accounted for (Zimmermann and Steudle 1998). Thus, there are inherent differences in root Lp_r that are related to changes in flow mechanisms. It has been suggested that the hydraulic conductivity of the material of the porous apoplast would increase with increasing flow (Steudle and Frensch 1996). In part, this increase may be due to changes in the water content of the apoplast. As was already pointed out, the changes are physiologically important, especially during plant responses to water stress.

The role of water channels in roots: Is there a fine regulation of water flow?

Water channels or aquaporins contribute to most of the hydraulic conductivity (water permeability) of plant cell membranes (Chrispeels and Maurel 1994, Steudle and Henzler 1995, Steudle 1997, Hertel and Steudle 1997, Maurel 1997, Schutz and Tyerman 1997, Schäffner 1998, Tyerman et al 1999). Water channels are transport proteins with a molecular weight of about 30 kDa. They span the membrane six times, forming a pore that is just wide enough to allow for the passage of water molecules one by one in a single file (Fig. 3). Water channels are quite selective for water, although there may be some slippage of small organic molecules (Henzler and Steudle 1995, Hertel and Steudle 1997). Mercurials such as mercuric chloride ($HgCl_2$) have been used to reversibly block the channel function. This has been demonstrated either in frog oocytes, where channel protein was expressed, or in *Chara* internodes (Chrispeels and Maurel 1994, Steudle and Henzler 1995, Schutz and Tyerman 1997). In root systems of tomato, Lp_r has been demonstrated to be reduced by treatment with mercurials, which suggested that water channels operate in roots, too (Maggio and Joly 1995). If true, this would be a possibility for regulating water uptake along the cell-to-cell passage, especially in older suberized roots where the apoplastic path is blocked. So, water channels may be looked at as a tool to provide some fine regulation of water uptake (Steudle 1997, Steudle and Peterson 1998). External factors such as high salinity, nutrient deprivation, annoxia, and temperature may affect regulation along the cell-to-cell path (Azaizeh and Steudle 1991, Azaizeh et al 1992, Birner and Steudle 1993, Carvajal et al 1996, Hertel and Steudle 1997, Henzler et al 1998). Regulation may be mediated by a phosphorylation of the transport proteins (Johansson et al 1996). Future work has to show whether this is true. This work should involve both the molecular characterization of transport proteins and the measurement of their function at the cell and root levels. In terms of root physiology, it is important to understand the mechanisms by which external and internal signals trigger water channel activity, that is, specify the gating properties of water channels (Tyerman et al 1999).

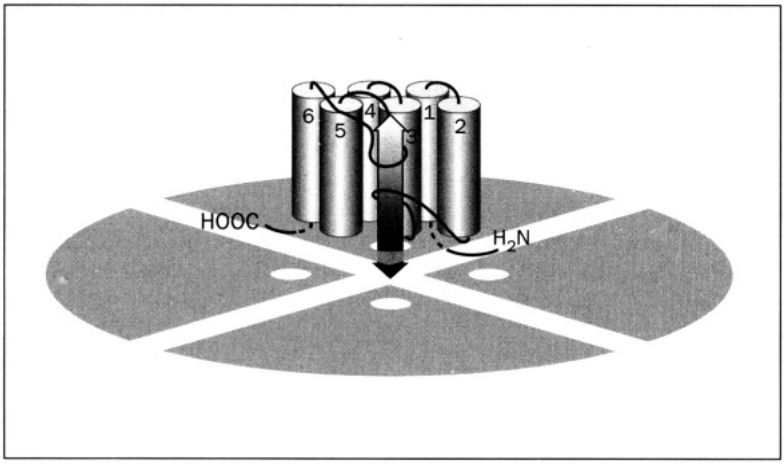

Fig. 3. Model of monomeric transport protein for water (aquaporin or water channel) arranged to form a pore ("hourglass model"). A single functional aquaporin is shown as a quarter of the tetrameric aggregate. Aquaporins consist of six transmembrane helices (1 to 6). The two loops between helices 2 and 3 (lower, intracellular loop) and helices 5 and 6 (upper, extracellular loop) form the channel, which represents a narrow pore selective for water and is penetrated by water in both directions (single-file passage). N- and C-terminals are on the apoplastic side of the membrane. (Adapted from Schaffner 1998.)

Experimental techniques

At the level of individual root cells, water transport (hydraulic conductivity, Lp) has been measured using the cell pressure probe (Steudle et al 1987, Zhu and Steudle 1991, Steudle 1993; Fig. 4). The tip of the probe was introduced into individual cells in different layers of the root, which allowed us to monitor cell turgor.

Cell Lp was measured after manipulating the equilibrium turgor to produce a water flow across the membrane, which was monitored as a "pressure relaxation." The half-time of the process is a measure of Lp. In the same or in a separate experiment, the root pressure probe has been employed to measure the overall hydraulic conductivity of roots (Steudle and Jeschke 1983, Steudle et al 1987, Zhu and Steudle 1991, Steudle 1993; Fig. 5). Figure 5 also shows how root Lp_r has been measured by alternative, steady-state techniques. With these techniques, either pressure gradients have been applied (pressure chamber, vacuum) or the osmotic pressure of the medium was varied (for details, see legend of Fig. 5). The root pressure probe has been used to separate the axial hydraulic resistance of xylem vessels from that related to flow across the root cylinder (Frensch and Steudle 1989, Melchior and Steudle 1993) and to measure the radial hydraulic resistances of individual root zones (Frensch et al 1996).

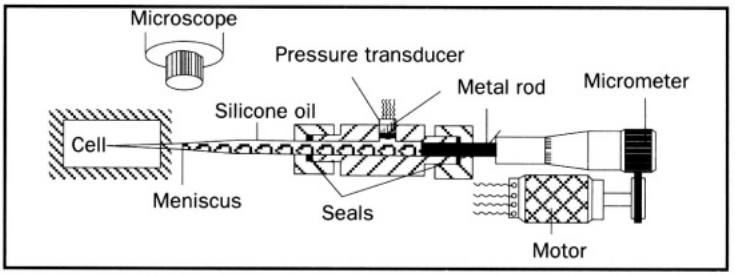

Fig. 4. Cell pressure probe for measuring the hydraulic conductivity (water permeability) of plant cell membranes. The tip of the microcapillary (diameter 2–7 μm) is introduced into the cell so that turgor causes a shift of the oil/water meniscus in the tip, which can be manipulated by moving the metal rod. Pressure relaxations are produced to work out the hydraulic conductivity of the membrane from half-times of the equilibration of turgor pressure. This requires us to also measure the elasticity of the cell (also done using the probe) and cell dimensions (cell surface area and volume).

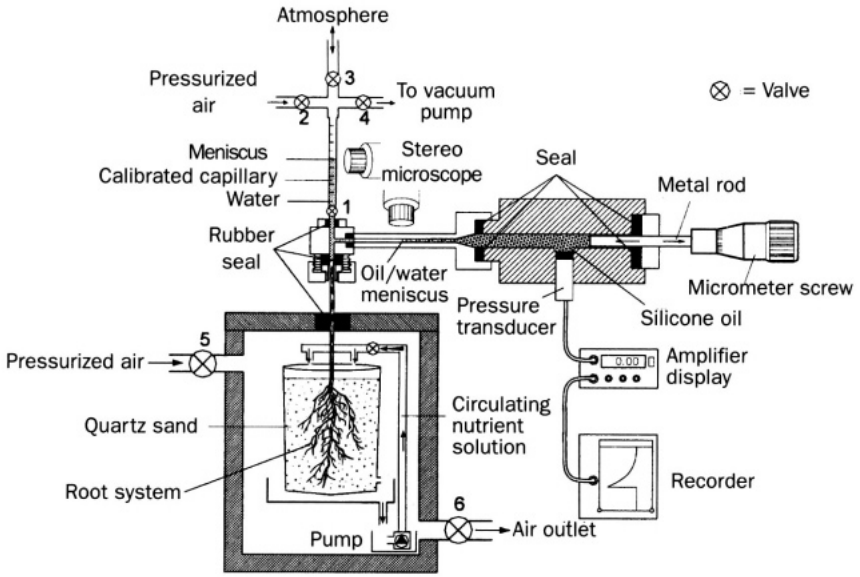

Fig. 5. Experimental set-up used to measure water transport and hydraulic conductivity of excised roots (root systems and individual roots). Different techniques can be used. In one procedure, root exudation is measured while pneumatic pressure is applied to the root system, which is tightly sealed in a pressure chamber. The protruding root base is connected to a calibrated capillary. With valves 1, 3, and 5 open and valves 2, 4, and 6 closed, the steady water flow across the root (root system) is measured in response to a pneumatic pressure applied to the root. Alternatively, a pressure gradient may be set up by applying a vacuum (valve 4 open) or pressurized air (valve 2 open) to the cut root base (xylem; valves 1, 5, and 6 open; valve 3 closed). In the absence of pressure gradients (all valves open, except 2, 4, and 5), water flow may also be measured in response to changes in the osmotic pressure of the root medium. With valves 1 and 5 closed and valve 6 open, the root pressure probe is employed to measure root Lp_r. In this type of experiment, root pressure builds up within the probe, which is then changed to induce a water flow, analogous to the procedure used for cells (Fig. 4). From the subsequent rate of equilibration of root pressure, root Lp_r is evaluated.

Recent results

Table 1 summarizes some results of root hydraulics. The table separates herbaceous and woody species. On the average, roots from herbs have a root Lp_r that is one order of magnitude larger than that of woody species because of differences in root structure, especially the stronger degree of suberization of woody roots. As for the water, the permeability of woody roots to solutes (permeability coefficients of salts, sugars, and the like) is substantially smaller than that of herbs. But root reflection coefficients of woody roots (which denote the passive selectivity of a root) were smaller than those of herbs. In terms of the composite transport model, this is explainable. In composite transport systems with parallel arrays, the components (cell-to-cell and apoplastic path) contribute to the overall reflection coefficient according to their hydraulic conductances. Because the permeability of woody roots is lower than that of herbs, the relative contribution of the apoplastic bypass is more significant for the former. In tree roots, differences of up to three orders of magnitude have been found between osmotic and hydraulic water flow (root Lp_r). This remarkable effect has been explained by the model but may, in addition, result from changes in the apoplastic hydraulic conductivity in the presence of pressure gradients (Steudle and Frensch 1996).

It should be noted that, besides the water, the apoplastic component of transport could also be important during root-to-shoot signaling. When the stress hormone abscisic acid (ABA) was applied to the root medium, there was a considerable bypass flow of ABA in young maize roots, that is, ABA was transported by solvent drag with the transpiration stream imitated by a vacuum applied to the cut end of the excised roots (Freundl et al 1998). In sunflower roots, the effect was much smaller. The results indicate that the uptake of ABA present in the soil solution in low concentrations could contribute to the xylem concentration of the hormone. Alternatively, and more likely, ABA produced in cells of stressed roots may be delivered to the root apoplast and then transferred to the shoot with the transpiration stream. In both cases, the apoplastic flow of ABA will compensate for the dilution caused by water uptake. Hence, the root-to-shoot signal of ABA may have an apoplastic component that is directly coupled to water uptake and transpiration.

Conclusions

The detailed data obtained so far for root hydraulics are in agreement with a composite transport model of the root. The model considers two parallel pathways—the apoplast and the cell-to-cell (protoplastic) path. Pathways are coupled to each other, that is, water and solutes are exchanged between pathways as they move across tissues. The model explains differences found between hydraulic and osmotic water flow and the variability of root Lp_r, which changes in response to the nature of the driving force and the intensity of flow. The model predicts that root Lp_r may vary according to conditions (as found). We propose that the variability in root Lp_r provides a coarse regulation of water uptake by roots. A fine regulation may be brought about by the

action of water channels, which affect the cell-to-cell pathway rather than the apoplastic pathway. Future work has to concentrate on the role of water channels during water uptake, that is, the activity of aquaporins has to be mapped in different root tissues. The modes by which external and internal signals trigger water channels have to be worked out (drought, salinity, temperature, nutritional state, heavy metals, etc.). The use of transgenic plants and of reverse genetics will play an important role in these future studies (Tyerman et al 1999). Another important point is the separation of series of hydraulic resistances in the root cylinder, especially those of the exodermis and endodermis, and how they would change during root development. Recent data from maize indicate that the development of an exodermis may strongly decrease overall root Lp_r. The relative contributions of the cell-to-cell and apoplastic components of radial water flow and how its relative importance would change during root development have to be further quantified.

References

Azaizeh H, Gunse B, Steudle E. 1992. Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings. Plant Physiol. 99:886-894.

Azaizeh H, Steudle E. 1991. Effects of salinity on water transport of excised maize (*Zea mays* L.) roots. Plant Physiol. 99:1136-1145.

Birner TP, Steudle E. 1993. Effects of anaerobic conditions on water and solute relations and active transport in root of maize (*Zea mays* L.). Planta 190:474-483.

Carvajal M, Cooke DT, Clarkson DT. 1996. Responses of wheat plants to nutrition deprivation may involve the regulation of water-channel function. Planta 199:372-381.

Chrispeels MJ, Maurel C. 1994. Aquaporins: the molecular basis of facilitated water movement through living plant cells. Plant Physiol. 105:9-15.

Cruz RT, Jordan WR, Drew MC. 1992. Structural changes and associated reduction of hydraulic conductance in roots of Sorghum bicolor L. following exposure to water deficit. Plant Physiol. 99:203-212.

Fiscus EL. 1975. The interaction of osmotic- and pressure-induced water flow in plant roots. Plant Physiol. 55:917-922.

Fiscus EL. 1986. Diurnal changes in volume and solute transport coefficients of *Phaseolus* roots. Plant Physiol. 80:752-759.

Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). Plant Physiol. 91:719-726.

Frensch J, Hsiao TC, Steudle E. 1996. Water and solute transport along developing maize roots. Planta 198:348-355.

Freundl E, Steudle E, Hartung W. 1998. Water uptake by roots of maize and sunflower affects the radial transport of abscisic acid and its concentration in the xylem. Planta 207:8-19.

Henzler T, Steudle E. 1995. Reversible closing of water channels in *Chara* internodes provides evidence for a composite transport model of the plasma membrane. J. Exp. Bot. 46:199-209.

Henzler T, Carvajal M, Smyth A, Cooke DT, Clarkson DT. 1998. Diurnal variation in root hydraulics and aquaporin gene-homologue expression in *Lotus japonicus.* J. Exp. Bot. 49S:10.

Hertel A, Steudle E. 1997. The function of water channels in *Chara:* the temperature dependence of water and solute flows provides evidence for composite membrane transport and for a slippage of small organic solutes across water channels. Planta 202:324-335.

Johansson I, Larsson C, Ek B, Kjellbom P. 1996. The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca^{2+} and apoplastic water potential. Plant Cell 8:1181-1191.

Kramer PJ, Boyer JS. 1995. Water relations of plants and soils. Orlando, Fla. (USA): Academic Press.

Maggio A, Joly R.J. 1995. Effects of mercuric chloride on the hydraulic conductivity of tomato root systems: evidence for a channel-mediated pathway. Plant Physiol. 109:331-335.

Maurel C. 1997. Aquaporins and water permeability of plant membranes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:399-429.

Melchior W, Steudle E. 1993. Water transport in onion (*Allium cepa* L.) roots: changes of axial and radial hydraulic conductivities during root development. Plant Physiol. 101:1305-1315.

Melchior W, Steudle E. 1995. Hydrostatic and osmotic hydraulic conductivities and reflection coefficients of onion (*Allium cepa* L.) roots. In: Baluska F, Ciamporova M, Gasparikova O, Barlow PW, editors. Structure and function of roots. Dordrecht (Netherlands): Kluwer Academic Press. p 209-213.

Miller DM. 1985. Studies of root function in *Zea mays.* III. Xylem sap composition at maximum root pressure provides evidence of active transport into the xylem and a measurement of the reflection coefficient of the root. Plant Physiol. 77:162-167.

Newman EI. 1973. Permeability to water of five herbaceous species. New Phytol. 72:547-555.

North GB, Nobel PS. 1991. Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of *Agave desertii* (Agavaceae). Am. J. Bot. 78:906-915.

Peterson CA. 1988. Exodermal Casparian bands: their significance for ion uptake by roots. Physiol. Plant. 72:204-208.

Peterson CA, Enstone DE. 1996. Functions of passage cells in the endodermis and exodermis of roots. Physiol. Plant. 97:592-598.

Peterson CA, Murrmann M, Steudle E. 1993. Location of major barriers to water and ion movement in young roots of *Zea mays* L. Planta 190:127-136.

Peyrano G, Taleisnik E, Quiroga M, de Forchetti SM, Tigier H. 1997. Salinity effects on hydraulic conductance, lignin content and peroxidase activity in tomato roots. Plant Physiol. Biochem. 35:387-393.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED. 1994. Hydraulic and osmotic properties of spruce roots. J. Exp. Bot. 45:1413-1425.

Schäffner AR. 1998. Aquaporin function, structure and expression: are there more surprises to surface in plant water relations? Planta 204:131-139.

Schütz K, Tyerman SD. 1997. Water channels in *Chara corallina.* J. Exp. Bot. 48:1511-1518.

Stavosky E, Peterson CA. 1993. Effects of drought and subsequent rehydration on the structure, vitality and permeability of *Allium cepa* adventitious roots. Can. J. Bot. 71:700-707.

Steudle E. 1989. Water flow in plants and its coupling to other processes: an overview. Meth. Enzymol. 174:183-225.

Steudle E. 1992. The biophysics of plant water: compartmentation, coupling with metabolic processes, and flow of water in plant roots. In: Somero GN, Osmond CB, Bolis CL, editors. Water and life: comparative analysis of water relationships at the organismic, cellular, and molecular levels. Heidelberg (Germany): Springer-Verlag. p 173-204.

Steudle E. 1993. Pressure probe techniques: basic principles and application to studies of water and solute relations at the cell, tissue and organ level. In: Smith JAC, Griffiths H, editors. Water deficits: plant responses from cell to community. Oxford (UK): Bios Scientific Publishers Ltd. p 5-36.

Steudle E. 1994a. Water transport across roots. Plant Soil 167:79-90.

Steudle E. 1994b. The regulation of plant water at the cell, tissue and organ level: role of active processes and of compartmentation. In: Schulze E-D, editor. Flux control in biological systems: from enzymes to population and ecosystems. San Diego, Calif. (USA): Academic Press, Inc. p 237-299.

Steudle E. 1997. Water transport across plant tissue: role of water channels. Biol. Cell 89:259-273.

Steudle E, Brinckmann E. 1989. The osmometer model of the root: water and solute relations of *Phaseolus coccineus*. Bot. Acta 102:85-95.

Steudle E, Frensch J. 1989. Osmotic responses of maize roots: water and solute relations. Planta 177:281-295.

Steudle E, Frensch J. 1996. Water transport in plants: role of the apoplast. Plant Soil 187:67-79.

Steudle E, Henzler T. 1995. Water channels in plants: Do basic concepts of water transport change? J. Exp. Bot. 46:1067-1076.

Steudle E, Heydt H. 1997. Water transport across tree roots. In: Rennenberg H, Eschrich W, Ziegler H, editors. Trees: contributions to modern tree physiology. Leiden (Netherlands): Backhuys Publishers. p 239-255.

Steudle E, Jeschke WD. 1983. Water transport in barley roots. Planta 158:237-248.

Steudle E, Meshcheryakov AB. 1996. Hydraulic and osmotic properties of oak roots. J. Exp. Bot. 47:387-401.

Steudle E, Murrmann M, Peterson CA. 1993. Transport of water and solutes across maize roots modified by puncturing the endodermis: further evidence for the composite transport model of the root. Plant Physiol. 103:335-349.

Steudle E, Oren R, Schulze ED. 1987. Water transport in maize roots. Plant Physiol. 84:1220-1232.

Steudle E, Peterson CA. 1998. How does water get through roots? J. Exp. Bot, 49:775-788.

Tyerman SD, Bohnert H, Maurel C, Steudle E, Smith JAC. 1999. Plant water channels: molecular biology meets biophysics. J. Exp. Bot. (In press.)

Wayne R, Tazawa M. 1990. Nature of water channels in the internodal cells of *Nitellopsis*. J. Membrane Biol. 116:31-39.

Zhu GL, Steudle E. 1991. Water transport across maize roots. Plant Physiol. 95:305-315.

Zimmermann HM, Steudle E. 1998. Apoplastic transport across young maize roots: effect of the exodermis. Planta 206:7-19.

Notes

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Physiological characterization of the rice plant for tolerance of water deficit

T. Hirasawa

Rice plants are grown in upland fields and in irrigated and nonirrigated fields. Soil moisture depletion suppresses the midday photosynthetic rate as well as leaf expansion and causes severe drought injury to panicles in the critical stages. The photosynthetic rate also decreases at midday and severe panicle dehydration sometimes occurs when the foehn blows at heading even under submerged soil conditions. A depletion in soil moisture also diminishes the photosynthetic rate because of senescence.

Compared with other crop plants, (1) rice plants suffer from water stress markedly when soil moisture decreases because of their small root system, (2) the stomata of rice plants close noticeably in response to a reduction in leaf water potential, causing a marked reduction in photosynthetic rate, and (3) leaf senescence of rice plants is promoted with a small reduction in soil moisture. Significant differences in root system development and drought resistance have been observed among rice cultivars. Water uptake capacity might depend on root system development and on root hydraulic conductivity. Many factors, such as the rate, duration, and direction of root elongation and root branching, might contribute to root system development. Root hydraulic conductivity changes with growth conditions and age. Some root functions might affect leaf senescence under drought. We need a better understanding of root system development, the path of water transport in roots, and root-shoot relationships and their genetics.

Rice plants are grown in various ecosystems defined by hydrological conditions. Drought often occurs in rice-growing areas and decreases the yield of upland and rainfed lowland rice (O'Toole and Chang 1979). Soil moisture depletion suppresses leaf expansion, tillering, and midday photosynthesis (Kramer and Boyer, 1995, Hanada 1990). It also causes a reduction in photosynthetic rate and leaf area because of senescence (Nooden 1988). All of these factors induce a reduction in dry matter accumulation and grain yield. Severe drought injury to panicles occurs when soil moisture diminishes at the panicle formation and heading stages (Tajima 1995).

Rice plants suffer from water stress even under submerged soil conditions. The photosynthetic rate also decreases at midday and in the afternoon on a clear day because of the reduction in leaf water potential (Ishihara and Saito 1987). A reduction in

vapor pressure deficit also decreases the photosynthetic rate without causing a reduction in leaf water potential (Hirasawa et al 1988). A rapid and severe panicle dehydration sometimes occurs when the foehn blows at heading, causing a white head (Tsuboi 1995). Resistance to water flow increased markedly at the panicle neck because of the formation of air bubbles in xylem vessels under these conditions, and this may cause panicle dehydration (Hirasawa et al 1996). A rapid and severe shoot dehydration is also observed in the middle of the ripening stage (Murayama 1995).

This chapter discusses ecophysiological properties for tolerance of water deficits in rice plants growing in imgated and rainfed conditions.

Comparing responses to soil moisture depletion

Growth and dry matter production

Upland rice represents about 0.5% of rice area in Japan. Upland rice plants are often affected by soil moisture depletion (Hirayama et al 1997, Tajima 1995). We compared growth and dry matter production of upland rice with those of other crop plants in a field (loamy soil) with a rainout shelter. Leaf expansion and dry matter production decreased significantly in rice plants compared with foxtail millet and common millet (Fig. 1; Fuse et al 1992). When plants were grown without irrigation in sandy soil in a greenhouse, only rice plants died before flowering although other crop plants (soybean, finger millet, groundnut, foxtail millet, and common millet) ripened (Hirasawa and Inada 1991).

Leaf water potential decreased significantly in rice plants compared with other crop plants (Table 1; Fuse et al 1992). This could result in the reduction in leaf expansion and dry matter production in rice plants. Leaf water potential recovered slowly during the night and was still low at predawn in rice plants (Table 1). Because rice

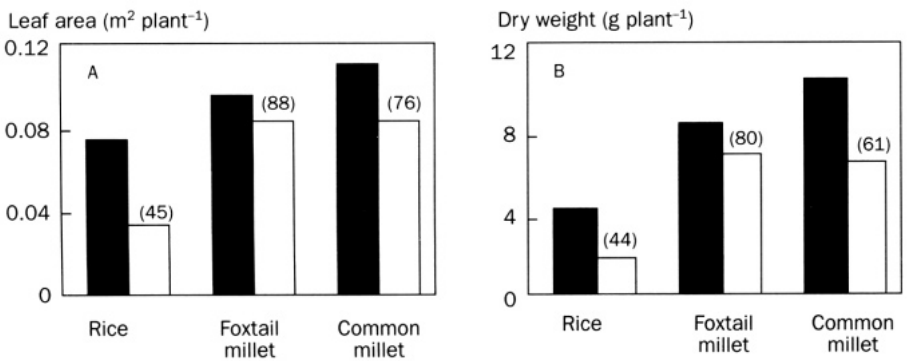

Fig. 1. Leaf area (A) and dry weight (B) of rice, foxtail millet, and common millet growing under sufficient (■) and deficient (□) soil moisture conditions 60 days after planting. Numbers in parentheses indicate the relative values of plants under deficient soil moisture conditions versus plants under sufficient soil moisture conditions. (Adapted from Fuse et al 1992.)

Table 1. Leaf xylem water potential (MPa) of rice, foxtail millet, and common millet growing in wet and dry soil in the field at midday, evening, and predawn. Data represent means ± standard deviations. (Adapted from Fuse et al 1992.)

Crop	Wet soil			Dry soil		
	Midday	Evening	Predawn	Midday	Evening	Predawn
Rice	–0.92 ± 0.04	–0.13 ± 0.01	–0.03 ± 0.01	–2.37 ± 0.06	–1.53 ± 0.24	–0.68 ± 0.13
Foxtail millet	–0.97 ± 0.16	–0.22 ± 0.02	–0.03 ± 0.01	–1.78 ± 0.12	–0.79 ± 0.03	–0.18 ± 0.05
Common millet	–0.48 ± 0.09	–0.15	–0.01 ± 0.00	–1.32 ± 0.32	–0.29 ± 0.05	–0.03 ± 0.01

plants have a poor root system, their leaf water potential decreased significantly with the depletion of soil moisture compared with other crop plants (Angus et al 1983, O'Toole and Chang 1979, Yoshida and Hasegawa 1982).

Effects of a reduction in leaf water potential on photosynthetic rate

The leaf photosynthetic rate decreases even with a slight reduction in leaf water potential in rice plants. As Figure 2 shows, photosynthetic rate started to decrease rapidly at a water potential of about –0.3 MPa for cv. Manryo (grown in irrigated conditions) and about –0.6 MPa for cv. Warabehatamochi (grown in upland conditions). On the other hand, a clear reduction in photosynthetic rate was not observed at a leaf water potential higher than –1.0 MPa in foxtail millet, common millet, wheat, and sunflower plants (Fig. 2). In the plants under water stress, the reduction in photosynthetic rate is usually induced by stomatal closure. As leaf water potential decreases further, nonstomatal factors also affect photosynthesis (Wakabayashi et al 1996). Therefore, researchers conclude that, in rice plants, the response to water potential is largely governed by stomatal behavior.

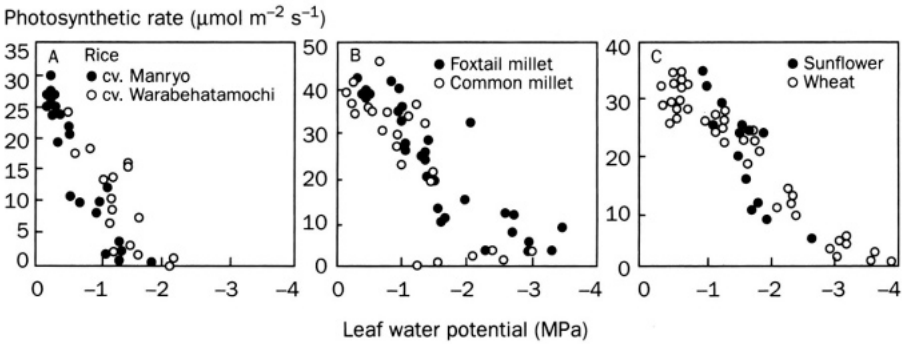

Fig. 2. Relationships between leaf water potential and photosynthetic rate in rice (A), foxtail millet and common millet (B), and sunflower and wheat (C). (Adapted from Fuse et al 1994, Hirasawa et al 1988, Wakabayashi et al 1996, Nakagami and Hirasawa, unpublished.)

Some differences in the relationship between water potential and turgor pressure were observed among leaves of different species (Fig. 3). Turgor pressure remained high at any reduction in leaf water potential in wheat plants compared with rice plants, but the relationship in rice plants did not differ significantly from that of foxtail millet and common millet. These results indicate that the difference in stomatal response to the reduction in leaf water potential among species cannot always be attributed to the maintenance of leaf turgor pressure.

Soil moisture depletion and leaf senescence

Soil moisture depletion also causes a reduction in photosynthetic rate because of senescence. The reduced photosynthetic rate usually does not recover even after irrigation. Leaf senescence progressed rapidly in plants grown in soil with lower moisture even when remarkable reductions in leaf water potential were not observed (Fig. 4). Irrigated (ponded) rice culture uses more water than other field crops. Intermittent irrigation is effective in decreasing irrigation water without reducing grain yield. But progressive leaf senescence was observed in irrigated fields under the treatment with intermittent irrigation. Even though the soil matric potential at a depth of 15 cm was kept at about –10 kPa during ripening, the leaf photosynthetic rate decreased significantly because of early senescence and the midday closure of stomata, causing a reduction in dry matter accumulation and grain yield (Lu et a1 1998).

Field crop plants usually perform well in soil with a matric potential lower than –10 kPa. Wheat plants maintained a higher leaf chlorophyll content and had a smaller reduction in photosynthesis during ripening even though the soil matric potential de-

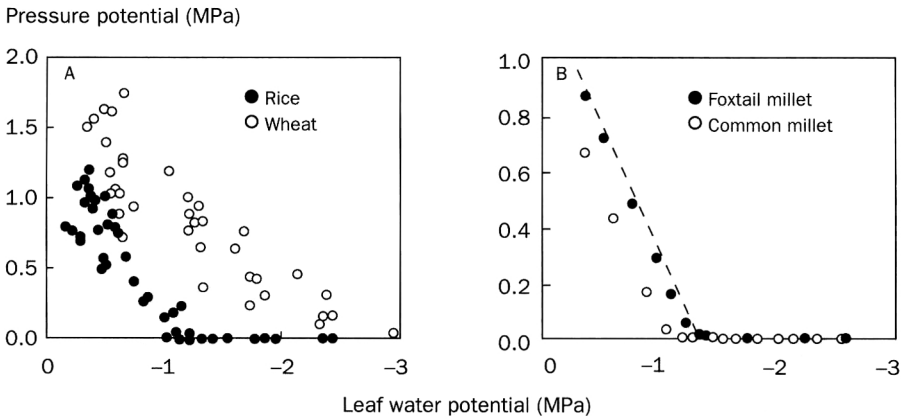

Fig. 3. Relationships between leaf water potential and pressure potential in rice and wheat (A) and foxtail millet and common millet (B). (Adapted from Hirasawa et al 1979, Tabei et al 1997, Nakagami and Hirasawa, unpublished.) In (A), water and osmotic potentials were measured with a thermocouple psychrometer and pressure potential was calculated. In (B), the relationship was determined by a pressure-volume technique. The broken line in B is for rice plants from Cutler et al (1980).

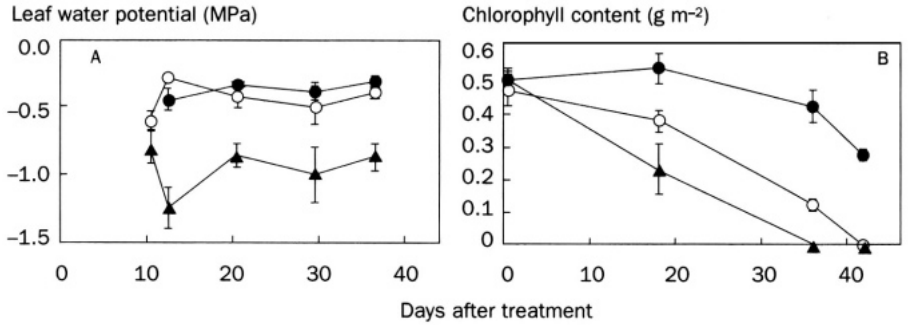

Fig. 4. Changes in leaf water potential during daytime (A) and leaf chlorophyll content (B) in rice plants growing in submerged soil (●), and in soil with moderately (○) and remarkably (p) reduced moisture. (Adapted from Hirasawa et al, unpublished.)

creased to −80Wa at a depth of 30 cm (Nakagami et al 1998). Leaf senescence of rice plants might be promoted with a small reduction in soil moisture compared with other crop plants. The effect of soil moisture depletion might result from the changes in chemical properties of soil after drainage as well as the reduction in plant water potential.

Characterizing tolerance of water deficits

We already have some general concepts of the characteristics that enable plants to escape or tolerate drought (e.g., Kramer and Boyer 1995, Loomis and Conner 1992). But we do not yet have clear ideas of the characteristics in rice and other crops for the genetic improvement of plants under conditions of water stress. We therefore tried to characterize the ecophysiological properties of rice plants for water uptake and leaf senescence for maintaining a high leaf photosynthetic rate under stress conditions.

Total root length and distribution in soil

Although rice plants tend to develop a poor root system compared with other field crops, large differences in root system development have been observed among rice cultivars (Ekanayake et al 1985, Hirayama et al 1997, O'Toole and Chang 1979, Yoshida and Hasegawa 1982). Plants with a larger root system can maintain a high rate of water uptake from the soil with a depletion in soil moisture and can therefore keep leaf water potential high (Hirasawa 1995, O'Toole and Chang 1997, Yoshida and Hasegawa 1982). A denser and deeper rooting habit is important for plant performance under deficient soil moisture conditions. At the same soil water potential, the total length or total area of root surface is important because the larger the area of root surface, the smaller the root resistance to water transport in general. Because moisture remains high in deeper soil when soil moisture decreases, the reduction in leaf water potential is small in plants with a deeper root system (Hirasawa et al 1994, Hirasawa 1995).

Table 2. Comparison of morphologlcal characteristics and resistance to water transport through the plant between Nipponbare and Akenohoshi grown in pots (30 cm in diameter, 100 cm in depth) under submerged soil conditions. (Adapted from Jiang et al 1988.)

Growth stage	Variety	Panicles (no.)	Top weight (g)	Leaf area (m^2)	Root weight (g)	Top-root weight ratio ($g\ g^{-1}$)	Leaf area-root weight ratio ($cm^2\ g^{-1}$)	Resistance to water transport (10^4 MPa s m^{-1})
Booting	Nipponbare	25.0	65.6	0.455	8.5	7.71	536	–
	Akenohoshi	20.3	68.4	0.449	9.8	7.01	459	–
Ripening	Nipponbare	25.0	121.8	0.383	11.0	11.04	317	11.5 ± 0.17[b]
	Akenohoshi	18.0	128.2	0.263[a]	16.0	8.01	165	8.6 ± 0.10[b]

[a]The decrease in leaf area of Akenohoshi was partly due to the death of nonproductive tillers at the ripening stage. [b]Means ± standard deviations.

The soil water potential at the root surface is quite different from the water potential of bulk soil, especially when the soil water potential decreases (Kramer 1983). This becomes quite complex when water movement in the soil at the root surface is estimated quantitatively. We can estimate the root water uptake capacity of plants growing in the field under submerged soil conditions because soil water potential can be regarded as 0 MPa and soil resistance can be ignored (Hirasawa and Ishihara 1991). Total resistance in plants to water transport is lower in cultivars with a larger length of roots because of lower root resistance (Table 2; Jiang et al 1988).

A significant difference in root system development has also been observed among plants grown under different conditions and among cultivars in wet soil. The extent of root system development also differed in dry soil between soybean cultivars (Hida et al 1995). The difference came from the rate of root branching in young plants (Hida et al 1996). Factors such as the rate and direction of elongation and branching contribute to root system development. We should examine the true status of differences in root system development among cultivars to improve this development when breeding drought-tolerant cultivars.

Root hydraulic conductivity

Root water uptake capacity is affected by hydraulic conductance per unit root surface or hydraulic conductivity and total area of root surface. Hydraulic conductivity decreases markedly with an increase in water uptake rate when the transpiration rate is low, and is constant at a transpiration rate larger than about 3.0 mmol H_2O m^{-2} s^{-1} in rice plants (Hirasawa and Ishihara 1991). Root conductivity decreased in plants where roots were treated with NaN_3 and in plants growing in reductive soil (Hirasawa et a1 1992a). It also decreased with age (Fig. 5). Radial resistance is usually significantly high in roots compared with axial resistance, except for the root apex (Frensch and Steudle 1989). The possible radial paths of water in roots have been proposed (Steudle 1994, Taura et al 1988). However, fundamental information on the radial path of

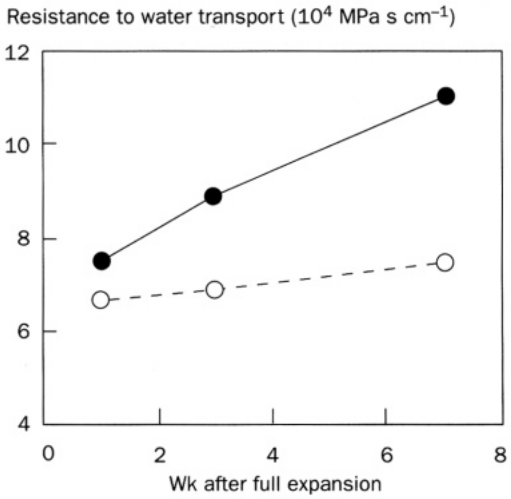

Fig. 5. Changes in resistance to water transport from roots to the 12th leaf (●) and resistance from the base of the stem to the leaf (O) in rice plants growing under submerged conditions. (Adapted from Hirasawa et al 1992b.)

water transport in rice is still lacking. We need to know the radial path of water and its characteristics in roots before we can know which properties should be improved for increasing water uptake capacity in roots.

Leaf senescence and root functions

The reduction in leaf photosynthetic rate because of senescence in the ripening stage was smaller for rice cv. Akenohoshi than for cv. Nipponbare under submerged conditions (Jiang et al 1988, Soejima et al 1992). The exudation rate at the base of the stem was higher in Akenohoshi. The total amount of cytokinins transported from roots to shoots was estimated to be significantly higher in Akenohoshi, based on the cytokinin content of the exudate (Soejima et al 1992, 1995). Cytokinins transported to shoots from roots were expected to be one factor in the slow leaf senescence during ripening in Akenohoshi.

Nitrogen and other mineral uptake decreased markedly with the reduction in soil moisture in rice plants (O'Toole and Baldia 1982). Nitrogen is a very important nutrient for keeping a high content of ribulose-l,5-bisphosphate carboxylase (Rubisco) and therefore a high rate of photosynthesis after leaf expansion of rice plants (Hidema et al 1991, Makino et al 1983). When leaf senescence was promoted with slightly decreased moisture, the exudation rate at the base of the stem had already decreased significantly (Table 3). The reduction of solute accumulation in xylem might contribute to the decrease in the exudation rate. We also found that leaf senescence of a wilting mutant, growing under submerged soil conditions, was slower than that of the wild type, growing under moderately deficient soil moisture conditions, even though the mutant had a greater leaf xylem resistance to water transport and, therefore, a lower midday leaf water potential (Hirasawa et al, unpublished). The root exudation rate was significantly higher in the former plants than in the latter plants.

Table 3. Exudation rate from the base of the stem In rice plants growing In submerged and moderately dry soils. Data represent means ± standard devlations. (Adapted from Hirasawa et al, unpublished.)

Item	Submerged soil	Dry soil
Leaf water potential[a]	–2.80 ± 0.60	–3.70 ± 1.00
Exudation rate (g hill^{-1})[b]	12.7 ± 0.4	1.3 ± 0.9

[a]Leaf water potential was measured at midday. [b]Exudation rate was measured for 24 h in the room.

These results suggest that root functions affect leaf senescence and that decreased soil moisture might promote leaf senescence by slowing root functions. Leaf senescence might be affected by the nitrogen supply from roots as well as cytokinins. Abscisic acid might also contribute to leaf senescence. The role of roots in leaf senescence should be investigated.

Final remarks

Rice plants are grown in soils with a wide range of moisture. They suffer from water stress even under submerged conditions. We are now forming a general concept on drought tolerance of plants. We need to clarify the true status of water deficits under these conditions. We also need to clarify characteristics relating to drought tolerance and define their genetics for any crop in order to achieve a definitive form of drought tolerance.

References

Angus JF, Hasegawa S, Hsiao TC, Liboon PS, Zandstra HG. 1983. The water balance of post monsoonal dryland crops. J. Agric. Sci. 101:699-710.

Cutler JM, Shahan KW, Steponkus PL. 1980. Alteration of the internal water relations of rice in response to drought hardening. Crop Sci. 20:307-310.

Ekanayake II, O'Toole JC, Garrity DP, Masajo TM. 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Sci. 25:927-933.

Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). Plant Physiol. 91:719-726.

Fuse Y, Hirasawa T, Ishihara K. 1992. Differences in the response to soil moisture depletion among foxtail millet, common millet and rice plants. Rep. Kanto Br. Crop Sci. Soc. Jpn. 7:43-44.

Fuse Y, Hirasawa T, Ishihara K. 1994. Differences in the response to soil moisture depletion between foxtail millet and common millet. Jpn. J. Crop Sci. 63(Extra issue 1):238-239.

Hanada K. 1990. Tiller. In: Matsuo T, Shimizu M, Tsunoda S, Murata Y, Kumazawa K, Futsuhara Y, Hoshikawa K, Maeda E, Yamazaki K, editors. Science of rice plant. 1. Morphology. Tokyo: Nobunkyo. p 173-203.

Hida Y, Hirasawa T, Ishihara K. 1995. Differences in dry matter production and root system development between soybean cultivars under deficient soil moisture conditions. Jpn. J. Crop Sci. 64:573-580.

Hida Y, Hirasawa T, Ishihara K. 1996. Comparison of root system development of soybean seedlings between cv. Enrei and Tachinagaha under different soil moisture conditions. Jpn. J. Crop Sci. 65(Extra issue 1):198-199.

Hidema J, Makino A, Mae T, Ojima K. 1991. Photosynthetic characteristics of rice leaves aged under different irradiances from full expansion through senescence. Plant Physiol. 97:1287-1293.

Hirasawa T, Ishihara K, Ogura T. 1979. Relationships among leaf water potential, osmotic potential and pressure potential in rice plants. Jpn. J. Crop Sci. 48(Extra issue 2):71-72.

Hirasawa T, Iida Y, Ishihara K. 1988. Effect of leaf water potential and air humidity on photosynthetic rate and diffusive conductance in rice plants. Jpn. J. Crop Sci. 57:112-118.

Hirasawa T, Ishihara K. 1991. On resistance to water transport in crop plants for estimating water uptake ability under intense transpiration. Jpn. J. Crop Sci. 60:174-183.

Hirasawa T, Tsuchida M, Ishihara K. 1992a. Relationship between resistance to water transport and exudation rate and the effect of the resistance on the midday depression of stomatal aperture in rice plants. Jpn. J. Crop Sci. 61:145-152.

Hirasawa T, Goto T, Ishihara K. 1992b. On resistance to water transport from roots to the leaves at the different positions on a stem in rice plants. Jpn. J. Crop Sci. 61: 153-158.

Hirasawa T, Inada K. 1991. Interspecific and varietal differences in water uptake capacity under depleted soil moisture conditions. Report of Cooperative Research in Arid-Land Research Center, Tottori University. p 3-4.

Hirasawa T, Tanaka K, Miyamoto D, Takei M, Ishihara K. 1994. Effects of pre-flowering soil moisture deficits on dry matter production and ecophysiological characteristics in soybean plants under drought conditions during grain filling. Jpn. J. Crop Sci. 63:721-730.

Hirasawa T. 1995. Water relations in plants. In: Matsuo T, Kumazawa K, Ishii R, Ishihara K, Hirata H, editors. Science of the rice plant. 2. Physiology. Tokyo Food and Agriculture Policy Research Center. p 434-460.

Hirasawa T, Tenmyo N, Suzuki M, Ishihara K. 1996. Mechanism on occurrence of white head of rice plants at heading stage under high temperature, low humidity and high wind velocity conditions: factors increasing stem resistance to water flow at the panicle base. Jpn. J. Crop Sci. 65(Extra issue 2):129-130.

Hirayama M, Nemoto H, Okamoto K, Miyamoto M, Sugo R. 1997. Relationship between drought tolerance and vertical distribution of roots in upland rice cultivars. Jpn. J. Crop Sci. 66(Extra issue 1):222-223.

Ishihara K, Saito K. 1987. Diurnal course of photosynthesis, transpiration, and diffusive conductance in the single-leaf of rice plants grown in the paddy field under submerged condition. Jpn. J. Crop Sci. 56:8-17

Jiang CZ, Hirasawa T, Ishihara K. 1988. Physiological and ecological characteristics of high yielding varieties in rice plants. II. Leaf photosynthetic rate. Jpn. J. Crop Sci. 57: 139-145.

Kramer PJ. 1983. Water relations of plants. New York (USA): Academic Press. 489 p.

Kramer PJ, Boyer JS. 1995. Water relations of plants and soils. San Diego, Calif. (USA): Academic Press. 495 p.

Loomis RS, Connor DJ. 1992. Crop ecology: productivity and management in agricultural systems. Cambridge (UK): Cambridge University Press. 538 p.

Lu J, Ookawa T, Hirasawa T. 1998. Effects of irrigation pattern on paddy rice production and its water use efficiency. Abstract of the 3rd Asian Crop Science Conference. p 52.

Makino A, Mae T, Ohira K. 1983. Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. Plant Physiol. 73: 1002- 1007.

Murayama N. 1995. Aogare (blue-green withering). In: Matsuo T, Kurnazawa K, Ishii R, Ishihara K, Hirata H, editors. Science of the rice plant. 2. Physiology. Tokyo: Food and Agriculture Policy Research Center. p 960-965.

Nakagami K, Ookawa T, Hirasawa T. 1998. Effects of soil moisture conditions from about one month before heading through ripening on dry matter production of wheat plants. Jpn. J. Crop Sci. 67(Extra issue 2):398-399.

Nooden LD. 1988. The phenomena of senescence and aging. In: Nooden LD, Leopold AC, editors. Senescence and aging in plants. San Diego, Calif. (USA): Academic Press. p 1-50.

O'Toole JC, Chang TT. 1979. Drought resistance in cereals—rice: a case study. In: Mussel H, Staples RC, editors. Stress physiology in crop plants. New York (USA): Wiley-Interscience. p 373-405.

O'Toole JC, Baldia EP. 1982. Water deficits and mineral uptake in rice. Crop Sci. 22:1144-1150.

Soejima HT, Sugiyama T, Ishihara K. 1992. Changes in cytokinin activities and mass spectrometric analysis of cytokinins in root exudates of rice plant (*Oryza sativa* L.). Plant Physiol. 100:1724-1729.

Soejima H, Sugiyama T, Ishihara K. 1995. Changes in the chlorophyll contents of leaves and in levels of cytokinins in root exudates during ripening of rice cultivars Nipponbare and Akenohoshi. Plant Cell Physiol. 36: 1105-1114.

Steudle E, Jeschke WD. 1983. Water transport in barley roots. Planta 158:237-248.

Steudle E. 1994. Water transport across roots. Plant Soil 167:79-90.

Tabei D, Hirasawa T, Ookawa T, Ishihara K. 1997. Water status of a leaf under depleted soil moisture conditions and characteristics of leaf water relations in foxtail and common millets. Jpn. J. Crop Sci. 66(Extra issue 2):239-240.

Tajima K. 1995. Occurrence and mechanism of drought damage. In: Matsuo T, Kumazawa K, Ishii R, Ishihara K, Hirata H, editors. Science of the rice plant. 2. Physiology. Tokyo: Food and Agriculture Policy Research Center. p 838-849.

Tsuboi Y. 1995. Wind damage and drought damage. In: Matsuo T, Kumazawa K, Ishii R, Ishihara K, Hirata H, editors. Science of the rice plant. 2. Physiology. Tokyo: Food and Agriculture Policy Research Center. p 813-823.

Wakabayashi K, Hirasawa T, Ishihara K. 1996. Analysis of photosynthesis depression of CO_2 exchange and O_2 evolution rates. Jpn. J. Crop Sci. 65:590-598.

Yoshida S, Hasegawa S. 1982. The rice root system: its development and function. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 97-114.

Notes

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Genotypic differences in physiological responses to water deficit in rice

G. Cabuslay, O. Ito, and A. Alejar

Several factors have limited progress in developing rice cultivars that can survive and yield favorably under rainfed conditions. These include the difficulty in identifying traits that would confer tolerance for water deficit and the fact that indices used by plant breeders are usually related to complex physiological mechanisms. In addition, high genotype by environment interaction makes it difficult to identify consistently superior genotypes. This study was carried out to (1) develop a system in which water-deficit conditions can be reproduced, (2) characterize rice cultivars popularly used for drought studies in rainfed ecosystems, and (3) identify physiological traits that confer tolerance for water deficit. In this study, a simple and repeatable method of simulating drought conditions using polyethylene glycol was effective in inducing water stress in plants. Variability in response to water deficit at the early seedling stage (2–3 wk after germination) was observed among 27 rice cultivars with diverse cultural adaptations. Visual scoring to assess damage provided a reliable measure of tolerance for water deficit and was highly correlated with transpiration. Visual scoring reflects dehydration of the plant tissue as shown by its relative water content. Stomatal closure is the immediate response of plants to avoid tissue dehydration during water stress. The stomata should remain partially open, however, to allow entry of CO_2 and maintain the supply of energy needed to sustain plant growth during water stress. Results showed that cultivars with high tolerance based on visual scoring had high transpiration rates under stress conditions, implying high stomatal conductance, and also maintained leaf area. The highly significant negative correlation between initial leaf area and relative transpiration suggests that high initial leaf area promotes excessive water loss at the onset of drought. Measurement of carbon isotope discrimination is easier than tedious daily weight change measurements involved in whole-plant transpiration studies. Carbon isotope discrimination was positively correlated with relative transpiration but negatively correlated with specific leaf weight after water-stress treatment. It seems that thinner leaves promote transpiration and carbon isotope discrimination in water-stressed plants. Levels of sugar and starch stored in leaf blades and leaf sheaths before the onset of drought did not correlate well with tolerance based on visual score. Osmotic adjustment did not play a significant role in tolerance for water deficit at the level and duration of stress used in this study.

Drought, defined as a period of low or below average rainfall or deficit irrigation that affects crop growth (Boonjung and Fukai 1996), has long been recognized as the primary constraint to rainfed rice production (Mackill et a1 1996). Rainfed lowlands are characterized by lack of water control, with floods and drought being potential problems (IRRI 1997). About one-fourth of the world's total rice land, or approximately 36 million ha, is rainfed (IRRI 1997). Reports of declining yield trends in well-managed irrigated rice lands (Cassman and Pingali 1995) and rapid land conversion for nonagricultural uses necessitate improvement of rice yields in rainfed and upland ecosystems.

Despite concerted efforts by scientists, the development of high-yielding drought-tolerant cultivars remains an elusive goal. Drought tolerance is poorly defined compared with other traits such as grain quality and disease resistance (Richards 1996). In addition, high genotype by environment (G × E) interaction makes it difficult to identify consistently superior genotypes. A method that is manageable and that gives a reproducible assessment of drought tolerance among rice cultivars must be developed to facilitate the identification of traits for drought tolerance.

Field experiments on plant response to water stress usually involve withholding irrigation. For many studies, however, water stress of soil-grown plants cannot be manipulated well enough (Kaufmann and Eckard 1971). Soil heterogeneity further complicates the interpretation of field data. The addition of nonionic osmotic agents such as polyethylene glycols to liquid nutrient media has been shown to closely mimic specific levels of soil water stress. Furthermore, drought avoidance by growing deep roots is not possible because roots of all cultivars are uniformly exposed to the same osmotic stress.

Several studies have focused on the use of polyethylene glycol (PEG) as an osmotic agent (Martin et a1 1986, Perez-Alfocea and Larher 1995, Bock et a1 1996). PEG imposes water stress on plants by decreasing the water potential of the rooting medium, and thus the water potential of the plant (Lawlor 1970). Because PEG is not readily taken up by the cells, it does not alter the ionic composition of the cell (Claes et a1 1990). The main concern about the use of PEG is the presence of toxic inorganic elements that presumably were residues of catalysts (such as aluminum) used in polymerization and organic contaminants such as formaldehyde (Yeo and Flowers 1984). However, these impurities can be effectively removed by dialysis or through ion-exchange columns.

Singh et al (1973) induced water deficit in barley seedlings with PEG 4000 and found no evidence of toxic or other effects on plant uptake of the PEG molecule. According to Michel (1971), Carbowax 6000 did not penetrate the cell walls of the cucumber hypocotyl as shown by the absence of plasmolysis despite shrinkage in cells treated with hypertonic Carbowax solutions. Janes (1974) compared PEG of molecular weights 400, 600, 1000, 1540, and 4000 as osmotic agents and concluded that PEG with molecular weights of 1000 or 1540 proved most satisfactory in decreasing the water potential of nutrient solutions. PEG 600 and 400 were absorbed in appreciable amounts, whereas PEG 4000 or larger may contain toxic impurities. In

another study by Lawlor (1970), PEG of l000 molecular weight or larger entered plants slowly; the larger the molecular weight, the slower the rate of entry. Leaf respiration studies, however, suggested that PEG did not affect metabolism but that the effect on plants is osmotic. The author suggested the use of PEG of large molecular weight, 1000 or 4000, to satisfactorily decrease the osmotic potential of nutrient solutions.

This chapter presents partial results of a study with the following objectives:

1. Assess the suitability of polyethylene glycol 1500 in simulating water deficit.
2. Characterize rice cultivars popularly used for drought studies in rainfed ecosystems.
3. Identify physiological traits that confer tolerance for water deficit.

Materials and methods

Plant material and growth conditions

Two experiments were conducted in the phytotron glasshouse of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines, maintained at 29/21 °C (day/night), 70% relative humidity, and natural light. One was done to measure osmotic adjustment in cultivars after drought treatment whereas the other sought to determine the effect of drought on transpiration and other growth parameters. Pregerminated seeds of 27 rice cultivars of diverse adaptation (Table 1) were sown on styropor seedbeds floating in full-strength nutrient solution prepared according to Yoshida et a1 (1976). The pH of the solution was adjusted to 5.0 daily and renewed once a week during the first 2 wk and twice a week thereafter for the control solution. Both experiments were laid out in a split-plot design with 5 replications.

Simulation of water stress

To measure osmotic adjustment, 3-wk-old seedlings were transferred from normal nutrient solution (without PEG) to that containing PEG 1500 giving –0.5 MPa of stress. The required amount of PEG 1500 was computed according to Lawlor (1970). A control plot remained in normal nutrient solution. Solutions of PEG 1500 were purified by mixing with ion-exchange resin prior to adding nutrients. Although plants were subjected to a sudden change in osmotic potential of the root medium, no apparent osmotic shock was observed in plants in terms of leaf rolling. The experiment was terminated after 11 d when differences among cultivars were visible. Plants were then scored visually for drought damage, which included leaf rolling and leaf drying, using a scale of 1, 3, 5, 7, and 9, where 1 corresponded to least damaged (drought-tolerant) and 9 to most damaged (drought-intolerant). Average daily solar radiation for the duration of the treatment was 15.2 MJ m^{-2} .

For the experiment on transpiration, cultivars and growth conditions were the same as in the measurement of osmotic adjustment. To measure the transpiration rates of stressed and untreated plants, 18-d-old seedlings were transferred into 50-mL plastic centrifuge tubes containing normal nutrient solution. One tube contained a

Table 1. IRRI germplasm bank accession number, cultivar identification/nomenclature, country of origin, adaptation, isozyme grouping, visual score, and relative water content of 27 rice cultivars grown in –0.5 MPa osmotic solution for 11 d.

Accession no.	Cultivar	Country of origin	Adaptation[a]	Isozyme group[b]	Visual score[c] (–0.5 MPa)	Relative water content (%)
10929	Mahsuri	Malaysia	RL	I (indica)	3.4	89.7
11354	C4-137	Philippines		I (indica)	3.4	88.5
11355	IR20	Philippines	IL	I (indica)	3.0	91.5
11462	Nam Sa-Gui 19	Thailand	DW	I (indica)	5.4	80.7
27748	Khao Dawk Mali 105	Thailand	RL	I (indica)	1.8	86.9
28506	IRAT9	Côte d'Ivoire	U	I (indica)	4.2	85.9
32583	IR442-2-58	Philippines	DW	I (indica)	3.0	86.0
38558	IRAT104	Côte d'Ivoire	U	VI (japonica)	2.2	81.9
50634	UPLRi 5	Philippines	U	I (indica)	3.8	87.1
53434	IR52	Philippines	RL	I (indica)	1.8	87.3
57020	IR6115-1-1-1	Philippines		I (indica)	1.8	92.1
67819	UPLRi 7	Philippines	U	I (indica)	2.6	86.8
72931	M55	Liberia	U	VI (japonica)	3.4	84.9
76330	IR72	Philippines	IL	I (indica)	1.0	87.7
77181	ITA130	Nigeria	U	VI (japonica)	2.6	88.5
77968	BPI RI 10	Philippines		I (indica)	1.8	91.0
	CT9993-5-10-1M	Colombia	U	VI (japonica)	3.4	87.9
	IR52561-UBN-1-1-2	Philippines		I (indica)	1.8	81.8
	IR58821-238-1-2-1	Philippines		I (indica)	1.8	87.5
	IR62266-42-6-2	Philippines		I (indica)	1.0	90.1
57011	IR5178-1-1-4	Philippines		I (indica)	4.2	90.7
80022	ITA119	Nigeria		I (indica)	3.4	91.7
55684	IRAT140	Côte d'Ivoire	U	VI (japonica)	3.4	91.0
5758	PI 163575	Philippines		I (indica)	7.0	79.8
8134	Lansaw	Philippines		I (indica)	7.4	70.4
8176	Ifugao rice	Philippines		I (indica)	2.6	80.9
8144	Qomenan	Philippines		I (indica)	1.0	86.7
$LSD_{0.05}$					0.56	2.5

[a] RL = rainfed lowland, IL = irrigated lowland, DW = deep water, U = upland. [b] Classification according to Glaszmann (1987). [c] Drought tolerance score: 1 = least damaged, 9 = most damaged.

single plant that was held in place by a styropor cover that fitted the lid of the plastic tube and had a hole bored at the center. The level of the solution was maintained daily. Water-deficit treatment was imposed 3 d later by changing the nutrient solution to that containing PEG 1500, giving –0.5 MPa of stress. A control plot was maintained. The weight of tubes with a plant was measured daily, and moisture lost was replenished. The experiment was terminated after 6 d when cultivars could be separated into drought-tolerant and intolerant according to visual score. Average daily solar radiation was 18.5 MJ m^{-2}, greater than that for the experiment on determination of osmotic adjustment (which took 11 d), which caused drought damage to manifest itself in only 6 d.

Measurements

Plants were sampled before the imposition of stress and at the end of treatment. At harvest, parameters such as plant height and root length were measured, and visual score was recorded. Plants were rinsed first with tap water and then with distilled water, blotted dry, and separated into leaf blade, leaf sheath, and root to determine fresh weights. Leaf area was measured in plants used for the transpiration experiment. Samples were then dipped into liquid nitrogen and stored at –20 °C prior to freeze-drying for 3 d. Freeze-dried samples were further dried overnight in an oven maintained at 70 °C, weighed, and stored at room temperature before grinding into a fine powder. Measurements were expressed on a per-plant basis.

Soluble sugars, starch, and nitrogen were analyzed by near-infrared spectrometry (IRRI/NSW Agriculture 1995), whereas a wet assay for constructing the calibration curves for sugar and starch was done following the procedure of Conocono et al (1998), modified by using ground samples instead of cut, dried samples and extracting the sugars by refluxing in a water bath maintained at 80–85°C.

Leaf osmotic potential (OP) of the second-youngest fully expanded leaves was measured by putting four leaves in a 5-mL disposable syringe, dipping in liquid nitrogen, and storing inside the freezer prior to measurement. After thawing, the leaf sap was expressed by increasing the pressure in the syringe. OP of the leaf sap was measured with a freezing point osmometer (Precision Systems, Inc. Model 5004).

To determine relative water content (RWC), 2 or 3 pieces of the second-youngest fully expanded leaf were weighed and cut into 2-cm segments and then allowed to rehydrate inside a refrigerator overnight. The following day, samples were blotted dry and turgid weight immediately determined. The leaves were then oven-dried for 3 d at 70 °C. Leaf RWC was then calculated according to Basnayake et a1 (1993).

Osmotic adjustment was determined as the difference in OP calculated to full turgor between the stressed plants and the unstressed control sampled at the same time. OA values were not standardized to the same RWC. OP at full turgor was obtained by substituting the measured RWC and OP in the equation described by Basnayake et al (1993).

For the transpiration experiment, specific leaf weight (SLW) was computed as the ratio of green leaf weight to leaf area. Water-use efficiency (WUE) was calculated by dividing shoot dry matter by water transpired. Carbon isotope composition of dried, ground leaf samples was analyzed using a continuous flow Isotope Ratio Mass Spectrometer (Europa Scientific Roboprep-CN coupled to VG Micromass 903). Carbon isotope discrimination (A) was computed according to Hubick et al (1986) with the isotope ratio of atmospheric CO_2 taken to be –7.60%*o* relative to PeeDee Belemnite.

Results and discussion

Visual scores and relative water content

In this study, which used an inert osmoticum, PEG 1500, to simulate water stress, whole roots were bathed uniformly with the osmoticum so that differences in root traits, such as the ability to grow deep roots when plants are grown in the field, may

not play a significant role in drought tolerance. It is therefore expected that results may differ slightly from those reported involving field- or pot-grown plants. The 27 cultivars studied exhibited significant differences in response to water deficit as shown by visual score (Table 1). IR72, IR62266, and Qomenan were the most tolerant; PI 163575 and Lansaw were rated intolerant. Most of the wetland cultivars belonged to the "unrolled" group, which is consistent with the observations of Turner et al (1986a) and Chang and Loresto (1986) as cited in Dingkuhn et al (1989a) that semidwarf rice has a less sensitive leaf rolling response.

Chang et al (1974) also used leaf rolling and leaf death in assessing levels of field tolerance for drought. O'Toole and Moya (1978) reported that visual scoring techniques, based on either leaf rolling or leaf tip drying, were highly correlated with maintenance of leaf water potential. A limitation on the use of visual scoring as an index for drought tolerance, however, is that it does not distinguish between tolerance and avoidance mechanisms (Ingram et al 1990). It is well known that one such avoidance mechanism involves growing deep roots to be able to gain access to water in the deeper soil horizons.

Water content is a commonly used measure of water status and is usually expressed as relative water content—the water content in proportion to that at full saturation. According to Lawlor (1995, an RWC of 100–90% is related to stomatal closure and decreased cell expansion and growth of organs, and 90–80% is related to changes in composition of tissues and some alterations in the relative rates of photosynthesis and respiration. Further, with an RWC below 80% (around water potential of –1.5 MPa), changes in metabolism become marked, with cessation of photosynthesis, much increased respiration, and accumulation of proline and abscisic acid.

In our investigation, a range of RWC—from 92% for IR6 115 to 70% for Lansaw—was obtained for the 27 cultivars subjected to water stress for 11 d (Table 1). These values indicate that, although a mild osmotic stress of –0.5 MPa was applied, prolonged duration of 11 d induced mild to severe water deficit, depending on how plants adapted to the treatment. Based on the preceding description by Lawlor (1995), we can infer that cultivars that are drought-tolerant based on visual score and that have a high RWC such as IR6115 and BPI RI 10 might have suffered only mild water stress involving stomatal limitation of photosynthesis, whereas intolerant ones such as PI 163575 and Lansaw could have suffered from severe stress involving nonstomatal effects on photosynthesis. This conclusion is supported by Dingkuhn et al (1989b), who reported that, even under continuous mild water stress under upland conditions, with leaf water potential values ranging from –0.5 to –1.7 MPa and minor leaf rolling, photosynthesis was constrained by both stomatal and nonstomatal factors. RWC was highly and negatively correlated with visual score ($r = –0.58^{**}$), which indicates that visual scores reflect the drying of tissues.

Osmotic adjustment

A mechanism for drought tolerance that is gaining wide acceptance is osmotic adjustment. Osmotic adjustment (defined as the decrease in osmotic potential resulting from

an accumulation of intracellular solutes) generates a more negative leaf water potential, thereby maintaining water movement into the leaf and, consequently, leaf turgor. By helping to maintain leaf turgor, OA also enables plants to keep their stomates open and continue taking up CO_2 for photosynthesis under conditions of moderate water stress (Hopkins 1995). OA has also been an important factor in the maintenance of root growth in drying soil (Sharp and Davies 1979). The metabolic cost of storing photosynthate and using it for OA is less than the cost of converting it to new biomass (McCree 1986). Potassium ions, sugars, proline, and glycinebetaine are important components of OA.

Hsiao et al (1984) observed lower leaf death in osmotically adjusted leaves of lowland rice IR36 and concluded that OA delays leaf rolling, thereby maintaining gas exchange and delaying leaf death. Nguyen et al (1997) showed that some rice genotypes lacked any capacity for OA, whereas others had values of up to 0.7 MPa at a leaf RWC of 75%. The researchers further noted that traditional upland cultivars generally tend to excel in root growth and soil moisture extraction capacity while lacking OA. During water deficit, these cultivars tend to maintain leaf water status by stomatal closure. Improved lowland cultivars, on the other hand, tend to lack root depth but generally excel in OA. Lilley and Ludlow (1996) found wide genotypic variation in OA (0.4–1.5 MPa) among 61 lines of *Oryza sativa* and 8 accessions of *Oryza* spp. Lines with a japonica background had poor dehydration tolerance and low OA, whereas indica lines had greater dehydration tolerance and greater OA. IR72 had the highest OA at 1.47 MPa.

As mentioned earlier, in our study OA was not standardized to the same RWC as was done by Lilley and Ludlow (1996) so that values obtained would therefore reflect the contribution of OA to drought tolerance as measured by visual score at the end of treatment. Osmotic adjustment ranged from 0.31 to 0.50 MPa. These values are close to those obtained by Turner et al (1986b) for field-grown rice cultivars—wetland cultivars IR20 and IR36—osmotically adjusted by 0.5-0.6 MPa, whereas upland cultivar IAC25 adjusted by less than 0.2 MPa. No significant differences in OA were found among the 27 cultivars and no correlation was obtained between OA and RWC, presumably because of the generally mild water deficit induced in plants.

Daily transpiration

Transpiration is a vital process in the life cycle of plants. Aside from its cooling effect, transpiration promotes water and nutrient absorption (O'Toole and De Datta 1986). Water uptake provides the physical driving force for cell enlargement (Acevedo et al 1971), which is one of the plant processes most sensitive to water stress (Hsiao 1973). The rate of water intake is determined largely by the rate of water loss (Kramer 1937). In our study, transpiration rate was markedly reduced to 35–50% of the control in all cultivars/lines after the first day of treatment. Cultivars rated drought-tolerant based on visual score, such as KDML105, had a higher relative daily transpiration than drought-intolerant ones such as PI 163575.

In general, cultivars with high relative transpiration (transpiration under water stress relative to that in the control) were rated tolerant on the basis of leaf rolling and leaf drying ($r = –0.52^{**}$), which further supports the role of transpiration in water uptake and cell enlargement. A high transpiration rate under conditions of water deficit also implies high stomatal conductance, which was associated with continued water extraction in cotton (Jordan and Ritchie 1971) and in sorghum (Sanchez-Diaz and Kramer 1971) in response to low leaf water potentials. Thus, our results on genotypic variation in relative transpiration among 27 rice cultivars suggest differences in stomatal conductance and that cultivars such as IR72 and KDML105, which are more tolerant of mild water deficit, maintained fairly open stomates.

Relative transpiration during water deficit was highly and positively correlated with relative leaf area, with 49% of the variation in relative transpiration explained by a change in leaf area. This is expected because the leaf is the organ for transpiration and any reduction in leaf growth will consequently cause a reduction in transpiration. Leaf expansion is much more sensitive to water stress than photosynthesis (Bansal 1994) and maintenance of leaf area is necessary under rainfed environments for interception of radiant energy and for reduction of soil evaporation (Van den Boogard et a1 1997). At the onset of drought and especially when solar radiation is high, however, having an initially large leaf area may be disadvantageous to plants because of high transpirational demand that is not met due to limited water supply. Initial leaf area was negatively correlated with relative transpiration (Fig. 1), which makes it possible to select drought-tolerant cultivars based on leaf area alone. It was also highly and positively correlated ($r = 0.50^{**}$) with visual score, which gives further credence to selection for small initial leaf area.

Having an initially small leaf area gives the advantage of having less leaf surface exposed to intense solar radiation so that, at the onset of drought, photorespiration and water loss from leaf tissues are minimized. This is consistent with the observation of Reitz (1974) that wheat and barley varieties with wide leaves tend to burn under water stress. Small radiation interception at the onset of stress was also one of the factors enumerated by Lilley and Fukai (1994) that enabled rice cultivar Todoroki-Wase to escape severe water stress at the vegetative stage. Courtois et a1 (1996) reported a highly significant negative correlation between biomass before stress and leaf drying, and recognized the importance of potential losses due to transpiration that increases with biomass.

Water-use efficiency

Evapotranspiration studies on rice revealed that from 759 to 1,150 kg of water is required to produce 1 kg of rough rice grain (Shih et a1 1982). Because water is limiting in rainfed environments, a desirable trait for rainfed-lowland-adapted rice cultivars is to have efficient water use. Water-use efficiency (WUE) of the whole plant, usually defined as the total dry matter produced per unit of water transpired (Boyer 1996), is an important aspect of plant adaptation to drought. An increase in WUE

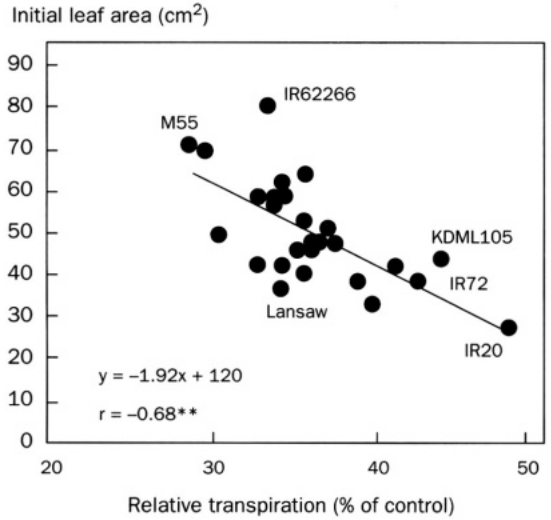

Fig. 1. Relationship between relative transpiration and initial leaf area for 27 rice cultivars/lines.

indicates that more carbon can be accumulated for growth with the use of less water. Thus, an increase in WUE is a water conservation measure (Nilsen and Orcutt 1996).

There was an increase in WUE in all cultivars (twofold in some entries) and wider genotypic variability when plants were grown under water stress (Table 2). This agrees with the results of Hubick and Farquhar (1989), who reported that mild water stress increased WUE in barley cultivars, and those of McCree and Richardson (1987), who showed increases of up to 100% in WUE of cowpea under moderate drought but a substantial decrease under extreme drought. There was a slight variation in genotype ranking between control and stress conditions although the correlation was high (r = 0.80**). Variability in WUE under stress, however, was not correlated with visual score. It is possible that, in some cultivars, an increase in WUE resulted from stomatal closure rather than from maintenance of photosynthetic activity.

Carbon isotope discrimination

Despite reports of genotypic variability in WUE among cultivars (Farquhar and Richards 1984, Hubick et al 1986) and the relatively few genetic loci controlling variability (Boyer 1996), plant breeders were reluctant to use WUE as a selection trait because of the time consumed in taking measurements. Recently, a new parameter called carbon isotope discrimination (A) was found to be a good estimate of WUE. This technique is based on the ratio of two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C, with the heavier isotope being less abundant, with a natural abundance of about 1.1% of total carbon present (Ebdon et al 1998). During fixation of C

Table 2. Water-use efficiency (WUE) and specific leaf weight (SLW) in control and in PEG and ^{13}C discrimination with cultivars arranged according to WUE under control conditions.

Cultivar	WUE (mg shoot g^{-1} H_2O)		Discrimination in PEG	SLW (g m^{-2})	
	Control	PEG	(‰)	Control	PEG
Lansaw	4.21	7.22	20.82	36	96
IRAT104	4.13	6.47	20.51	36	126
IRAT140	4.11	6.40	20.32	41	111
ITA119	4.06	5.09	20.84	34	99
IR52	4.01	7.33	20.99	36	52
Khao Dawk Mali 105	3.85	6.69	20.86	40	58
BPI RI 10	3.76	6.02	21.05	35	74
ITA130	3.71	5.45	20.09	42	119
IR62266-42-6-2	3.64	4.96	21.30	35	52
PI 163575	3.64	5.30	20.53	36	129
RAT9	3.63	6.27	20.78	40	102
IR5178	3.63	7.18	20.82	44	82
IR442-2-58	3.61	4.95	21.83	32	51
IR6115-1-1-1	3.58	6.65	20.78	33	65
M55	3.58	5.67	20.11	34	124
IR72	3.56	6.25	21.07	43	73
IR52561-UBN-1-1-2	3.56	5.42	21.03	38	61
C4-137	3.48	4.11	20.69	37	87
UPLRi7	3.40	5.92	20.48	34	70
IR58821-23-B-1-2-11	3.37	5.07	21.16	36	70
CT9993-5-10-1M	3.34	3.42	20.90	37	99
IR20	3.32	5.51	21.60	37	50
Nam Sa-Gui 19	3.25	5.44	20.88	42	94
Ifugao rice	3.25	4.28	21.20	40	78
Mahsuri	3.16	4.69	21.01	35	71
Qomenan	3.12	3.65	21.05	37	87
UPLRi5	2.35	0.86	20.61	32	105
$LSD_{0.05}$	0.30	0.69	0.26	1.4	12.4

by photosynthesis, the heavier isotope (^{13}C) is discriminated against, resulting in a smaller ratio of ^{13}C to ^{12}C in plant tissues compared with the source air. The ratio of ^{13}C to ^{12}C in plants varies because of the differential diffusion rate through stomata and because Rubisco, the primary fixation enzyme in C_3 plants, discriminates against $^{13}CO_2$. Carbon isotope discrimination decreases as water stress increases because the ratio of ^{13}C to ^{12}C increases in stressed C_3 leaves and Rubisco has less opportunity to discriminate against the heavier isotope ($^{13}CO_2$).

Significant variation in A was found among genotypes grown under water stress (Table 2). Carbon isotope discrimination ranged from 20.1% for ITA130 to 21.8‰ for IR442-2-58. These values are comparable with the range of A (19.8‰ to 21.5‰) obtained by Dingkuhn et a1 (1991) for 28 upland rice cultivars grown under mild water stress. Carbon isotope discrimination was negatively correlated, although loosely, with

visual score (r = –0.38*), which implies that Δ is also an indicator of plant adaptation to water deficit.

WUE and carbon isotope discrimination

Farquhar (1991) predicted a negative linear dependence of WUE on Δ, based on (1) the negative relationship between WUE and the ratio of intercellular to atmospheric partial pressures of CO_2, p/p_a, and (2) the positive relationship between Δ and p/p_a. The negative relationship between transpiration efficiency and Δ was shown as predicted in peanut by Hubick et al (1988). In wheat, Condon et al (1990) found a negative correlation between WUE and Δ under both well-watered conditions and gradually increasing water stress. In rice, however, a negative relationship between WUE and Δ was demonstrated among only japonica and aus rice but not among indica rice cultivars (Dingkuhn et al 1991). Turner (1993) also reported the absence of correlation between WUE and Δ in three wheat cultivars measured in the field. In a review, Boyer (1996) pointed out that, under relatively favorable conditions, the relationship between WUE and A tends to become less negative or even positive. In peanut, 68% of the variance of WUE was explained when WUE was regressed on Δ; 32% of the variation in WUE remained unexplained (Wright et al 1988).

In our study, Δ was not related to WUE under stress but was strongly correlated with relative transpiration (r = 0.55**). It was mentioned earlier that plants with high relative transpiration would also have high stomatal conductance. Transpiration is a function of both leaf area and stomatal conductance (Lafitte, personal communication) and only about 50% of the change in relative transpiration is explained by leaf area. Hence, high Δ in cultivars grown under moderate water stress implies high stomatal conductance. The positive correlation obtained by Ehleringer (1990) between Δ and stomatal conductance in common bean supports the assumption that plants with more open stomates can better discriminate against ^{13}C, presumably because of greater fractionation by Rubisco. Because stomatal closure is the main limitation to transpiration and photosynthesis during water stress, the ability to monitor stomatal conductance by carbon isotope discrimination will give relevant information on how the plant adapts to water deficit.

The prediction of stomatal conductance by Δ, if it can be extended to other crops, will provide another role for selection for high discrimination during mild water stress independent of WUE. Application of carbon isotope discrimination in plant breeding should not be limited to prediction of WUE alone because improved WUE may actually restrict growth, as when WUE is increased by partial closure of stomata (Hubick et al 1986). Ehleringer et al (1990) reported that, in addition to leaf conductance, other plant characters such as earliness and dry matter production tend to be correlated with Δ under field conditions, which implies that these characters represent specific adaptive combinations.

Selection for high discrimination has a high potential in breeding programs because significant genotypic variability is obtained during drought stress as shown in this study and in other reports (Condon et al 1990, Dingkuhn et al 1991). High broad-

sense heritability of 81% with no significant G × E interaction was obtained in field-grown peanut cultivars (Hubick et al 1988). In leaves of field-grown cowpea, abroad-sense heritability of 76% was obtained with similar heritabilities under wet and dry conditions (Hall et al 1990). Reports of stable and significant quantitative trait loci (QTL) for Δ (Quarrie 1996) suggest that marker-assisted selection can be applied successfully for the trait in a breeding program.

Specific leaf weight and carbon isotope discrimination

Water deficit caused marked increases in specific leaf weight (SLW) of up to four times in M55 and PI 163575 (Table 2), confirming the general observation of an increase in leaf thickness in plants subjected to water stress (Kramer 1969). A negative relationship was found between SLW and Δ ($r = –0.78^{**}$), which was similar to that reported by Wright et al (1988) using peanut cultivars under nonlimiting water conditions. A highly significant negative relationship was also found between SLW and transpiration relative to the control ($r = 4.56^{**}$). These results indicate that thinner and more expanded leaves promote transpiration and carbon discrimination in water-stressed plants (hence increased stomatal conductance) during drought. Turner (1993) recommended selection for low SLW (in addition to early vigor) rather than for increased transpiration efficiency because high transpiration efficiency may be linked with reduced early growth and leaf area development. Specific leaf weight also offers a cheaper alternative for measuring isotope discrimination in plants.

Role of sugar and starch

Simple linear correlation coefficients of initial sugar and starch contents in the leaf blade and leaf sheath with visual scores were not significant, indicating that the initial contents of sugar and starch or nonstructural carbohydrates have no significant role in achieving tolerance for water deficit at the seedling stage. This is in contrast with observations in submerged environments, where an initially high carbohydrate level in the tissues is mentioned as an adaptive trait by serving as a source of metabolic activity during submergence (Chaturvedi et al 1996). It is possible that submergence and water deficit differ in degree of carbohydrate depletion. Because the stomates remain partially open, allowing continued assimilation under mild water deficit, dependence on initial carbohydrates may not be as crucial under water deficit as in submergence, where the main effect on the plant tissues is carbohydrate depletion (Mackill et al 1996).

A significant negative correlation ($r = –0.38^{*}$) was obtained between visual score and relative sugar in the leaf. Cultivars with high sugar relative to the control (such as IR72 and KDML105) had less severe leaf stress symptoms. Relative sugar in the leaf was strongly correlated ($r = 0.56^{**}$) with relative transpiration, which indicates that sugar content was largely determined by photosynthetic activity and that cultivars tolerant of mild water deficit (such as IR72) maintained fairly open stomates. This allowed physiological processes such as transpiration and assimilation to proceed. A similar trend was observed for starch.

Conclusions and implications for germplasm improvement

PEG 1500 is a suitable osmoticum for lowering the water potential of liquid nutrient media. Significant differences in drought tolerance based on visual score were found among genotypes. Results such as the level of osmotic adjustment and the range in leaf Δ of stressed plants were similar to those observed by other investigators who conducted field experiments involving mild water stress.

Low initial leaf area, low SLW at the end of stress, and high transpiration rate and Δ during periods of mild water stress are desirable characteristics that enable cultivars to function well during periods of moderate water stress. This study demonstrated the complexity of plant responses to water deficit and the difficulty of identifying a single trait that confers tolerance. The use of liquid nutrient media and the methodology described herein, however, have the advantage of focusing on shoot-related adaptation. This is not so with pot or field studies where it is difficult to determine whether adaptation is due to shoot or root character or both. In the present setup, all roots from the base to the tip are subjected to the same degree of stress, with no possibility of drought escape, because the ability to grow deep roots to explore more soil and to develop thick roots to penetrate compacted soil layers does not alleviate the effects of water deficit. Phenotypic selection for tolerance for water deficit could therefore be related to the shoot. This facilitates the identification of genes that are responsible for shoot- and root-related adaptations separately. Plant breeders can then combine both in a pyramiding approach to improve the performance of cultivars under rainfed lowland conditions.

This study points to lowland cultivars as donors of desirable shoot traits. But these cultivars lack the deep root character of traditional upland cultivars that enables plants to have access to water in the lower soil horizons and thus escape from drought in upland and rainfed areas. Germplasm improvement for rainfed lowland areas should therefore focus on incorporating deep root characteristics into improved lowland rice cultivars. This task may not be easy because of the apparent association between plant height and root length. Within a species, large plants have greater root lengths than small plants (Taylor and Klepper 1978). In rice, Yoshida et al (1982) found that the deep root system is correlated with tall plant stature and low tiller number. Armenta-Soto et al (1983) also observed a positive correlation ($r = 0.58^{**}$) between deep and thick roots and plant height in rice. Breeding tools are now available, however, that make it possible to develop root traits in plants that are independent of shoot characters.

Ekanayake et al (1985) found that, although tall plants tend to have deeper root systems, it appears possible to obtain short- or intermediate-statured and moderate-tillering segregants with deep root systems in semidwarf × tall crosses. Champoux et al (1995) identified QTLs that were associated with root thickness, root/shoot dry matter ratio, root dry weight per tiller, and deep root weight. Courtois et al (1996) are developing near-isogenic lines of the semidwarf cultivar IR64 introgressed with the deep root trait from Azucena, a traditional tropical japonica from the Philippines. Their data suggest that shoot and root mechanisms will likely be disconnected and be

possible to improve separately. Current work by Zhang et al (this volume, "Progress on the molecular mapping of osmotic adjustment and root traits in rice") aims to identify QTLs for root and osmotic adjustment traits in rice. To achieve this, they developed a doubled-haploid line population from a cross between a japonica type (CT9993) that has a higher root penetration ability and an indica type (IR62266) that has a higher OA. Four QTLs were identified for OA.

It remains to be seen whether the shoots of semidwarf cultivars can adequately supply the assimilate requirements of deep roots and still maintain acceptable yields under sufficient and limiting water conditions. According to Nguyen et al (1997), up to 60% of the carbon in rice plants is invested in the root system. Under conditions of limited water supply, the large root mass can either function as a source of stored assimilates that can be partitioned to the shoot, especially to the developing panicles, or act as a sink and compete with the panicles for photosynthates. The authors also phenotyped four mapping populations from which 3–6 QTLs for root penetration ability were identified.

References

Acevedo E, Hsiao TC, Henderson DW. 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. Plant Physiol. 48:631-636.

Armenta-Soto J, Chang TT, Loresto GC, O'Toole JC. 1983. Genetic analysis of root characters in rice (*Oryza sativa* L.). Soc. Adv. Breed. Res. Asia Oceania J. 15: 103-1 16.

Bansal KC. 1994. Role of water in plants. Indian Farming 44(7):3-6.

Basnayake J, Ludlow MM, Cooper M, Henzell RG. 1993. Genotypic variation of osmotic adjustment and desiccation tolerance in contrasting sorghum inbred lines. Field Crops Res. 35:51-62.

Bock C, Jacob A, Erst GO, Leibfritz D, Mayer A. 1996. Metabolic changes of the Antarctic green alga *Prasiola crispa* subjected to water stress investigated by in vivo ^{31}P NMR. J. ExP. Bot. 47:241-249.

Boonjung H, Fukai S. 1996. Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. I. Growth during drought. Field Crops Res. 48:37-45.

Boyer JS. 1996. Advances in drought tolerance in plants. Adv. Agron. 56:187-218.

Cassman KG, Pingali PL. 1995. Intensification of irrigated rice systems: learning from the past to meet future challeges. GeoJournal 35(3):299-305.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Chang TT, Loresto GC, Tagumpay O. 1974. Screening rice germplasm for drought resistance. SABRAO J. 6:9-16.

Chaturvedi GS, Ram PC, Singh AK, Ram P, Ingram KT, Singh BB, Singh RK, Singh VP. 1996. Carbohydrate status of rainfed lowland rices in relation to submergence, drought and shade tolerance. In: Singh VP, Singh RK, Singh BB, Zeigler RS, editors. Physiology of stress tolerance in rice. Proceedings of the 1994 International Conference on Stress Physiology of Rice. Uttar Pradesh (India): Narendra Deva University of Agriculture and Technology, and Manila (Philippines): International Rice Research Institute. p 103-122.

Claes B, Dekeyser R, Villaroel R, Van den Bulcke M, Bauw G, Van Montagu M, Caplan A. 1990. Characterization of a rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell 2: 19-27.

Condon AG, Farquhar GD, Richards RA. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat: leaf gas exchange and whole plant studies. Aust. J. Plant Physiol. 17:9-22.

Conocono EA, Egdane JA, Setter TL. 1998. Estimation of canopy photosynthesis in rice by means of daily increases in leaf carbohydrate concentrations. Crop Sci. 38:987-995.

Courtois B, Chaitep W, Moolsri S, Prasad K, Sinha PK, Trebuil G, Yadav R. 1996. Drought resistance and germplasm improvement: ongoing research of the upland rice consortium. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. Proceedings of the 1996 Upland Rice Consortium Workshop. Manila (Philippines): International Rice Research Institute. p 154-175.

Dingkuhn M, Cruz RT, O'Toole JC, Dörffling K. 1989a. Net photosynthesis, water use efficiency, leaf water potential and leaf rolling as affected by water deficit in tropical upland rice. Aust. J. Agric. Res. 40:1171-1181.

Dingkuhn M, De Datta SK, Dörffling K, Javellana C. 1989b. Varietal differences in leaf water potential, leaf net CO_2 assimilation, conductivity and water use efficiency in upland rice. Aust. J. Agric. Res. 40:1183-1192.

Dingkuhn M, Farquhar GD, De Datta SK, O'Toole JC. 1991. Discrimination of ^{13}C among upland rices having different water use efficiencies. Aust. J. Agric. Res. 42: 1123- 1131.

Ebdon JS, Petrovic AM, Dawson TE. 1998. Relationship between carbon isotope discrimination, water use efficiency, and evapotranspiration in Kentucky bluegrass. Crop Sci. 38:157-162.

Ehleringer JR. 1990. Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. Plant Physiol. 93:1422-1425.

Ehleringer JR, White JW, Johnson DA, Brick M. 1990. Carbon isotope discrimination, photosynthetic gas exchange, and transpiration efficiency in beans and range grasses. Acta Oecologia 11:611-625.

Ekanayake IJ, O'Toole JC, Ganity DP, Masajo TM. 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Sci. 25:927-933.

Farquhar GD. 1991. Use of stable isotopes in evaluating plant water use efficiency. In: Stable isotopes in plant nutrition, soil fertility and environmental studies. Vienna: International Atomic Energy Agency. p 475-489.

Farquhar GD, Richards RA. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11:539-552.

Glaszmann JC. 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. 74:21-30.

Hall AE, Mutters RG, Hubick KT, Farquhar GD. 1990. Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci. 30:300-305.

Hopkins WG. 1995. Introduction to plant physiology. New York (USA): John Wiley and Sons, Inc. p 429.

Hsiao TC, 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24:519-570.

Hsiao TC, O'Toole JC, Yambao EB, Turner NC. 1984. Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). Plant Physiol. 75:338-341.

Hubick KT, Farquhar GD, Shorter R. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (Arachis) germplasm. Aust. J. Plant Physiol. 13:803-816.

Hubick KT, Shorter R, Farquhar GD. 1988. Heritability and genotype x environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Aust. J. Plant Physiol. 15:799-813.

Hubick K, Farquhar G. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. Plant Cell Environ. 12:795-804.

Ingram KT, Real JG, Maguling MA, Obien MA, Loresto GC. 1990. Comparison of selection indices to screen lowland rice for drought. Euphytica 48:253-260.

IRRI (International Rice Research Institute). 1997. Rice almanac. 2d ed. Manila (Philippines): IRRI, with Centro Intemacional de Agricultura Tropical and West Africa Rice Development Association. 181 p.

IRRI (International Rice Research Institute)/NSW Agriculture. 1995. Analysis of nitrogen and starch in tropical rice crops using NIR spectroscopy. Manila (Philippines): IRRI, with Yanco (NSW Australia): NSW Agriculture. Unpublished.

Janes BE. 1974. The effect of molecular size, concentration in nutrient solution, and exposure time on the amount and distribution of polyethylene glycol in pepper plants. Plant Physiol. 54:226-230.

Jordan WR, Ritchie JT. 1971. Influence of soil water stress on evaporation, root absorption, and internal water status of cotton. Plant Physiol. 48:783-788.

Kaufmann MR, Eckard AN. 1971. Evaluation of water stress control with polyethylene glycols by analysis of guttation. Plant Physiol. 47:453-456.

Kramer PJ. 1937. The relation between rate of transpiration and rate of absorption of water in plants. Am. J. Bot. 24:10-15.

Kramer PJ. 1969. Plant and soil water relationships. New Delhi: Tata McGraw Hill Publishing Company Ltd. p 360-361.

Lawlor DW. 1970. Absorption of polyethylene glycols by plants and their effects on plant growth. New Phytol. 69:501-513.

Lawlor DW. 1995. The effects of water deficit on photosynthesis. In: Smirnoff N, editor. Environment and plant metabolism: flexibility and acclimation. UK. BIOS Scientific Publishers Ltd. p 129-160.

Lilley JM, Fukai S. 1994. Effect of timing and seventy of water deficit on four diverse rice cultivars. II. Physiological responses to soil water deficit. Field Crops Res. 37:215-223.

Lilley JM, Ludlow MM. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. Field Crops Res. 48: 185- 197.

Mackill DJ, Coffman WR, Gamty DP. 1996. Rainfed lowland rice improvement. Manila (Philippines): International Rice Research Institute. 242 p.

Martin BA, Schoper JB, Rinne RW. 1986. Changes in soybean (*Glycine max* [L.] Merr.) glycerolipids in response to water stress. Plant Physiol. 81:798-801.

McCree KJ. 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. Aust. J. Plant Physiol. 13:33-43.

McCree KJ, Richardson SG. 1987. Salt increases the water use efficiency in water stressed plants. Crop Sci. 27:543-547.

Michel BE. 1971. Further comparisons between Carbowax 6000 and mannitol as suppressants of cucumber hypocotyl elongation. Plant Physiol. 48:513-516.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37:1426-1434.
Nilsen ET, Orcutt DM. 1996. The physiology of plants under stress: abiotic factors. New York (USA): John Wiley and Sons, Inc. p 351.
O'Toole JC, De Datta SK. 1986. Drought resistance in rainfed lowland rice. In: Progress in rainfed lowland rice. Manila (Philippines): International Rice Research Institute. p 145-158.
O'Toole JC, Moya TB. 1978. Genotypic variation in maintenance of leaf water potential in rice. Crop Sci. 18:873-876.
Perez-Alfocea F, Larher F. 1995. Sucrose and proline accumulation and sugar efflux in tomato leaf discs affected by NaCl and polyethylene glycol 6000 iso-osmotic stresses. Plant Sci. 107:9-15.
Quarrie SA. 1996. New molecular tools to improve the efficiency of breeding for increased drought resistance. Plant Growth Reg. 20:167-178.
Reitz LP. 1974. Breeding for more efficient water use: Is it real or a mirage? Agric. Meteorol. 1413-11.
Richards RA. 1996. Defining selection criteria to improve yield under drought. Plant Growth Reg. 20:157-166.
Sanchez-Diaz MF, Kramer PJ. 1971. Behavior of corn and sorghum under water stress and during recovery. Plant Physiol. 48:613-616.
Sharp RE, Davies WJ. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta 147:43-49.
Shih SF, Rahi GS, Harrison DS. 1982. Evapotranspiration studies on rice in relation to water use efficiency. Trans. ASAE 25:702-712.
Singh TN, Paleg LG, Aspinall D. 1973. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. Aust. J. Biol. Sci. 26:45-56.
Taylor HM, Klepper B. 1978. The role of rooting characteristics in the supply of water to plants. Adv. Agron. 30:99-128.
Turner NC. 1993. Water use efficiency of crop plants: potential for improvement. In: Buxton DR, Shibles R, Forsberg RA, Blad BL, Asay KH, Paulsen GM, Wilson RF, editors. International crop science. I. Madison, Wis. (USA): Crop Science Society of America, Inc. p 75-82.
Turner NC, O'Toole JC, Cruz RT, Namuco OS, Ahmad S. 1986a. Responses of seven diverse rice cultivars to water deficits. I. Stress development, canopy temperature, leaf rolling and growth. Field Crops Res. 13:257-271.
Turner NC, O'Toole JC, Cruz RT, Yambao EB, Ahmad S, Namuco OS, Dingkuhn M. 1986b. Response of seven diverse rice cultivars to water deficits. II. Osmotic adjustment, leaf elasticity, leaf extension, leaf death, stomatal conductance, and photosynthesis. Field Crops Res. 13:273-286.
Van den Boogard R, Alewijnse D, Veneklaas EJ, Lambers H. 1997. Growth and water-use efficiency of 10 Triticum aestivum cultivars at different water availability in relation to allocation of biomass. Plant Cell Environ. 20:200-210.
Wright GC, Hubick KT, Farquhar GD. 1988. Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field-grown peanut cultivars. Aust. J. Plant Physiol. 15:815-825.
Yeo AR, Flowers TJ. 1984. Nonosmotic effects of polyethylene glycols upon sodium transport and sodium-potassium selectivity by rice roots. Plant Physiol. 75:298-303.

Yoshida S, Bhattacharjee DP, Cabuslay GS. 1982. Relationship between plant type and root growth in rice. Soil Sci. Plant Nutr. 28:473-482.

Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory manual for physiological studies of rice. Manila (Philippines): International Rice Research Institute. p 61-66.

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Characteristics of the root system and water uptake in upland rice

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The importance of root system morphology, especially deep rooting zone and root density at depth, in ensuring water capture under drought is established in upland rice. To improve the efficiency of the root system, environmental factors and genotype × environment interactions that affect plasticity of the root system must be emphasized, as well as genetic factors.

Because the development of nodal roots basically determines the rooting zone, it is important to understand the dynamics of the nodal root system in relation to tillering habit. Stimulating the growth of nodal roots from early internodes in early tillers is an important mechanism for forming a deep rooting zone. We also need to further understand genetic and environmental effects on morphological characteristics of lateral roots in rice in relation to their function in water uptake.

Results from field experiments indicated that root length density at depth is important in determining the water extraction rate across layers in the soil profile. The limited water-capturing capacity of rice under severe stress can be attributed to (1) the low morphological response to the stress in increasing root density, especially in deep layers, and (2) limited water absorption rate per length in the deep soil layer.

Because of the global water scarcity, improving the productivity of rice under water-limited conditions, such as in uplands, is becoming more important. Improving root capacity to capture water from soil is critical in maintaining shoot water potential and stabilizing growth, and hence the productivity of upland rice. Past research clarified the importance of root density in deep soil layers in determining water uptake rate and water potential in shoots under stress in varietal comparisons of rice (Yoshida and Hasegawa 1982, Lilley and Fukai 1994) and in comparisons between rice and other upland cereals (Fukai and Inthapan 1988). Information on genetic diversity in the root system, especially in nodal root traits, has been accumulated since the 1970s (Yoshida and Hasegawa 1982). Much progress has been made recently in genetic control of root traits that are related to the deep rooting zone (Champoux et al 1995, Yadav et al 1997).

In addition to information on genetic factors, an understanding of environmental effects on the root system is also essential to maximizing genetic potential in forming the deep root system because the root system is a very plastic organ exposed to different soil conditions with a variety of nutrient, water, and physical properties. Under diverse soils and cultural conditions, genotype × soil environment interactions are

critical to the expression of genotypic characters in root traits. The effects of the spatial distribution of nutrients and water and their changes over time on different root components and mutual interactions among root components should be considered. The capacity of the root system for water capture is determined by root morphological growth and physiological ability to absorb water. The plant is exposed to various types of water stress. The ideal root system for improving water capture should be sought according to the type of stress. This paper examines (1) the characteristics of the upland rice root system and environmental effects and (2) the characteristics of rice roots in extracting water under stress conditions.

Characteristics of the rice root system

The mature root system of rice is composed of one seminal root, nodal roots (crown root, adventitious root), lateral roots (branch root), and root hairs. The basic structure of the rice root system is in common with that of other cereal crops. Because rice has fewer and less developed seminal roots than other cereals, the contribution of the seminal root to the overall root system function is probably less important in rice. The rooting zone of rice is basically determined by the development of nodal roots in their formation, elongation, angle, and duration. The development and survival of lateral roots largely affects the density of roots at depth. In an improved upland cultivar (UPLRi-5) planted in a pot fertilized with N and P, total root length per plant at panicle initiation was 2,846 m under wet soil conditions ((0.03 MPa) in our observations (Table 1). Roots that are thinner than 220 μm and 570 μm in diameter accounted for 54% and 93% of total root length, respectively. These figures indicate the importance of lateral roots in the length and surface area of the rice root system. A similar result was obtained in a mature lowland cultivar in which the lateral roots occupied 97% of the length, 78% of the surface area, and 28% of the volume of the total root system under submerged conditions (Kawashima 1988b). We assume that the main part for the uptake of water and nutrient is the lateral roots, whereas nodal roots exploit the root zone and transport water and nutrient to the shoot, although clear evidence for

Table 1. Root growth and diameter distribution in rice (UPLRi-5) grown in wet and dry soil in a pot for 82 d.

Soil water	Nodal roots (no. plant^{-1})[a]	Root dry weight (g plant^{-1})	Root/ shoot ratio	Root length/ leaf area (m cm^{-2})	Total root length (m plant^{-1})	Root length (%) by diameter		
						<220	220–570 (μm)	>570
Wet soil (–0.03 MPa)	815 (262)	9.9 (1.4)	0.23 (0.01)	119 (23)	2,846 (741)	54	39	7
Dry soil (–0.1 MPa)	473 (89)	1.7 (0.3)	0.17 (0.03)	62 (14)	537 (95)	47	46	7

Values in parentheses are standard error.

these different functions should be accumulated. Under drier soil conditions (–0.1 MPa), nodal root number, root dry weight, and total root length were much lower than under wet soil conditions. Root/shoot and root length/leaf area were also lower under drier soil conditions than under wet soil conditions, indicating a high sensitivity of rice roots to low soil water.

Formation of the rice root system is characterized by synchronous development of roots and shoots. In the formation of the rice plant body, the emergence and elongation of nodal roots are closely linked with the development of leaves, tillers, and nodes (Nemoto et al 1995). New nodal roots and tillers emerge on the third node lower than the node with the newly emerging leaf. The nodal roots elongate rapidly from their emergence until new nodal roots emerge from the internode unit of two or three orders higher (Kawashima et al 1973). Because the number of nodal roots per tiller is related to the number of internode units and leaves, early tillers have a larger number of nodal roots. Nodal roots from the main tiller and primary tillers are generally longer than the roots from late tillers. This tiller order-nodal root length relationship explains the correlation between the deep root trait and type with less tillers in general (Yoshida and Hasegawa 1982).

Within a tiller, nodal roots on basal internodes tend to be longer than nodal roots from higher internodes in upland conditions. Nodal root length was higher in the 1st-3rd and 4th internodes than in higher internodes in both deep-rooted variety Moroberekan and medium deep-rooted variety UPLRi-5 (Table 2). The difference between the two varieties in root length was most pronounced in the lower internodes. The total number of primordia of the nodal roots in internodes depends on the position of the internodes and cultural conditions, such as N and light (Kawata et al

Table 2. Number and average length of nodal roots from different internode units in the main tiller in rice grown in a root box at 112 d after seeding.

Variety	Internode unit[a]							Total
	1st-3rd	4th	5th	6th	7th	8th	9th	
Moroberekan								
Number of nodal roots	21.7 (7.1)	8.7 (2.2)	8.0 (1.4)	7.3 (0.4)	6.7 (2.3)	1.3 (0.8)	0.3 (0.4)	54.0 (7.6)
Average length of nodal roots (cm)	76.6 (2.4)	81.3 (13.3)	65.6 (9.3)	38.4 (10.7)	58.4 (10.7)	42.9 (3.2)	12.6 (0.7)	69.8 (9.2)
UPLRi-5								
Number of nodal roots	15.0 (1.4)	7.5 (1.1)	4.5 (0.3)	4.5 (1.0)	2.5 (1.0)	2.5 (1.7)	1.0 (0.9)	37.5 (2.1)
Average length of nodal roots (cm)	43.8 (5.1)	55.7 (4.9)	38.5 (3.3)	31.9 (2.2)	26.2 (4.2)	11.5 (0.0)	2.7 (0.2)	39.7 (2.5)

[a]Values in parentheses are standard error.

Table 3. Tiller number and number of nodal roots per internode unit in the main tiller in rice grown in hydroponic culture for 38 d.

Variety	Tiller number (plant^{-1})	Number of nodal roots per internode unit									Total
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th and higher	
IRAT216	8	4	8	8	7	7	4	7	4	1	50
Azucena	7	4	5	6	7	3	5	6	7	4	47
UPLRi-5	17	4	13	11	8	8	8	8	7	5	72
IR20	13	4	13	12	16	11	13	10	3	1	83
IR72	26	4	13	15	12	8	7	6	5	0	70
IR43	13	4	17	21	13	12	12	5	5	1	90

1978). Nodal roots often don't develop from their primordia in tillers or internode units of a high order so that the number of developed nodal roots decreases as the order of the tiller or internode becomes higher. One factor that affects the development of roots from primordia may be the supply of assimilate to the primordia. The number of dormant primordia of nodal roots on the main tiller was decreased by defoliation of leaves on other tillers to depress their growth, resulting in an increase in the number of nodal roots on the main tiller, especially in higher internode units (Kawata and Harada 1980).

Because suppression by shading was more pronounced for the emergence and elongation of new nodal roots than for the growth of older nodal roots, the development of new nodal roots depends more on newly assimilated carbohydrate than on translocation from accumulated assimilate in shoots compared with old nodal roots (Tatsumi and Kono 1980). Nodal root number per internode or nodal roots per tiller varies among genotypes (Table 3) and by cultural condition. If the allocation of assimilate for the formation of nodal roots and thickness of roots is constant, nodal root length is negatively correlated with nodal root number. Genotypic variability in nodal root number per internode indicates the possibility for genetic manipulation of the association between tillering type and nodal root length in designing a deep-rooted plant type.

Two types of lateral roots have long been recognized in rice: thick and long lateral roots with higher-order branching and thin and short lateral roots without higher-order branching (Kawata and Shibayama 1965, Kono et al 1972). These two types of lateral roots are also found in other cereals. Anatomical observation clarified that thick and long lateral roots (L-type) have an inner structure similar to that of seminal roots and nodal roots, whereas short lateral roots (S-type) have a simpler vascular bundle (Yamauchi et al 1987b). Although this morphological difference is clearly distinct, the difference in function between these two types of lateral roots is not well known. Because L-type lateral roots are long and have higher-order branches, these roots account for an important portion of total root length and surface area (Yamauchi et al 1987a). We assume that the density of L-type lateral roots largely affects root

Table 4. Density of thick lateral roots on nodal root axis (no. cm^{-1}) from different internodes and tillers. (No statistical results are provided.)

Variety	Nodal roots from lower internodes in main tiller	Nodal roots from higher internodes in main tiller	Nodal roots from tillers	Av in whole plant
IRAT216	0.54	0.33	0.30	0.40
UPLRi-5	0.70	0.53	0.48	0.54
Azucena	0.63	0.67	0.63	0.66
Kinandang patong	0.91	0.60	0.59	0.70
IR20	0.58	0.52	0.39	0.47
IR72	0.86	0.67	0.51	0.62
Vandana	0.52	0.50	0.42	0.48
IR43	0.77	0.64	0.60	0.65
Moroberekan	0.83	0.71	0.73	0.76
Dular	0.79	0.66	0.58	0.70
IR64	0.87	0.61	0.51	0.61
IR65598-112-2	0.63	0.43	0.46	0.51
Mean	0.72	0.57	0.52	0.59

length density at depth. The density of lateral roots was the highest in the middle portion of the nodal root axis (Kawashima 1988a). Upper nodal roots have a higher density than lower nodal roots when compared within the same internode unit (Kawata et al 1980).

The root environment, such as oxygen supply and soil physical stress, affects the development of different types of lateral roots in different ways. Aerobic conditions stimulate the length of thin first-order lateral roots and the number of second-order lateral roots rather than the number of thick lateral roots (Abe et al 1994). The density of L-type lateral roots is higher with NO_3 than with NH_4 as an N source. There is still limited evidence on genotypic variability in lateral root traits and response to water stress. It appears that rice has genotypic variability in the density of thick L-type lateral roots on the nodal axis (Table 4). The density of L-type lateral roots is higher on nodal roots from lower internodes than on those from higher nodes in the main tiller or in other tillers. Varietal differences were consistently found in all portions and these were larger than the differences among portions. Some studies indicate that the major portion of water is taken into the roots through the lateral roots in maize (Varney and Canny 1993). The implication of morphological differences in lateral root traits for their function in water uptake needs to be clarified in relation to plant response to drought.

Root growth under field conditions and environmental effect

Root length and dry weight generally reach their maximum from the panicle initiation (Lilley and Fukai 1994) to flowering stage (Cruz et al 1986, Beyrouty et al 1988). Root growth, in terms of length and dry weight, proceeded more quickly than shoot

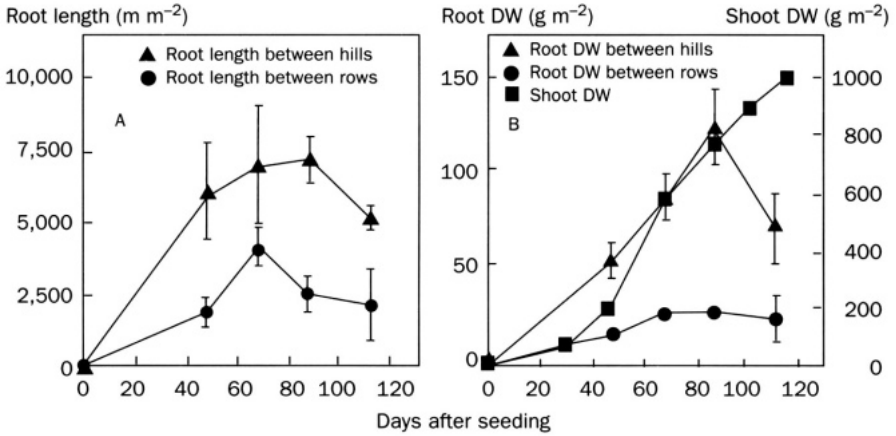

Fig. 1. Change in (A) root length and (B) root and shoot dry weight (DW) during rice growth (UPLRi-5) (unpublished data).

growth in the early stages and declined after panicle initiation between plant rows and after flowering between plant hills in our observations in the wet-season crop (Fig. 1). The rooting zone expanded both vertically and horizontally from crop establishment to the tillering stage and slightly more horizontally from tillering to panicle initiation. Maximum rooting depth was attained at the early tillering stage. Root length distribution became slightly shallower from the middle tillering stage to panicle initiation, which is indicated by the large increase in root length between plant rows. This is probably related to the increase in shallower nodal roots emerging from late tillers. A close linkage between tiller and nodal root development is reflected in the spatial expansion of the rooting zone.

The rice root system is characterized by a high distribution in the shallow layer compared with other upland crops (Angus et al 1983), mainly because of numerous and shallow nodal roots. Nodal roots of rice are distributed deeper in aerobic uplands than in submerged soil. Aerobic conditions decrease the number of nodal roots per internode and stimulate the elongation of nodal roots. To investigate root distribution and its variability in tropical upland rice, root growth (in terms of length and dry weight) and distribution of roots in improved upland cultivar IRAT 216 were investigated under different P and N management and plant density in rainfed field conditions at five locations in the Philippines and Thailand in the 1995 to 1997 wet-season crop. Root length density usually decreased exponentially as depth increased. Total root length in the 0–90-cm depth measured by the intersection method (Newman 1966) varied from 2,154 m to 15,661 m m^{-2} (Table 5). On average across experimental sites and management, root length depth index, above which 50% of root length

Table 5. Growth and distribution of roots in field conditions at flowering stage (IRAT216).

Parameter	Av	Max.	Min.	n
Total root dry weight (g m^{-2})	127	221	51	52
Total root length (m m^{-1})	6,790	15,661	2,154	52
Shoot dry weight (g m^{-2})	400	836	123	52
RLDI[a](cm)	20.3	27.1	12.1	52
RMDI[b](cm)	16.9	21.6	10.3	52
Root length % deeper than 30 cm	21.9	36.8	6.6	52
Root mass % deeper than 30 cm	15.4	25.5	3.3	50

[a] Root length depth index, above which 50% of root length exists. [b] Root mass depth index, above which 50% Of root mass exists.

exists, was 20 cm. Some 22% of root length was distributed below 30-cm depth. Root dry weight was distributed shallower than length, which indicated that roots are thinner in the deeper layer. Those figures confirmed the shallow root distribution in rice. But root growth and distribution varied substantially as affected by nutrient and water conditions.

The application of P increased root length and weight from the surface to the lower layers in general and led to deeper root distribution, especially in P-deficient acidic soils. A root box experiment signified that the deeper root distribution with Pin the field is associated with a change in the development of nodal and lateral roots. Phosphorus increased total nodal root number by increasing the number of tillers and the number of nodal roots per tiller (Table 6). Early and long nodal roots from the lower internodes in early tillers increased with a higher P level, which led to an increase in root density in the deep layers. Phosphorus status in the plant and hence the development of main and primary tillers during the early vegetative stage largely affect root distribution after the reproductive stage. In lateral roots, the application of P in the surface layer modifies root branching in the whole soil profile. Phosphorus applied in the 0–20-cm depth increased the proliferation of fine lateral roots, which is expressed as a high proportion of roots thinner than 220 μm, not only at the surface but also in lower layers below 20 cm (Table 7). Localized stimulation of lateral root proliferation by NH_4, NO_3, P, and K is well known (Drew 1975, de Jager 1982). The results imply that nutrient supply in a part of the root system affects lateral root development in the whole root system.

A high dose of N application substantially inhibits the elongation of nodal roots, which results in a shallow root system in submerged conditions (Kawata et al 1977). N reduced the length of nodal roots from all tillers. In aerobic soil, there was no obvious inhibition of nodal root elongation. This different response to N in submerged and aerobic soil is probably due to the difference in the major form of N—NO_3 in aerobic soil and NH_4 in submerged soil—and the interaction with oxygen supply. High NH_4 at 20 mg N L^{-1} increased nodal root number and tended to increase total

Table 6. Effect of P level on number of nodal roots per tiller and number of long nodal roots (>30 cm) in internode units in main tiller in Moroberekan and UPLRi-5 grown in root box.

Variety and P level	Nodal roots per tiller (no.)		Long (>30 cm) nodal roots (no.) in internode unit							
	Main tiller	Primary tillers	1st-3rd	4th	5th	6th	7th	8th	9th	10th
Moroberekan										
No P	16 (3)[a]	2 (1)	5.3	2.9	2.3	1	0.9	0.4	0	0
0.5 g box^{-1}	50 (5)	19 (4)	12.8	7.8	5.3	3.9	3.4	2.2	1.2	0.2
1.0 g box^{-1}	53 (9)	19 (3)	17.7	8.2	5.1	4	2.3	0.1	0	0
UPLRi-5										
No P	14 (3)	2 (1)	2.6	1.6	1.4	1.4	1.3	0.8	0	0
0.5 g box^{-1}	38 (5)	7 (2)	5.8	4.6	4.1	1.7	0.6	0.1	0.2	0
1.0 g box^{-1}	44 (4)	10 (3)	7.6	5.2	4.2	2.1	1.3	0.2	0	0

Values in parentheses are standard error.

Table 7. Effect of P on diameter of roots in soil layers in UPLRI-5 grown in a pot for 82 d.

Treatment and soil depth	Diameter (μm)			
	<80	80–220	220-570	>570
	(%)			
No P				
0–20 cm	5.1 (0.5)[a]	42.6 (0.3)	42.1 (0.2)	9.4 (0.1)
20-40 cm	4.3 (0.6)	38.7 (1.0)	47.2 (1.0)	9.3 (0.9)
40–60 cm	10.9 (4.6)	33.6 (5.1)	41.4 (4.7)	14.2 (3.0)
220 mg P pot^{-1}				
0–20 cm	8.0 (1.9)	55.3 (1.7)	31.1 (2.9)	5.6 (0.7)
20–40 cm	4.5 (0.3)	46.6 (1.4)	42.4 (0.4)	6.5 (0.8)
40–60 cm	5.5 (0.5)	43.7 (5.4)	44.7 (4.0)	6.0 (1.7)

[a]Values in parentheses are standard error.

nodal root length compared with low NH_4 at 2 mg N L^{-1}, but inhibited root elongation as indicated by a lower maximum root length (Table 8). High NO_3 increased nodal root number compared with low NO_3, but to a lesser extent than NH_4, and it had no inhibitory effect on maximum root length. Under field conditions, a medium level of N (90 kg ha^{-1}) increased root length density from the surface to 20-cm depth compared with no N (Fig. 2). Root length density also tended to be higher in the 20-60-cm depth in medium N than with no N. N would not necessarily lead to shallow root distribution in uplands, unlike in submerged soil. There was no increase in root length density with a higher N level (180 kg ha^{-1}) compared with a medium N level.

Genotypic variability in root system morphologies is broadly reviewed in relation to drought tolerance among crops by O'Toole and Bland (1987). Large environ-

Table 8. Effect of N source and level on shoot dry weight and roots in rice (UPLRi-5) grown in hydroponic culture for 38 d.

N source and level	Shoot dry weight[a] (g plant^{-1})	Tiller number (plant^{-1})	Nodal root number (plant^{-1})	Max. root length (cm)	Total nodal root length (cm)
Low NO_3 (3 mg N L^{-1})	1.89 c	5 d	100 d	59.2 a	206 b
High NO_3 (20 mg N L^{-1})	6.31 b	12 b	179 b	72.7 a	313 a
Low NH_4 (2 mg N L^{-1})	2.74 c	8 c	138 c	61.7 a	245 ab
High NH_4 (20 mg N L^{-1})	8.38 a	19 a	261 a	38.2 c	293 ab

[a]Values followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

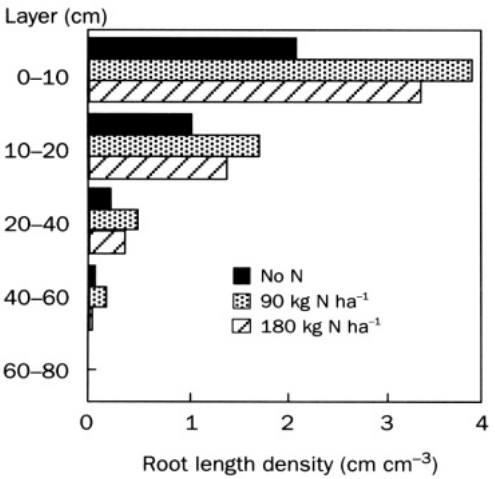

Fig. 2. Effect of N on root length density in the soil profile in upland rice (UPLRi-5) (unpublished data).

mental effects often complicate the characterization of genotypic traits in root morphology. Genetic and environmental effects on root architecture integrate effects in each root component. The responses of each root component to the environment are often mutually related or compensatory. Root architecture, especially of nodal root structure, is related to the development of shoot components, such as tillering habit and leaf emergence. Plasticity of the root system as affected by environmental and genetic factors and their interactions can be considered in two ways: the direct effect on each root component and the effect through mutual links among root and shoot components. For response to N, for example, the root system of different genotypes responds to N in different ways because of different responses in elongation of the individual root axis (Tanaka et al 1993) and differences in tillering habit. An analysis

of these two aspects would help us understand comprehensively the environmental effect and environment × genetic interactions in the plasticity of root architecture.

Water capture by roots under stress

Water-capturing capacity is considered to be determined by three factors: rooting zone or depth, root length at depth, and plant resistance. Understanding the importance of these factors under different types of stress is important in designing an efficient plant and root system. To characterize the capacity of rice roots to extract water from soil layers, the pattern of water extraction from soil layers was investigated under different intensities of water stress: mild stress (irrigation two times per week at 56% of potential evaporation) and severe stress (no irrigation), both around the panicle initiation stage in a comparison with maize. The effects of a different N application rate (0, 90, and 180 kg N ha^{-1}) on the water extraction pattern were also analyzed.

Under mild stress when soil water potential was higher than –0.42 MPa in the 0-20-cm depth and –0.06 MPa below 20 cm, a major portion of water was extracted from the 0–20-cmdepth by both rice and maize 8–13d after the stress began (Fig. 3). Extraction from the 0-20-cm layer contributed more to total extraction in rice than in maize. This difference in extraction pattern between rice and maize is reflected in root distribution. Rice had less root distribution than maize in the layer below 20-cm depth (Fig. 4). Water extraction from the 0–20-cm to the 40–60-cm layer was in-

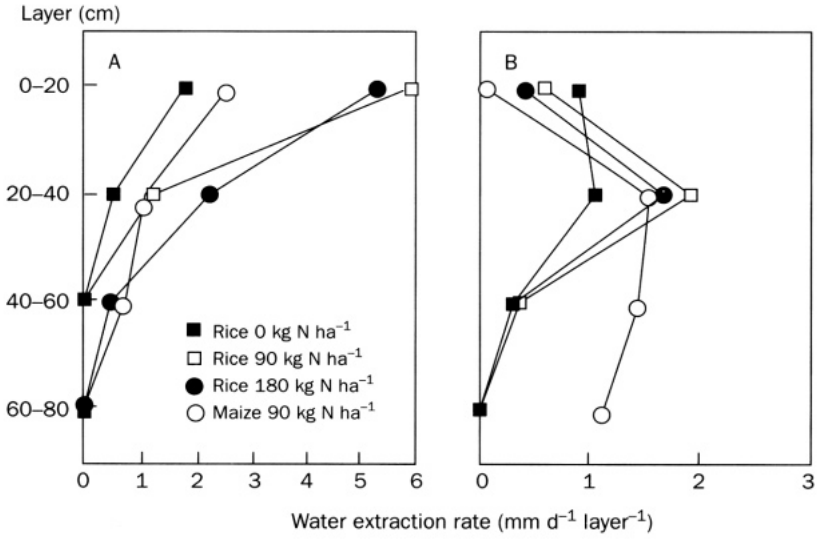

Fig. 3. Water extraction from soil by rice (UPLRi-5) and maize under (A) mild stress and (B) severe stress (unpublished data).

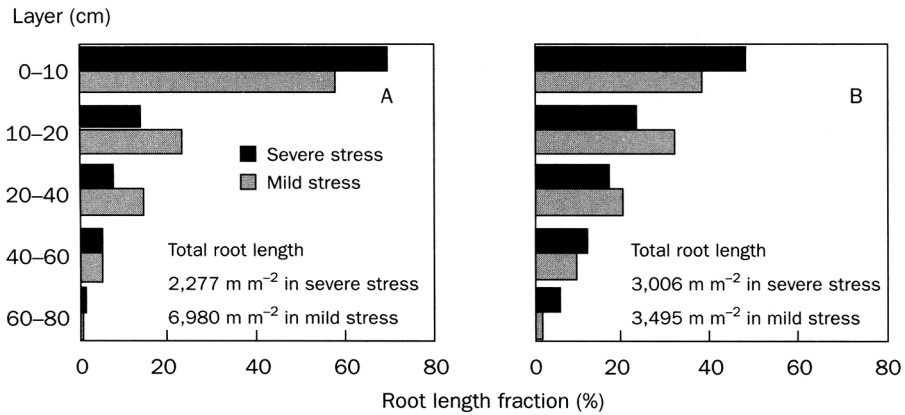

Fig. 4. Root length distribution in the soil profile after severe stress and mild stress in (A) rice and (B) maize (unpublished data).

creased with N compared with no N. This increase in water extraction with N was partly explained by the increase in root length density caused by N. In addition, the maximum extraction rate per unit length of root was also increased with N in the 0-60-cm depth. With higher N, the extraction rate per unit length of root increased in the deeper layer (Table 9). The water extraction rate per unit length of root can be flexible to some extent possibly because of the transpiration demand of the shoot. These results indicate that proper nutrient supply can help exploit more water in and below the surface layer under mild stress or favorable soil water conditions. Rice showed a higher maximum extraction rate per unit length of root than maize. The maximum water extraction rate per unit length of root was higher in the layer below 20 cm than in the surface 0–20 cm in both rice and maize, perhaps because of the low water potential in the surface 0–20 cm, which was lower than –0.1 MPa, or the lower proportion of active roots to absorb water in the surface layer.

Under severe stress when the soil was drying rapidly in the surface layer, total transpiration declined rapidly in the first 7 d by 44% compared with before the stress in rice, while transpiration was similar for 13 d in maize. The peak of water extraction moved downward as the surface became drier in both rice and maize (Fig. 3). Water extraction from the layers below 40 cm was clearly more limited in rice than in maize. With a higher N rate, the depletion of water in the surface layer was faster and extraction from the layers below 20 cm increased more than with no N in rice. At the medium N rate (90 kg ha^{-1}), rice leaves were totally rolled before soil water potential in the 20–40-cm and 40-60-cm layers decreased to –0.09 MPa and –0.02 MPa, respectively.

Table 9. Maximum water extraction rate per unit length of root (cm^3 m^{-1} d^{-1}) during mild stress in rice and maize.

Crop and N level		Layer		
		0–20 cm	20–40 cm	40–60 cm
Rice	0 kg ha^{-1}	1.27	2.45	0.00
Rice	90 kg ha^{-1}	1.99	2.49	2.26
Rice	180 kg ha^{-1}	2.29	3.99	5.00
Maize	90 kg ha^{-1}	1.09	1.53	2.27

Limited water extraction in rice can be attributed primarily to reduced root length density at depth under severe stress. In maize, total root length decreased slightly, but the proportion of root length below 40 cm tended to increase under severe stress (Fig. 4). On the other hand, there was a large reduction in total root length and no increase in root distribution below 40 cm in rice. The growth of rice roots seems to be sensitive to soil drying. Besides constitutive root traits expressed under nonstress conditions, increasing root length in deeper layers in response to soil drying is considered to be an important trait that ensures water capture under severe stress (Hurd 1974, Nobel et a1 1993). In responding to decreasing soil water, stimulation of growth in higher-order branching to increase root length was recognized in upland crops (Jupp and Newman 1987, Morita and Okuda 1994). The formation and elongation of new nodal roots continued under low soil water content to maintain total nodal root length in maize (Paradales and Kono 1990). The elongation of nodal roots was maintained under low water potential conditions by the increased elasticity of the cell wall in maize (Wu et a1 1994).

Partitioning of assimilate to roots was higher in drought-tolerant species than in sensitive species of forage crops (Ogata et a1 1985). The response of rice roots in the deep layer to intense soil drying in the surface layer and its genotypic variability should be studied, in terms of growth of lateral and nodal roots and partitioning of assimilate to the root system, to improve water uptake under severe stress.

Although the water extraction rate per unit length of root increased slightly in the 20-40- and 40–60-cm layers to compensate for the decrease in extraction in the surface 0–20 cm, total transpiration declined rapidly and leaf water potential could not be maintained in rice under severe stress. Soil water potential was higher than –0.01 MPa in both rice and maize below 40 cm 8 to 13 d after stress began. The water extraction rate per unit length of root in the layer below 40-cm depth in rice was comparable to or lower than that of maize during this period, whereas soil water potential was lower in maize than in rice. Leaf water potential was lower in rice than in maize. Soil resistance at the observed range of soil water potential is likely to be minor in the total resistance of soil and plant (Newman 1969). Therefore, plant resistance from roots to leaves seemed to be higher in rice than in maize.

There are variable indications on the relative importance of shoot and root resistance to water flow in plants. Shoot resistance is comparable to or higher than root resistance (Blizzard and Boyer 1980, Eavis and Taylor 1979, Adeoye and Rawlins 1981) or shoot resistance is lower than root resistance (Dube et al 1974, Allaway et al 1981, Meyer and Ritchie 1980). In rice, the resistance of roots increases as their age increases and becomes important in total plant resistance, whereas shoot resistance is relatively stable regardless of age under submerged soil conditions (Hirasawa et al 1992). The importance of root axial resistance relative to root radial resistance was suggested in rice (Hasegawa and Yoshida 1982) and other crops (So 1979, Richards and Passioura 1981). Although it is possible that genetic variability in xylem number and size in rice (Terashima et al 1987) would provide an opportunity for improving axial resistance, experimental evidence on the advantages of a large xylem in water uptake is not available. It is essential to understand the dynamics of water potential in leaves, stems, and roots under stress to clarify the relative magnitude of shoot and root resistance and the opportunity for improving plant resistance in rice.

References

Abe J, Yamamoto S, Morita S, Akita S. 1994. Effects of soil moisture on the development of lateral roots in rice (*Oryza sativa* L.). Jpn. J. Crop Sci. 63(Extra Issue 1):200-201.

Adeoye KB, Rawlins SL.1981. A split-root technique for measuring root water potential. Plant Physiol. 68:44-47.

Allaway WG, Pitman MG, Storey R, Tyerman S. 1981. Relationship between sap flow and water potential in woody and perennial plants on islands of the Great Barrier Reef. Plant Cell Environ. 4:329-337.

Angus JFS, Hasegawa S, Hsiao TC, Liboon SP, Zandstra HG. 1983. The water balance of post-monsoonal dryland crops. J. Agric. Sci. 101:699-710.

Beyrouty CA, Wells BR, Norman RJ, Marvel JN, Pillow JA. 1988. Root growth dynamics of a rice cultivar grown at two locations. Agron. J. 80:1001-1004.

Blizzard WE, Boyer JS. 1980. Comparative resistance of the soil and the plant to water transport. Plant Physiol. 66:809-814.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Cruz RT, O'Toole JC, Dingkuhn M, Yambao EB, Thangaraj M, De Datta SK. 1986. Shoot and root responses to water deficits in rainfed lowland rice. Aust. J. Plant Physiol. 13:567-575.

de Jager A. 1982. Effect of localized supply of H_2PO_4, NO_3, SO_4, Ca and K on the production and distribution of dry matter in young maize plants. Neth. J. Agric. Sci. 30:193-203.

Drew MC. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot in barley. New Phytol. 75:479-490.

Dube PA, Stevenson KR, Thurtell GW, Neumann HH. 1974. Steady state resistance to water flow in corn under well watered condition. Can. J. Plant Sci. 55:941-948.

Eavis BW, Taylor HM. 1979. Transpiration of soybeans as related to leaf area, root length, and soil water content. Agron. J. 71:441-445.
Fukai S, Inthapan P. 1988. Growth and yield of rice cultivars under sprinkler irrigation in south-eastern Queensland. 3. Water extraction and plant water relations: comparison with maize and grain sorghum. Aust. J. Exp. Agric. 28:249-252.
Hasegawa S, Yoshida S. 1982. Water uptake by dryland rice root system during soil drying cycle. Soil Sci. Plant Nutri. 28:191-204.
Hirasawa T, Gotou T, Ishihara K. 1992. On resistance to water transport from roots to leaves at different positions on a stem in rice plants. Jpn. J. Crop Sci. 61: 153-158.
Hurd EA.1974. Phenotype and drought in wheat. Agric. Meteorol. 14:39-55.
Jupp AP, Newman EI. 1987. Morphological and anatomical effects of severe drought on the roots of *Lolium perenne* L. New Phytol. 105:393-402.
Kawashima T, Ishihara H, Ogura T. 1973. On the development of crown roots in various growth stages of the rice plant. Proc. Crop Sci. Soc. Jpn. 42:197-206.
Kawashima T. 1988a. Root system formation in rice plant. I. Development of lateral roots on the primary roots of the main stem. Jpn. J. Crop Sci. 57:8-18.
Kawashima T. 1988b. Root system formation in rice plant. III. Quantitative studies. Jpn. J. Crop Sci. 57:26-36.
Kawata S, Shibayama S. 1965. On the lateral root primordia formation in the crown roots of rice plants. Proc. Crop Sci. Soc. Jpn. 33:423-431.
Kawata S, Maruyama S, Soejima M. 1977. Root formation in rice plant and levels of nitrogen supply. Jpn. J. Crop Sci. 46:193-198.
Kawata S, Harada J, Yamazaki K. 1978. On the number and the diameter of crown root primordia in rice plants. Jpn. J. Crop Sci. 47:644-654.
Kawata S, Harada J. 1980. The effect of defoliating tiller leaves on the crown root formation in the main stem of rice plants. Jpn. J. Crop Sci. 49:587-592.
Kawata S, Saaski O, Yamazaki K. 1980. On the lateral root formation in relation to the diameter of crown roots in rice plants. Jpn. J. Crop Sci. 49:103-111.
Kono Y, Igeta M, Yamada N. 1972. Studies on development physiology of the lateral roots in rice seminal roots. Proc. Crop Sci. Soc. Jpn. 41:192-204.
Lilley JM, Fukai S. 1994. Effect of timing and severity of water deficit on four diverse rice cultivars. I. Rooting patterns and soil water extraction. Field Crops Res. 37:205-214.
Meyer WS, Ritchie JT. 1980. Resistance to water flow in the sorghum plant. Plant Physiol. 65:33-39.
Morita S, Okuda H. 1994. Effects of soil water condition on root development of wheat seedling with special reference to branching of primary seminal root. Jpn. J. Crop Sci. 63:418-422.
Nemoto K, Morita S, Baba T. 1995. Shoot and root development in rice related to the phyllochron. Crop Sci. 35:24-29.
Newman EI. 1966. A method of estimating the total root length in a sample. J. Appl. Ecol. 3:139-145.
Newman EI. 1969. Resistance to water flow in soil and plant. I. Soil resistance in relation to amount of root: theoretical estimates. J. Appl. Ecol. 6:1-12.
Nobel PS, Huang B, Garcia-Moya E. 1993. Root distribution, growth, respiration, and hydraulic conductivity for two highly productive agaves. J. Exp. Bot. 44:747-754.

Ogata S, Saneoka H, Matsumoto K. 1985. Nutrio-physiological evaluation of the drought resistance of warm season forage species. II. Comparative studies in growth, water and nutrient absorption of warm season forage species grown under various soil water levels. J. Jpn. Grassl. Sci. 31:43-51.

O'Toole JC, Bland WL. 1987. Genetic variation in crop plant root system. Adv. Agron. 41:91-145.

Paradales JR, Kono Y. 1990. Development of sorghum root system under increasing drought stress. Jpn. J. Crop Sci. 59:752-761.

Richards RA, Passioura JB. 1981. Seminal root morphology and water use of wheat. I. Environmental effects. Crop Sci. 21:249-252.

So HB. 1979. Water potential gradients and resistances of a soil-root system measured with the root and soil psychrometer. In: Harley JL, Russel RS, editors. The soil-root interface. London (UK): Academic Press. p 99-113.

Tanaka S, Yamauchi A, Kono Y. 1993. Response of the seminal root elongation to NH_4-nitrogen in several rice cultivars. Jpn. J. Crop Sci. 62:288-293.

Tatsumi J, Kono Y. 1980. Effects of shading on respiration and ammonium uptake of rice roots: comparison of activity in roots from different nodes. Jpn. J. Crop Sci. 49:66-74.

Terashima K, Hiraoka H, Nishiyama I. 1987. Varietal difference in the root of rice plant. 1. Varietal difference in the morphology of crown root. Jpn. J. Crop Sci. 56:521-529.

Varney GT, Canny MJ. 1993. Rates of water uptake into the mature root system of maize plant. New Phytol. 123:775-786.

Wu Y, William G, Shrap RE, Hetherington PR, Fry SC. 1994. Root growth maintenance at low water potentials. 1. Increased activity of xyloglucan endtransglycosylase and its possible regulation by abscisic acid. Plant Physiol. 106:607-615.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Yamauchi A, Kono Y, Tatsumi J. 1987a. Quantitative analysis on root system structures of upland rice and maize. Jpn. J. Crop Sci. 56:608-617.

Yamauchi A, Kono Y, Tatsumi J. 1987b. Comparison of root system structures of 13 species of cereals. Jpn. J. Crop Sci. 56:618-631.

Yoshida S, Hasegawa S. 1982. The rice root system: its development and function. In: Drought resistance in crops with emphasis on rice. Manila (Philippines): International Rice Research Institute. p 97-114.

Notes

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Perspectives on molecular genetics

Genetic improvement of rice for drought and salt tolerance: molecular breeding and transgenic strategies

A.R. Reddy, V. Ramanathan, N. Seetharama, S. Bajaj, and R. Wu

Genetic enhancement of rice for improved performance under water-limited environments and high salt is of paramount importance. Producing improved rice lines for stress environments requires the rigorous application of molecular breeding and biotechnology. In this chapter, we discuss current efforts in this direction and present information on the status of research on DNA marker technologies in the improvement of crops for drought and salt tolerance. To improve rice further using marker-assisted selection and map-based cloning, we require critical information on component traits, accurate phenotyping, the identification of candidate genes and quantitative trait loci (QTLs), the relationship between QTLs and genes, the contribution of individual QTLs to the phenotype, and their variability across different locations and different crop seasons. Another important route to producing stress-tolerant rice varieties is transformation, which offers a powerful means of incorporating exotic or even synthetic genes with a profound ability to up or down-regulate specific metabolic steps. Rapid progress has been made in developing transformation technologies for rice and gene transfer will be done routinely soon. Developments in new strategies of isolation and characterization of novel genes and promoters and their successful transfer into rice provide new avenues for metabolic engineering for stress tolerance. We examine the usefulness of the transgenic approach for improving stress tolerance in rice. We describe the potential genes and promoters that are associated with various stress-response pathways in plants and other organisms. Such novel genes and promoters offer unique opportunities in genetic engineering of rice for stress environments. Marker-aided selection and transgenic approaches are two powerful tools to accelerate plant breeding to produce rice varieties with improved drought and salt tolerance.

Drought and increased soil salinity are two major factors that limit plant growth and productivity (Yancey et al 1982). In spite of a surge in literature on drought tolerance in crops during the past two decades, practical progress in breeding for drought tolerance has not been significant. Drought therefore requires an analytical approach of dissecting and studying the contribution of trait components using the quantitative trait loci (QTLs) model. This approach is particularly suited to crops like rice for which dense genetic linkage maps with a variety of DNA markers are already available. Most studies on drought tolerance deal with evaluating specific traits logically

related to crop performance under drought. A molecular genetic analysis is more effective if conducted on individual traits (and even their components) before crop performance is considered. Thus, it is important to phenotype for specific traits or responses under managed nursery or greenhouse conditions, besides subsequent field evaluations under drought in target regions. A strong demonstrated linkage between traits in relation to crop performance in the target environment is a crucial step before advocating marker-assisted selection (MAS).

A second approach is to create a novel and functionally known type of variability in plant stress response by genetic transformation. The transgenic approach offers a powerful means of incorporating a broad spectrum of genes with profound ability to up- or down-regulate specific metabolic steps associated with stress response. But transformation with any single gene or group of genes for a particular pathway may not be adequate for conferring drought tolerance because the products from several pathways are required to ensure drought tolerance (Ingram and Bartels 1996). In general, many proteins and low-molecular-weight osmolytes accumulate under stress. It is not clear which factor(s) among many changes contributes to the development of stress tolerance because so many changes occur. The transgenic approach is useful, however, to obtain valuable information. By transferring a single gene into a plant, and then studying the response of the resulting plants to drought or salt stress, we can demonstrate whether or not a given gene contributes to increased tolerance for a particular stress condition, and what related changes occur. This approach will thus be useful in identifying candidate genes for stress tolerance or its components with significant developments in gene transfer technologies for rice and rapid progress in gene isolation and manipulation. In this chapter, we examine the utility of DNA marker technologies in the genetic enhancement of drought tolerance in rice and highlight progress made and new opportunities in QTL studies. Further, we analyze the contribution of the transgenic approach to improve rice for water-limited environments and high salt. We emphasize the utility of certain genes and promoters from plants and other organisms that may have potential to confer drought or salt stress tolerance in rice.

Molecular breeding

Identifying and characterizing RFLPs and QTLs for component traits

There are some fundamental similarities and a few differences in the mechanisms that contribute to drought tolerance and yield formation in different crops. The similarities are expected to be more in molecular mechanisms than in the morphological or physiological manifestation of drought effects. Therefore, rice scientists can learn much from drought research on other crops and vice versa. Therefore, we highlight and compare progress made with different crops using the molecular marker approach. As expected, most studies on molecular marker detection deal with specific components that are likely to be significant for crop growth and yield under drought such as the ability of roots to penetrate deeper layers of the soil (Ray et al 1996). They include morphological (e.g., root and shoot characteristics), developmental (anthesis to silking

Table 1. Summary of quantitative trait loci (QTLs) related to drought tolerance in some crops.

Trait	Crop	Mapping population	Reference
Root characteristics			
1. Morphology	Rice	203 RILs from Co39 x Moroberekan	Champoux et al (1995)
2. Distribution	Rice	DH population: 105 lines	Yadav et al (1997)
Leaf/shoot characteristics			
1. Leaf and stomatal conductance	Rice	178 F_2s	Price and Tomas (1997)
2. Seedling vigor	Rice	118 F_2s; both parents are japonica	Redona and Mackill (1996)
Physiological			
1. Relative water content and osmotic potential	Barley	187 RILs	Teulat et al (1997, 1998)
2. Anthesis silking interval	Maize	272 F_2s	Ribaut et al (1996)
3. Preflowering stress	Sorghum	98 RILs	Tuinstra et al (1996)
4. Postflowering stress, stay-green trait	Sorghum	98 RILs	Tuinstra et al (1998)
5. Biochemical accumulation of ABA	Maize	81 F_2s	Quarrie et al (1997)
Water-use efficiency (WUE)			
1. WUE	Wheat	150 DH lines	Quarrie et al (1995)
2. WUE, ^{13}C discrimination	Tomato	F_3s and BC_1F_1 families, 11 high and 8 low families selected	Martin et al (1989)
Yield and yield components; G x E interactions			
1. Yield, yield components	Maize	150 F_2:F_3 lines	Veldboom and Lee (1994)
2. G x E interactions	Maize	272 F_2s	Ribaut et al (1997)
Combination with other economic traits			
1. Heterosis	Maize	264 backcross families	Stuber (1997)
2. Nitrogen-use efficiency	Maize	100 RILs	Bertin et al (1997)

interval, ASI), physiological (osmotic adjustment, OA), metabolic (ABA accumulation), and agronomic (water-use efficiency, WUE) characteristics (Table 1). Several studies also include yield or yield components. Identifying QTLs for both physiological components of drought-tolerance traits and final yield and yield components un-

der drought in the same study is rare. Sometimes, the populations used for mapping and QTL detection are of different generations. Rarely is the QTL detected in one population verified in other crosses. It is somewhat difficult to compare results obtained with different types (doubled haploids, DH, recombinant inbred lines, RILs, etc.; Table 1) and sizes of mapping populations (varying from as low as 19 to 272 or more).

The number of QTLs detected for a drought-tolerance trait generally varied between 1 and 4, and QTLs were spread across the genome on several linkage groups. Measuring integrated responses by means of traits such as water-use efficiency also involved only a few QTLs (4–5 in soybean; Milan et al 1998). In a few cases, there were useful QTLs for more than one trait on the same linkage group (e.g., osmotic adjustment and dehydration tolerance in the region associated with root morphology in rice; Lilley et al 1996). Phenotypic variation for the measured trait accounted for by an individual QTL was generally about 10%. Some exceptions are noted, however. For example, the QTL for root length at 28 d of growth accounted for 30% of phenotypic variation (Price and Tomas 1997; Table 1), but such observations can only be taken seriously if they are repeatable. The best model (with about 3 QTLs) rarely accounted for more than 50% of the observed phenotypic effect. In several cases, association between QTLs for different traits existed (e.g., for yield components; Bertin et al 1997).

The relationship between QTL and yield was negative in some cases, but in others, such as with ASI (below), the drought-tolerance trait did not decrease yield under well-watered conditions (Ribaut et al 1996). QTLs such as those for ASI, which are stable over years and stress levels, are the immediate candidates for use in MAS. Stability of QTLs across environments or stress levels is rarely studied except for yield or yield components. In the case of maize, in spite of >10-fold differences in yields, the QTLs for yield were consistent across environments (Stuber 1997). Evidence of crossover-type interactions across generations tested under different stress levels was only slight, and most of the interactions were in the form of a change in magnitude of QTL effects (Austin and Lee 1998).

The above observations suggest that, while QTL analysis of drought-tolerance traits is encouraging, the challenge of breeding for drought tolerance using DNA markers remains. Progress will come in incremental steps, but at least in the near future it will be confined to a few specific crops (Table 1) under well-defined stress patterns. For correct interpretation of QTL data, measurement of phenotypic values in relation to specific characteristics in the test environments is as important as genotypic data.

Molecular breeding strategies for parents, mapping populations, and markers

Most maps are developed with very contrasting and diverse parents. In rice, most of the initial mapping populations are between indica and japonica types. There are only a few exceptions. Ideally, we would like to choose parents with extreme phenotypic

values, which may or may not meet the immediate needs of breeders. In most practical breeding programs, we would normally use one donor parent and the other from the local pool of materials, which is also useful for many other locally important agronomic traits to make marker-assisted breeding cost-effective. With the availability of a large number of markers, and experience with different types of mapping populations of rice, finding sufficient DNA polymorphism between the parents and mapping populations is not a serious problem in rice, unlike in many other self-pollinated crops. Once the QTLs are identified in an early generation, further selection within the family can result in isogenic lines for fine mapping, and for verifying the effect of individual QTLs as shown for sorghum (Tuinstra et a1 1998). For detailed studies on the effect of individual QTLs, generating advanced-backcross RILs (or contig lines) is a useful approach. Selective genotyping and bulked segregant analysis (BSA) normally bring down the cost of mapping and identifying closely linked QTLs considerably. Such pools can be further used to screen even with dominant markers such as random amplified polymorphic DNA (RAPDs), or even amplified fragment length polymorphisms (AFLPs). But the identification of pools for BSA is very risky for mapping drought-tolerance traits because we are dealing with batteries of QTLs. Therefore, a skeleton map may always be needed before techniques such as BSA can be attempted to saturate specific map regions.

Most marker-assisted breeding for drought tolerance is restricted to a few selected characters. To make MAS for drought tolerance a reality, we could consider incorporating several QTLs simultaneously. As a first attempt, we could use the best subset of QTLs for different traits as suggested for maize (Ribaut et a1 1997). For the production of F_1 hybrids of rice, QTL mapping results obtained with different testers may be examined in the near future. Results with maize, however, indicate that the consistency of QTL results across testers is disappointingly low (Lubberstedt and Melchinger 1997).

Phenotyping and co-localization of QTLs with other traits of agronomic interest and known functional genes

QTLs for many traits can be identified from the mapping population (Table l), and many such QTLs can be found in close proximity on the same chromosome. For example, Champoux et a1 (1995) found that 12 of the 14 chromosomal regions harbor QTLs for drought tolerance and root traits, and some of the co-located QTLs could be negatively associated (such as osmotic adjustment and dehydration tolerance; Lilley et a1 1996). If such negative associations are strong, we face a problem in selection unless a large population is used. If the QTLs for a specific drought-tolerance trait are located near those for general adaptive traits such as maturity and yield components, plant breeders will promptly include such QTLs in their selection program. Physiologists need to review the causal relationship between the co-localized QTLs and the associated traits using the tools of molecular genetics. If reasonable assumptions on the relationships among the component traits can be made, interpretation may not be difficult. Further, those assumptions dealing with specific deleterious responses (such

as pollen or spikelet sterility) are easier to interpret than those for productivity under drought. The mapping/QTL approach offers an excellent opportunity to verify the genetic basis and usefulness of specific component traits (Prioul et a1 1997). For instance, Ottaviano et a1 (1991) have shown that *hsp* 70 (coding for heat-shock protein) levels are not associated with drought tolerance in maize.

Which trait is useful, and which parent contributes?

One of the interesting revelations of the QTL analysis is that both parents (drought-tolerant and -sensitive) contribute useful alleles for the trait of interest (e.g., Lilley et a1 1996, Quarrie et a1 1997). Traditional grouping of drought-tolerance traits into four distinct classes (phenological, morphological, physiological, and biochemical) may still be relevant for identifying QTLs. Because phenological and morphological traits can be scored easily in appropriately managed screening nurseries, the QTLs identified for these traits may be more reliable. Physiological traits such as osmotic adjustment or water-use efficiency are cumbersome to measure in the field, and can be relied on only under a carefully defined set of conditions. Biochemical traits, on the other hand, defy simple characterization, and most often they may only be the symptoms of stress. But they will have an increasing role in increasing our understanding of drought-tolerance mechanisms. The association between biochemical traits and QTLs is too speculative at the moment.

Drought stress intensity and identification of QTLs

It is crucial to evaluate how QTLs vary in their location when tested across different levels of stress in natural environments. Many of the QTLs associated with drought-tolerance traits may or may not be associated with yield potential. Further, there may be major inconsistencies in genomic positions in QTLs across water regimes as noted for maize (Ribaut et a1 1997). In maize (Stuber 1997), the yields of test materials (RI lines backcrossed to each parent) were tenfold less under stress than under control, but the QTLs for yield were nearly the same. Breeders could rely on mapping data from favorable environments for breeding for materials adapted to stress environments. Similar conclusions were also drawn by Bertin et a1 (1997) for breeding for yield under different nitrogen (N) levels; the QTLs under N stress were a subset of QTLs detected under high N level. They also concluded that it is more difficult to find QTLs for N stress, part of which could be due to a higher experimental error under low N. A similar argument probably applies for QTLs detected under severe drought stress. Part of this could be due to the high levels of variability in the phenotypic data collected under severe stress. Austin and Lee (1998) found little evidence of cross-over-type interactions across generations and stress levels. Instead, the interactions seemed to be in the form of a change in magnitude of effects, although only 17% of the QTLs were common across stress environments. This observation is promising for the use of QTLs. Further, common QTLs across generations and stress levels could be selected during early generations, whereas in later generations only field evaluations may be used to select for specific adaptability.

Relating QTLs to genes or gene products

If the QTLs map to a region close to genes of known function, the physiologist's goal of designing a drought-tolerant plant and the breeder's goal of finding the most-effective DNA markers are met to a significant extent. In the study of Bertin et al (1997), three out of five QTLs detected for seed size were probes for glutamine synthase, a correspondence difficult to explain by chance alone. Similarly, two major QTL regions for malting quality are located close to the beta-amylase gene in barley (Han et al 1997). For drought, a short ASI is related to greater nitrate reductase efficiency. Quarrie et a1 (1997) found changes in the frequencies of cDNAs coding for two proline-rich proteins with maize populations differing in drought tolerance. Thus, using probes of known genes could be very useful for studying the physiological and genetic meaning of the detected QTLs, and for finding markers linked to QTLs. This approach can be compared with the efforts of plant pathologists to map putative disease-tolerance genes. Localization and co-localization of QTLs for different measured traits can be easily interpreted, such as those for relative water content and number of leaves per tiller in barley (Teulat et al 1997), even when not all QTLs for each trait are located in the same region.

Only a few studies deal with identifying QTLs across different mapping populations. Not all QTLs were shared between them even when one parent was common (Milan et al 1998, Yadav et al 1997). Initial applications of marker-assisted selection may be on a selective basis and may even be alternated with empirical selection across segregating generations as in barley (Han et al 1997). Considering the syntenic relationships among different cereals, it is desirable to use a set of the same or similar anchor probes in different crops so that opportunities to examine the relevance of selected genomic regions in one crop can be readily tested in others (Van Deznze et al 1998). With the current rate of progress in gene cloning, it will be possible to isolate QTLs across crops and test these with suitable systems. In the long run, the identification of QTLs for complex traits associated with drought tolerance will enable us to re-evaluate 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 developed during such collaboration will also help us to better understand physiological and developmental phenomena in rice.

Transformation technologies for rice

The biolistic method

Following the first report of rice transformation via a particle gun (Cao et al 1990), spectacular advances have been made in increasing transformation efficiency, including transforming rice genotypes belonging to both japonica and indica groups. Some of the recent advances made in this direction using the biolistic method are reviewed by Christou (1997). Sivamani et al (1996) developed a procedure for transforming rice calli derived from mature embryos. This procedure generated a highly homogeneous population of embryogenic subcultured calli by selectively propagating a small number of regenerable calli from the seed. Thousands of such calli were recovered

from 50 seeds within 10 wk, and a transformation efficiency of 2–4% was reported using this procedure.

Zhang et al (1996) reported enhanced transformation efficiency of indica rice embryogenic cell suspensions by osmotic treatment. A similar increase in transformation efficiency in an elite indica rice cultivar was reported by Jain et al (1996). Recently, Biswas et al (1998) obtained high-frequency transformation of rice using embryogenic cell clusters. A reduction in both the amount of tissue to be handled for transformation and the time required for the recovery of transformed plants was reported by Vain et al (1998) using green fluorescent protein as a screenable marker in conjunction with low levels of antibiotic selection. Further, it has been shown that transgenic plants generated via a particle gun exhibited negligible genomic changes, indicating that transgenic rice plants retained their genomic integrity and superior agronomic traits (Arencibia et al 1998).

Agrobacterium-mediated method

Agrobacterium tumefaciens is the predominant agent of choice for transforming dicotyledonous plants. This method is efficient, easy, and cost-effective. Transformation of monocotyledons using *Agrobacterium* remained an impossible task until the early 1990s, when there were a few reports of stable transformation of monocotyledons using *Agrobacterium* (Raineri et al 1990, Gould et al 1991). But the proof for transformation was equivocal in these studies.

Chan et al (1993) reported stable transformation of a japonica rice variety at low frequencies. They demonstrated stable inheritance of the integrated genes in the progeny. They infected injured immature embryos and used a potato extract to improve transformation. Hiei et al (1994) described a method for high-frequency transformation of japonica varieties using scutellum-derived calli as the starting material. They preinduced *Agrobacterium* with acetosyringone and also supplemented the co-cultivation medium with the inducer. Their study showed that certain virulence genes (*virB, virG,* and *virC*) from the supervirulent strain Bo542 augment rice transformation. Several other groups repeated this method for transforming japonica, indica, and javanica rice varieties (Aldemita and Hodges 1996, Rashid et al 1996, Dong et al 1996). Park et al (1996) described an alternative approach. They infected isolated shoot meristems of rice and obtained stably transformed plants, albeit at a low frequency. In rice varieties that are exceptionally difficult to regenerate from calli, this approach would be useful. Also, in this method the rice tissues never go through a callusing phase and are hence less likely to accumulate somaclonal variations.

Genes conferring tolerance for drought and salt stress

Genes that confer osmotic stress tolerance in tobacco and other plants *Mannitol-1-phosphate dehydrogenase (mtlD).* Tobacco plants transformed with *Escherichia coli mtlD* showed mannitol accumulation. These plants showed increased tolerance for high salinity relative to control plants (Tarczynski et al 1993). Thomas

et al (1995) reported enhanced seed germination under high-salinity conditions in transgenic *Arabidopsis* plants. Karakas et al (1997), however, reported that transgenic plants showed a marginal increase in dry weight upon salt stress although no difference in growth could be observed between transgenic and control plants upon drought stress. Targeting mannitol biosynthesis to chloroplasts in transgenic tobacco plants also resulted in increased tolerance for oxidative stress (Shen et al 1997).

Proline. The enzyme Dl-pyrroline-5-carboxylate synthetase (P5CS) catalyzes the conversion of glutamate to D1-pyrroline-5-carboxylate, which is then reduced to proline. Overexpression of a gene encoding for mothbean P5CS in transgenic tobacco plants resulted in an accumulation of proline and facilitated maintenance of osmotic potential during water stress (Kavi Kishor et al 1995). This group also showed that transgenic plants had enhanced biomass production and flower development under salt stress conditions.

Glycine betaine. Glycine betaine is accumulated in the cells of several halophytes and bacteria as an adaptive response to saline or water stress conditions. The bacterial choline oxidase (*codA*) gene isolated from *Arthrobacter globiformis,* which converts choline to glycine betaine, was introduced in *Arabidopsis.* The transgenic plants accumulated glycine betaine and showed enhanced tolerance for salt and cold stress (Hayashi et al 1997, Alia et al 1998). Previously, the cyanobacterium *Synechococcus* sp. PCC7942, when transformed with the *codA* gene, exhibited enhanced tolerance for salt stress (Deshnium et al 1995).

Trehalose. The yeast trehalose-6-phosphate synthetase gene (*TPS1*) was introduced in tobacco. Trehalose-accumulating plants exhibited multiple phenotypic alterations and improved drought tolerance (Romero et a1 1997). Pilon-Smits et al (1998) introduced bacterial trehalose-6-phosphate synthase (*otsA*) and trehalose-6-phosphate phosphatase (*otsB*) in tobacco. The transgenic lines showed better growth in terms of dry weight with larger leaves under drought stress. Detached leaves from young, well-watered transgenic plants showed better capacity to retain water when air-dried than the wild-type plants.

Fructans. Fructans are polyfructose molecules that are produced by many plants and bacteria. Owing to their solubility, they may help plants survive periods of osmotic stress. Pilon-Smits et al (1995) introduced a gene encoding for bacterial fructan (*sacB*) isolated from *Bacillus subtilis* in tobacco. The transgenic plants performed significantly better under PEG-mediated water stress than the wild-type tobacco. Drought tolerance correlated well with the amount of fructan accumulated.

D-ononitol. Expression of a cDNA-encoding myo-inositol O-methyltransferase (*IMT1*) in tobacco during salt and drought stress resulted in the accumulation of methylated inositol (D-ononitol), which in turn conferred tolerance for both stresses (Sheveleva et al 1997).

Polyamines. Polyamines have been implicated in a variety of stress responses in plants. Polyamines accumulate under several abiotic stress conditions, including salt and drought. Cultivars demonstrating a higher degree of tolerance for salt contained higher levels of polyamines (reviewed in Galston et al 1997). Further, exogenous

application of polyamines gave oat leaves protection against osmotic stress (Besford et al 1993). Minocha and Sun (1997) showed that transgenic carrot cell lines overexpressing mouse ornithine decarboxylase, which converts ornithine to the diamine putrescine, can withstand salt and osmotic stress over a short period of 0–48 h.

Oxidative stress-related genes. Moran et al (1994) showed that drought induced oxidative stress in pea plants. They observed major reductions in photosynthesis and transpiration under stress. Analysis of four drought-tolerant varieties of tobacco by Van Rensburg and Kruger (1994) revealed that oxidative stress-related genes were induced. They found elevated levels of glutathione reductase, superoxide dismutase, and ascorbate peroxidase and, to a lesser extent, catalase activities. They also associated these activities with the integrity of photosynthetic pigments. These observations suggest that abiotic stresses primarily affect plants through oxidative damage.

Many groups have developed transgenic plants expressing oxidative-stress-induced genes, but their results are not easy to interpret. Tepperman and Dunsmuir (1990) developed transgenic tomato and tobacco plants overexpressing Cu/Zn superoxide dismutase (*Sod*) and failed to see any protection against superoxide toxicity. Bowler et al (1991) observed that targeting the constitutively overexpressed *Mn-SOD* into chloroplasts and mitochondria reduced cellular damage. Gupta et al (1993) found that transgenic tobacco plants overexpressing *Cu/Zn-SOD* retained 90% photosynthesis under chilling and high-light stress compared with control plants (nontransgenics), which suffered badly under both types of stresses. Van Camp et al (1996) reported overexpression of *Fe-SOD* in tobacco plants. Though they found elevated levels of oxidative-stress-related enzymes in the transgenic plants, the plants were not any more tolerant of salt stress than the control plants. Different groups have used different classes of SODs derived from various plant species. This makes comparison of results and drawing a general inference difficult.

Roxas et al (1997) constitutively overexpressed Nt107 cDNA (which encodes a glutathione-S-transferase with additional peroxidase activity) in tobacco. They found that transgenic seedlings showed better growth than the control plants under cold and salt stress. More work needs to be done to understand the various oxidative-stress-related genes and the interactions of their products during stress.

Genes that have been shown to confer osmotic stress tolerance in rice

Late embryogenesis abundant (LEA) protein gene Hval. A barley group 3 LEA protein HVAl was found to be specifically expressed in the aleurone layers and embryos during late seed development correlating with seed desiccation (Hong et al 1988). ABA and several stress conditions, including dehydration, salt, and extreme temperatures, rapidly induced the expression of this gene in young seedlings (Hong et al 1992). Xu et al (1996) produced transgenic rice plants expressing the barley *HVA1* gene, driven by a constitutive promoter from the rice Actin 1 gene. The accumulation of barley HVAl protein in root and leaf tissues of transgenic rice plants conferred increased tolerance for water deficit and salt stress and the extent of tolerance correlated with the level of HVAl protein accumulation.

Proline. Zhu B et al (1998) produced transgenic rice plants overexpressing the mothbean PSCS cDNA under the control of an ABA-inducible promoter (Su et al 1998). The transgenic rice plants accumulated up to 2.5-fold more proline than the control plants under stress conditions. Preliminary results showed that the stress-inducible expression of the PSCS transgene in second-generation transgenic rice plants showed an increase in biomass under salt stress and water stress conditions compared with the nontransformed control plants. The extent of salt tolerance could be correlated with the levels of proline accumulated.

Glycine betaine. Sakamoto et al (1998) have shown that transgenic plants overexpressing the *codA* gene are more tolerant for salt and low-temperature stress. In transgenic plants, the levels of glycine betaine were as high as 1 and 5 mmol g^{-1} fresh weight of leaves in two types of transgenic plants in which choline oxidase was targeted to the chloroplasts and to the cytosol, respectively. The transgenic plants recovered earlier than the nontransgenic plants after 150 mM NaCl stress.

Polyamines. Recently, preliminary data from Capell et al (1998) showed an improved performance of transgenic rice plants overexpressing oat arginine decarboxylase in terms of chlorophyll loss under drought stress. Constitutive overexpression of this gene, however, severely affected developmental patterns in vitro. Further, more data have yet to confirm these results. Recently, we (R. Wu's group) have begun transforming rice with genes that encode polyamine biosynthesis or catabolic enzymes driven by an ABA-inducible promoter. We have recovered morphologically normal transgenic plants and they set normal amounts of seeds (Bajaj, Roy, and Wu, unpublished results).

Strategies for producing highly stress-tolerant transgenic rice plants

A very important consideration in the development of functional transgenic plants is in selecting the expression of the desired trait at an optimal level and effectively regulating its expression in specific organs at appropriate developmental stages. This can be achieved by choosing the appropriate promoter and other control elements to drive the expression of the transgene. To date, only a few constitutive and inducible promoters have been used to drive transgene expression in rice (Tables 2 and 3). Also, a wide variety of promoters from different plant sources have been fused to a reporter gene and their regulation has been studied using rice as the model system (Table 2). Another worthwhile consideration is whether to target the transgene or the polypeptide it encodes to subcellular compartments. A protein could be targeted to an organelle if it is expected to give protection to components of the organelle (e.g., photosystem components inside the chloroplast; protection against membrane lipid peroxidation by reactive radicals in the chloroplast and mitochondria). It is essential to use the appropriate transit signals for organelle targeting. For targeting SOD to mitochondria, Bowler et al (1991) used a cDNA from *Arabidopsis* that has the signal peptide and the SOD coding region. To target the same polypeptide to the chloroplast,

Table 2. Promoters used to drive transgene expression in rice.

Promoter/gene	Activity	Reference
CaMV35S/rice		
Chi1.1, crylA(b)	Constitutive	Lin et al (1995), Alam et al (1998)
Rice *Act1/Lea3*	Constitutive	Xu et al (1996)
Maize *Ubi1/gusA*	Constitutive	Cornejo et al (1993)
Maize *Emu1/gusA*	Constitutive	Chamberlain et al (1994)
Wheat histone *H3*	Cell division-dependent in meristematic cells; cell division-independent expression in anther wall, pistil, coleoptile, and mature embryo	Terada et al (1993)
Maize *Adh1*	Semiconstitutive; elevated expression under anaerobic conditions; largely root-specific	Kyozuka et al (1991)
Rice & tomato		
RbcS	Light-regulated, mesophyll-specific	Kyozuka et al (1993)
Pepc	Exclusively in mesophyll of leaf blade and leaf sheath	Matsuoka et al (1994)
Pinus *Cab*	Light-dependent	Yamamoto et al (1994)
Rice *Osg6b*	Tapetum-specific	Yokoi et al (1997)
Barley *Ltp2*	Aleurone-specific	Kalla et al (1994)
Potato *PinII*-Act1Int/PinII	Induced by wounding, methyl jasmonate, and abscisic acid	Duan et al (1996)
Barley ABRC-Act1/*P5CS*	ABA-inducible	Zhu B et al (1998)

Table 3. Expression of novel genes conferring salt and drought tolerance in rice transgenics.

Gene	Enzyme/protein	Source	Response	Reference
HVA1	LEA group	Barley	Drought, salt	Xu et al (1996)
P5CS	D'pyrroline-5-carboxylate synthetase	Mothbean	Drought, salt	Zhu B et al (1998)
Bet4	Choline dehydrogense/ betaine aldehyde dehydrogenase	*E. coli*	Drought, salt, temperatures	Takabe et al(1998)
CodA	Choline oxidase	*A. globiformis*	Drought, salt	Murata et al(1998)
ADC	Arginine decarboxylase	Oat	Drought	Capell et al (1998)

they replaced the original signal peptide with the transit peptide region from pea *rbcS.* With the corresponding signals, they found the polypeptide to be localized in the appropriate compartment. The transit peptide region from pea *rbcS* will make a good choice for chloroplastic localization of polypeptides (Bowler et al 1991, Shen et al 1997).

The transgene itself could be targeted into the genome of the chloroplast for the following reasons: (1) if the gene is of bacterial origin, then expressing it in a compartment like a chloroplast is beneficial owing to the similarity in codon usage and translation apparatus between prokaryotes and the chloroplast, (2) if overproduction of a foreign protein is expected, it can be easily achieved in the several chloroplasts present in each cell, with their own capacity to supply energy and many essential metabolites for protein synthesis, and (3) if the gene is integrated into the genome of the chloroplast, then it cannot be spread to plants of the same or other species via pollen (Daniell et al 1998).

To be successful, transgenic plants that harbor a single transgene must give stable and high-level expression. High-level expression can be achieved in the following ways: (1) by using a tobacco matrix attachment region sequence to flank the gene of interest in order to increase the level of expression (Spiker and Thompson 1996), (2) by selecting those transgenic plants that have only one copy of the transgene to minimize the problem of gene silencing, (3) by testing second- and third-generation transgenic plants to confirm a high level of expression of the transgene, and (4) by using only homozygous lines for greenhouse and field tests for drought and salt tolerance.

The commonly observed inverse relationship between stress tolerance and the absolute levels of yields under stress can be overcome, or at least minimized, by regulating transgene expression. Use of a stress-inducible promoter (such as an ABA-inducible promoter; Su et al 1998) to drive the expression of the transgene in rice can ensure that the transgene is expressed only when needed, that is, when the plants undergo stress. Thus, there is no wasteful consumption of energy in synthesizing the transcript and the protein corresponding to the transgene. But cross interactions between such genes in transgenic plants should be analyzed. This might eventually help in improving the yield of transgenic plants under stress conditions. Further, a multigene approach of simultaneously transferring more than one useful gene into the same transgenic plant might also be an effective strategy. Because of the complexity of stress physiology, amelioration of stress symptoms is more likely to be achieved using multiple genes rather than any given single gene. Several well-characterized genes that confer stress tolerance in different organisms (Table 4) are now available for introducing into rice. Simultaneous transfer should be preferable over sequential transfer (by multiple rounds of transformation and crossing) because it facilitates the inheritance of all the transgenes as a single locus and it saves time.

Once a gene has been individually introduced into transgenic plants, and the plants have shown an increased tolerance for drought or salt stress, the next step is to test whether combinations of several such genes can further enhance stress tolerance. The best approach is to include several genes, each with a different promoter and terminator, in the same plasmid. It would be helpful to include at least one gene in the stress-induced signal-transduction pathway so that overexpression of this gene can activate several endogenous genes to increase stress tolerance. When this plasmid is integrated into the transgenic plants, the expression of these genes will be coordi-

Table 4. Genes with potential for improving salt or drought tolerance.

Gene	Source	Pathway	Stress	Reference
MnSOD	*N. plumbognifolia*	Oxidative stress	Drought	McKersie et al (1996)
TPS1	Yeast	Trehalose pathway	Drought	Holmstrom et al (1996)
rd22, rd29a	*Arabidopsis*	Dehydration response	Drought	Yamaguchi-Shinozaki and Shinozaki (1993), Shinozaki and Yamaguchi-Shinozaki (1997)
MtlD	*E. coli*	Mannitol pathway	Salinity	Tarczynski et al(1993)
DREB2	*Arabidopsis*	Dehydration response	Drought, salt	Liu and Zhu (1998)
Cer (Eceriformis)	*Arabidopsis*	Epicuticular wax biosynthesis	Drought	Aarts et al (1995)
Myc and Myb	Maize, *Arabidopsis*	Transcription activators	Drought, salt	Abe et al (1997), Reddy et al (1998)
sos, cos, hos, los	*Arabidopsis*	K and Na^+ transport	Osmotic stress	Zhu J-K et al (1998)
SacB	*Bacillus subtilis*	Fructan	Drought	Pilon-Smits et al (1995)
HAL1	Yeast	Na/K transport	Salt	Gaxiola et al (1992), Bordas et al (1997)
HAL2	Yeast	Na/K transport	Salt	Glaser et al (1993)
HAL3	Yeast	Na/K transport	Salt	Ferrando et al (1995)
SAG	*Arabidopsis*	Senescence associated	Drought	Weaver et al (1998)
Sod2	Yeast	Na^+/K antiport	Salt	Jia et al (1992), Young and Zheng (1991)

nated and, once stabilized, the genes will not further segregate in subsequent generations.

In summary, MAS and transgenic approaches are complementary as each has some advantages and some shortcomings. Therefore, in practical terms, both tools will be handy in prebreeding, and the products of prebreeding can be further combined in a specific manner to optimize drought and other responses at a target location. MAS relies on only the genes available within rice germplasm, and there is a limit to which the expression of a trait can be enhanced. Transformation technologies,

on the other hand, offer unique opportunities to transfer a wide spectrum of functionally relevant genes from organisms that are known for superior adaptations to a range of stress environments. In fact, transgenic strategies are beginning to show potential for producing rice plants with improved performance under different stress conditions. It is expected that lab-screening data on transgenics will hold true for fields also, at least for a few well-characterized target traits. This approach is poised for exploitation as new efforts to integrate transgenics with breeding sciences are to begin soon. Thus, a multidisciplinary approach consisting of physiology, genetics and marker technology, molecular biology, and transgenic technology is needed to produce and breed rice varieties tolerant of drought stress and high salt levels.

References

Aarts MGM, Keijer CJ, Stiekema WJ, Pereira A. 1995. Molecular characterization of the CERI gene of *Arabidopsis* involved in epicuticular wax biosynthesis and pollen fertility. Plant Cell 7:2115-2127

Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K. 1997. Role of *Arabidopsis MYC* and *MYB* homologs in drought and abscisic acid-regulated gene expression. Plant Cell 9:1859-1868

Alam MF, Datta K, Abrigo E, Vasquez A, Senadhira D, Datta SK. 1998. Production of transgenic deepwater indica rice plants expresing a synthetic *Bacillus thuringiensis cryIA(b)* gene with enhanced resistance to yellow stem borer. Plant Sci. 135:25-30.

Aldemita RR, Hodges TK. 1996. *Agrobacterium tumefaciens*-mediated transformation of japonica and indica rice varieties. Planta 199:612-617.

Alia, Hayashi H, Chen THH, Murata N. 1998. Transformation with a gene for choline oxidase enhances the cold tolerance of Arabidopsis during germination and early growth. Plant Cell Environ. 21:232-239.

Arencibia A, Gentinetta E, Cuzonni E, Castglione S, Kohli A, Vain P, Leech M, Christou P, Sala F. 1998. Molecular analysis of the genome of transgenic rice (*Oryza sativa* L.) plants produced via particle bombardment or intact cell electroporation. Mol. Breed. 4:99-109.

Austin D, Lee M. 1998. Detection of quantitative loci for grain yield and yield components in maize across generations in stress and nonstress environments. Crop Sci. 38:1296-1308.

Bertin P, Charcosset A, Gallais A. 1997. Physiological and genetic basis of nitrogen use efficiency in maize. In: Tsaftaris A, editor. Genetics, biotechnology and breeding of maize and sorghum. Cambridge (UK): The Royal Society of Chemistry. p 59-64.

Besford RT, Richardson C, Campes JL, Tiburcio AF. 1993. Effect of polyamines on the stabilization of molecular complexes in thylakoid membranes of osmotically stressed oat leaves. Planta 189:201-206.

Biswas GCB, Chen D-F, Elliot MC. 1998. A routine system for generation of transgenic rice (*Oryza sativa* L.) plants by microprojectile bombardment of embryogenic cell clusters. Plant Sci. 133:203-210.

Bordas M, Montesinos C, Dabauza M, Salvador A, Roig LA, Serrano R, Moreno V. 1997. Transfer of yeast salt tolerance gene *HAL1* to *Cucumis melo* L. cultivars and in vitro evaluation of salt tolerance. Transgenic Res. 6:41-50.

Bowler C, Slooten L, Vandenbranden S, Rycke RD, Botterman J, Sybesma C, Van Montagu M, Inze D. 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J. 10:1723-1732.

Cao J, Wang Y-C, Klein TM, Sanford J, Wu R. 1990. Transformation of rice and maize using the particle gun method. In: Lamb CJ, Beachy RN, editors. Plant gene transfer. New York: Wiley-Liss. p 21-33.

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P. 1998. Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns in vitro and results in putrescine accumulation in transgenic plants. Theor. Appl. Genet. 97:246-254.

Chamberlain DA, Brettell RIS, Last DT, Witrzens B, McElroy D. Dolferus R, Dennis ES. 1994. The use of the emu promoter with antibiotic and herbicide resistance genes for the selection of transgenic wheat callus and rice plants. Aus. J. Plant Physiol. 21:95-112.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Haung N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Chan M-T, Chang H-H, Ho S-L, Tong W-F, Yu S-M. 1993. *Agrobacterium*-mediated production of transgenic rice plants expressing a chimeric a-amylase promoter/b-glucuronidase gene. Plant Mol. Biol. 22:491-506.

Christou P. 1997. Rice transformation: bombardment. Plant Mol. Biol. 35: 197-203.

Cornejo MJ, Luth D, Blankenship KM, Anderson OD, Blechl AE. 1993. Activity of maize ubiquitin promoter in transgenic rice. Plant Mol. Biol. 23:567-581.

Daniell H, Datta R, Varma S, Gray S, Lee SB. 1998. Containment of herbicide resistance through genetic engineering of the chloroplast genome. Nature Biotech. 16:345-348.

Deshnium P, Los DA, Hayashi H, Mustardy L, Murata N. 1995. Transformation of *Synechococcus* with a gene for choline oxidase enhances tolerance to salt stress. Plant Mol. Biol. 29:897-907.

Dong J, Teng W, Buchholz WG, Hall TC. 1996. *Agrobacterium*-mediated transformation of javanica rice. Mol. Breed. 2:267-276.

Duan X, Lee X, Xue Q, Abo-El-Saad M, Xu D, Wu R. 1996. Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. Nature Biotech. 14:494-498.

Ferrando A, Kron SJ, Rios G, Fink GR, Serrano R. 1995. Regulation of cation transport in *Saccharomyces cerevisiae* by the salt tolerance gene HAW. Mol. Cell Biol. 15:5470-5481.

Galston AW, Kaur SR, Altabella T, Tiburcio AF. 1997. Plant polyamines in reproductive activity and response to abiotic stress. Bot. Acta 110:197-207.

Gaxiola R, de Larrino IF, Villalba JM, Serrano R. 1992. A novel and conserved salt induced protein is an important determinant of salt tolerance in yeast. EMBO J. 11:3157-3164.

Glaser H-U, Thomas D, Gaxiola R, Montrichard F, Surdin-Kerjan Y, Serrano R. 1993. Salt tolerance and methionine biosynthesis in *Saccharomyces cerevisiae* involve a putative phosphatase gene. EMBO J. 12:3105-3110.

Gould J, Devery M, Hasegawa O, Ulian EC, Peterson G, Smith RH. 1991. Transformation of *Zea mays* L. using *Agrobacterium tumefaciens* and the shoot apex. Plant Physiol. 95:426-434.

Gupta AS, Heinen JL, Holaday AS, Burke JJ, Allen RD. 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA 90:1629-1633.

Han F, Romagosta I, Ulrich SE, Jones BL, Hayes PM, Wesenberg DM. 1997. Molecular marker-assisted selection for malting quality traits in barley. Mol. Breed. 3:427-437.

Hayashi H, Alia, Mustardy L, Deshnium P, Ida M, Murata N. 1997. Transformation of *Arabidopsis thaliana* with the *codA* gene for choline oxidase: accumulation of glycine betaine and enhanced tolerance to salt and cold stress. Plant J. 12:133-142.

Hiei Y, Ohta S, Komari T, Kumashiro T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. 6:271-282.

Holmstrom KO, Manyla E, Welin B, Mandal A, Palva ET. 1996. Drought tolerance in tobacco. Nature 379:683-684.

Hong B, Barg R, Ho T-HD. 1992. Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol. Biol. 18:663-674.

Hong B, Uknes SJ, Ho T-HD. 1988. Cloning and characterization of a cDNA encoding an mRNA rapidly induced by ABA in barley aleurone layers. Plant Mol. Biol. 11:495-506.

Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Mol. Biol. 47:377-403.

Jain RK, Jain S, Wang B, Wu R. 1996. Optimization of biolistic method for transient gene expression and production of agronomically useful transgenic Basmati rice plants. Plant Cell Rep. 15:963-968.

Jia 2-P, McCullough N, Martel R, Hemmingsen S, Young PG. 1992. Gene amplification at a locus encoding a putative Na^{+}/H^{+} antiporter confers sodium and lithium tolerance in fission yeast. EMBO J. 11:1631-1640.

Kalla R, Shimamoto K, Ptter R, Nielson PS, Linnestad C, Olsen O. 1994 The promoter of the barley aleurone-specific gene encoding putative 7 kDa lipid transfer protein confers aleurone-cell specific expression in rice. Plant J. 62:849-860

Karakas B, Ozias-Akins P, Stushnoff C, Suefferheld M, Rieger M. 1997. Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. Plant Cell Environ. 20:609-616.

Kavi Kishor PB, Hong Z, Miao G-H, Hu C-AA, Verma DPS. 1995. Overexpression of 1–pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108: 1387-1394.

Kyozuka J, Fujimoto H, Izawa T, Shimamoto K. 1991. Anaerobic induction and tissue-specific expression of maize *Adh1* promoter in transgenic rice plants and their progeny. Mol. Gen. Genet. 228:40-48.

Kyozuka J, McElroy D, Hayakawa T, Xie Y, Wu R, Shimamoto K. 1993. Light-regulated and cell-specific expression of tomato *rbcS-gusA* and rice *rbcS-gusA* fusion genes in transgenic rice. Plant Physiol. 102:991-1000.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Lin W, Anuratha CS, Datta K, Potrykus I, Muthukrishnan S, Datta SK. 1995. Genetic engineering of rice for resistance to sheath blight. Bio/Technology 13:686-691.

Liu J, Zhu J-K. 1998. A calcium sensor homolog required for plant salt tolerance. Science 280:1943-1945.

Lubberstedt T. Melchinger A. 1997. QTL analysis of forage maize traits: comparison across testers. In: Tsaftaris A, editor. Genetics, biotechnology and breeding of maize and sorghum. Cambridge (UK): The Royal Society of Chemistry. p 71-75.

Martin B, Nienhuis J, King G, Shaefer A. 1989. Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science 243:1725-1728.

Matsuoka M, Kyozuka J, Shimamoto K, Kano-Murakami Y. 1994 The promoters of two carboxylases in a C_4 plant (maize) direct cell-specific, light-regulated expression in a C_3 plant (rice). Plant J. 6:311-319.

McKersie BD, Bowley SR, Harjanto E, Leprince O. 1996. Water-deficit tolerance and field performance of transgenic alfalfa over-expressing superoxide dismutase. Plant Physiol. 111:1177-1181.

Milan MAR, Aswey DA, Boerma HR. 1998. An additional QTL for water-use-efficiency in soyabean. Crop Sci. 38:390-393.

Minocha SC, Sun D-Y. 1997. Stress tolerance in plants through transgenic manipulation of polyamine biosynthesis. Plant Physiol. Suppl. 297.

Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV, Aparicio Tejo P. 1994. Drought induces oxidative stress in pea plants. Planta 194:346-352.

Murata N, Hayashi H, Sakamoto A, Alia. 1998. Genetic enhancement of tolerance to multiple stress by metabolic engineering of biosynthesis of glycine betaine. In: Proceedings of international workshop on breeding and biotechnology for environmental stress in rice held 26-29 Oct. 1998, Japan.

Ottaviano E, Gorla MS, Pe E, Frova C. 1991. Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor. Appl. Genet. 81:713-719.

Park SH, Pinson SRM, Smith RH. 1996. T-DNA integration into genomic DNA of rice following *Agrobacterium* inoculation of isolated shoot apices. Plant Mol. Biol. 32:1135-1148.

Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek PJ, Smeekens SCM. 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol. 107: 125- 130.

Pilon-Smits EAH, Terry N, Sears T, Kim H, Zayed A, Hwang S, Van Dun K, Voogd E, Verwoerd TC, Krutwagen RWHH, Goddijn OJM. 1998. Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. J. Plant Physiol. 152:525-532.

Price AH et al. 1997. Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). New Phytol. 137:233-91.

Prioul, J-L, Quarrie S, Causse M, de Vienne D. 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. J. Exp. Bot. 48:1151-1163.

Quarrie S, Lazic-Jancic V, Ivanovic M, Pekic C, Heyl A, Landi P, Lebreton C, Steed A. 1997. Molecular marker methods to dissect drought resistance in maize. In: Tsaftaris A, editor. Genetics, biotechnology and breeding of maize and sorghum. Cambridge (UK): The Royal Society of Chemistry. p 52-58.

Quarrie SA, Steed A, Semikhodski A, Lebreton C, Calestani C, Clarkson DA, Tuberosa R, Sanguineti MC, Melchiorre R, Prioul JL. 1995. Identification of quantitative trait loci regulating water- and nitrogen-use efficiency in wheat. In: II STRESSNET Conference, 21-23 Sept. 1995, Salsomaggiore, Italy. European Commission, Rep FII.3-MOR/OCO2.

Raineri DM, Bottino P, Gordon MP, Nester EW. 1990. *Agrobacrerium*-mediated transformation of rice (*Oryza sativa* L.). Bio/Technology 8:33-38.

Rashid H, Yokoi S, Toriyama K, Hinata K. 1996. Transgenic plant production mediated by *Agrobacterium* in Indica rice. Plant Cell Rep. 15:727-730.

Ray JD, Yu L, McCouch SR, Champoux, MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor. Appl. Genet. 6:627-636.

Reddy VS, Scheffler BC, Weinand U, Wessler SR, Reddy AR. 1998. Cloning and characterization of the rice homologue of the maize *C1* anthocyanin regulatory gene. Plant Mol. Biol. 36:497-498.

Redona ED, Mackill DJ. 1996. Molecular mapping of quantitative trait loci in japonica rice. Genome 39:395-403.

Ribaut JM, Hoisington DA, Deutsch JA, Jiang C, Gonzalez de Leon D. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. I. Flowering parameters and anthesis-silking interval. Theor. Appl. Genet. 92:905-914.

Ribaut JM, Jiang C, Gonzalez de Leon D, Edmeades GO, Hoisington DA. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor. Appl. Genet. 94:887-896,

Romero C, Belles JM, Vaya JL, Serrano R, Culianez-Macia FA. 1997. Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta 201:293-297.

Roxas VP, Smith RK Jr, Allen ER, Allen RD. 1997. Overexpression of glutathione-S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotech. 15:991-998.

Sakamoto A, Alia, Murata N. 1998. The metabolic engineering of rice leading to biosynthesis of glycine-betaine and tolerance to salt and cold. Plant Mol. Biol. 38:1011-1019.

Shen B, Jensen RG, Bohnert HJ. 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol. 113:1177-1183.

Sheveleva E, Chmara W, Bohnert HJ, Jensen RG. 1997. Increased salt and drought tolerance by D-ononitol production in transgenic *Nicotiana tabacum* L. Plant Physiol. 115: 1211-1219.

Shinozaki K, Yamaguchi-Shinozaki K. 1997. Gene expression and signal transduction in water stress response. Plant Physiol. 115:327-334.

Sivamani E, Shen P, Opalka N, Beachy RN, Fauquet CM. 1996. Selection of large quantities of embryogenic calli from indica rice seeds for production of fertile transgenic plants using the biolistic method. Plant Cell Rep. 15:322-327.

Spiker S, Thompson WF. 1996. Nuclear matrix attachment regions and transgenic expression in plants. Plant Physiol. 110:15-21.

Stuber C. 1997. Years of searching and manipulating QTLs in maize. In: Tsaftaris A, editor. Genetics, biotechnology and breeding of maize and sorghum. Cambridge (UK): The Royal Society of Chemistry. p 43-58.

Su J, Shen Q, Ho T-HD, Wu R. 1998. Dehydration-stress-regulated transgene expression in stably transformed rice plants. Plant Physiol. 117:913-922.

Takabe T, Hayashi Y, Tanaka T, Kishitanu S. 1998. Evaluation of glycine betaine accumulation for stress tolerance in transgenic rice plants. In: Proceedings of International workshop on breeding and biotechnology for environmental stress in rice, 26-29 Oct. 1998, Japan.

Tarczynski MC, Jensen RG, Bohnert HJ. 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science 259:508-510.

Tepperman JM, Dunsmuir P. 1990. Transformed plants with elevated levels of chloroplastic SOD are not more resistant to superoxide toxicity. Plant Mol. Biol. 14:501-511.

Terada R, Nakayama T, Iwabuchi M, Shimamoto K. 1993. A wheat histone H3 promoter confers cell division-dependent and -independent expression of the *gusA* gene in transgenic rice plants. Plant J. 3:241-252.

Teulat B, Monneveux P, Wery J, Borries C, Souyris I, Charrier A, This D. 1997. Relationships between relative water content and growth parameters under water stress in barley: a QTL study. New Phytol. 137:99-107.

Teulat B, This D, Khairallah M, Bomes C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A. 1998. Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 96:688-698.

Thomas JC, Sepahi M, Arendall B, Bohnert HJ. 1995. Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana.* Plant Cell Environ. 18:801-806.

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G. 1996. Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. Crop Sci. 36: 1337-1344.

Tuinstra MR, Ejeta G, Goldsbrough P. 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. Crop Sci. 38:835-842.

Vain P, Worland B, Kohli A, Snape JW, Christou P. 1998. The green fluorescent protein (GFP) as a screenable marker in rice transformation. Theor. Appl. Genet. 96:164-169.

Van Camp W, Capiau K, Van Montagu M, Inze D, Slooten L. 1996. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. Plant Physiol. 112:1703-1714.

Van Deznze, AV, Sorrells ME, Park, WD, Ayres NM, Fu, H, Cortinhour AW, Paul E, McCouch SR. 1998. Anchor probes for comparative mapping of grass genera. Theor. Appl. Genet. 97:356-369.

Van Rensburg L, Kruger GHJ. 1994. Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. J. Plant Physiol. 143:730-737.

Veldboom LR, Lee M. 1994. Molecular-marker-facilitated studies of morphological traits in maize. II. Determination of QTLs for grain yield and yield components. Theor. Appl. Genet. 89:451-458.

Weaver LM, Gan S, Quirino B, Amasino RM. 1998. A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. Plant Mol. Biol. 37:455-469.

Xu D, Duan X, Wang B, Hong B, Ho T-HD, Wu R. 1996. Expression of a late embryogenesis abundant protein gene, *HVA1,* from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol. 110:249-257.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Yamaguchi-Shinozaki K, Shinozaki K. 1993. The plant hormone abscisic acid mediates the drought-induced expression but not the seed-specific expression of *rd22,* a gene responsive to dehydration stress in *Arabidopsis thaliana.* Mol. Gen. Genet. 238: 17-25.

Yamamoto N, Tada Y, Fujimura T. 1994 The promoter of a pine photosynthetic gene allows expression of a ß-glucuronidase reporter gene in transgenic rice plants in a light-dependent but tissue-specific manner. Plant Cell Physiol. 35:773-778.

Yokoi S, Tsuchiya T, Toriyama K, Hinata K. 1997. Tapetum-specific expression of the Osg6B promoter ß-glucuronidase gene in transgenic rice. Plant Cell Rep. 16:363-367.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. 1982. Living with water stress: evolution of osmolyte system. Science 217:1214-1222.

Young PC, Zheng PJ. 1991. Gene conferring salt tolerance. International patent WO91/06651.
Zhang S, Chen L, Qu R, Marmey P, Beachy RN, Fauquet CM. 1996. Regeneration of fertile transgenic indica (group 1) rice plants following microprojectile transformation of embryogenic suspension culture cells. Plant Cell Rep. 15:465-469.
Zhu B, Su J, Chang MC, Verma DPS, Fan Y-L, Wu R. 1998. Overexpression of a Dl-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water- and salt-stress in transgenic rice. Plant Sci. 139:41-48.
Zhu J-K, Xiong L, Ishitane M, Liu J, Li H, Stevenson B, Shi W. 1998. Identification of genes important for environmental stress tolerance in plants. In: Proceedings of international workshop on breeding and biotechnology for environmental stress in rice. Oct 26-29, 1998, Sapporo, Japan.

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DNA markers and QTL mapping in rice

Zhikang Li

The advent of DNA marker technology in 1980 has energized the most exciting era in biological sciences, genomics. Wide applications of DNA markers and resulting molecular linkage maps allow the molecular dissection of genetic variation of complex phenotypes through the design, execution, and analysis of quantitative trait loci (QTL) mapping experiments. To date, QTLs identified in rice appear to include two types. The first type represents major genes that affect quantitative traits, which are detected with large LOD scores (>10). The second type includes most of the QTLs identified in rice, which have relatively small effects and tend to be involved in epistasis and/or genotype x environment interactions. We have addressed several important issues in QTL mapping, such as background genetic variation control, epistasis, and QTL x environment interaction, by model comparison and computer simulation. Our results indicated that control of background genetic variation with a significant maia-effect and epistatic QTLs was required to obtain accurate and precise estimates of QTL parameters, particularly in the presence of linkage. Based on a mixed linear model, software (QTLMAPPER v. 1.0) was developed for the reliable detection and quantification of maineffect and epistatic QTLs and QTL x environment interactions in doubled-haploid, recombinant inbred, and backcross populations. Furthermore, the use of experimental designs and advanced backcross QTL identification are discussed briefly. Finally, we anticipate that accurate genetic and physical mapping of QTLs coupled with new developments in rice structural genomics and genome research technology will lead to a golden era of functional genomics. In this era, the comprehensive integration of genomic technologies, bioinformatics, and conventional breeding techniques will occur. This will allow the functional assignment of large numbers of genes/QTLs and quick identification of allelic diversity of important genes/QTLs and multilocus genotypes associated with desirable phenotypes.

As an important component of biotechnology, DNA markers have played an increasingly important role in genome research, genetics, and breeding of crop plants. The merging of DNA marker technology with principles of genetics has resulted in one of the most exciting areas in life science — genome mapping. As a generally applicable approach, genome mapping offers unique opportunities to study the repertoire of genetic information that directs the growth and development of plants. Technically, genome mapping includes several related areas such as the construction of a "genome map" or molecular linkage map, gene mapping and tagging, QTL mapping, and physical mapping of specific genomic regions or whole genomes.

DNA markers

DNA markers are defined as linear landmarks in the DNA molecules or chromosomes where genotypic differences can be detected by various molecular tools. Several major types of DNA markers classified largely according to specific molecular techniques by which DNA differences are detected are described briefly as follows.

RFLP (restriction fragment length polymorphism). This technique uses cDNA or random genomic DNA as probes (labeled with radioactivity or conjugated enzymes) to detect DNA fragment length differences generated by enzyme digestion and electrophoresis (Botstein et al 1980). RFLP is often co-dominant and highly reliable. In rice, RFLP is powerful in detecting DNA differences at subspecific and specific levels.

RAPD (randomly amplified polymorphic DNA). RAPD is one type of DNA marker produced by PCR (polymerase chain reaction) technology (Williams et a1 1990). In RAPD analysis, numerous short (200~2,000 bp) DNA sequences are amplified from a small amount of genomic DNA using a single random primer (usually a l0mer). Amplified DNA is then separated electrophoretically on agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. Most RAPD markers are scored as the presence or absence of specific amplified bands and are thus dominant in nature. This technology has low requirements for time and training, and amounts of start DNA, and is thus suitable for automation and quick genotyping of large numbers of samples. The most serious problem of this technology, however, is inconsistency because PCR reactions are very sensitive to many factors, such as annealing temperature, template DNA concentration, etc. In rice, RAPD markers have the power to detect DNA polymorphism similar to RFLP markers (unpublished data).

STS (sequence-tagged sites). Originally coined by Olsen et al (1989), STS refers to a class of known loci (genes, cDNA, or genomic clones) directly amplified by PCR primer sets designed from the sequences of these specific loci. Although the power of STS to detect DNA polymorphism is generally low, it can be improved by techniques such as enzyme digestion of PCR products and/or by denaturing gradient electrophoresis (Lizuka et al 1992). In rice, STS markers for more than 350 mapped anchor RFLP markers have been developed and used as a tool in genome mapping and marker-aided selection (MAS) experiments (Robenbiol et al 1996). STS markers are usually dominant, but a recent technical development using duplex analysis of STS allows the detection of single co-dominant nucleotide polymorphism (SNP) in STS, making this PCR-based technology much more powerful in genomic research (Hauser et al 1998).

Microsatellites or SSLPs (single sequence length polymorphism). Microsatellites are a class of PCR-based markers that detect simple sequence repeats (SSRs) of different lengths flanked by unique DNA sequences (which are used to design PCR primers). Because of their apparent advantages such as co-dominance, technical simplicity, lower costs, small amounts of DNA required, rapid turnaround time, and high power to detect DNA polymorphism, microsatellites are rapidly replacing RFLP and

RAPDs. In rice, microsatellite markers include di- and tri-nucleotide motifs such as poly (GA, GT, CAT, CTT) sequences, which are randomly distributed in the rice genome (Chen et al 1997).

AFLP (amplified fragment length polymorphism). AFLP is a type of PCR-based marker that detects DNA differences by selectively amplifying digested (by restriction enzymes) genomic DNA sequences ligated with adapters (Vos et al 1995). Because it combines the advantages of both RFLP and RAPD, AFLP is very powerful in detecting DNA polymorphism, even within closely related rice accessions (Maheswaran et al 1996). But AFLP markers are often dominant.

Because of their unique genomic positions and behaviors as single Mendelian transmission factors, DNA markers have been used widely in developing molecular linkage maps for different genomes. In these molecular linkage maps, the linear orders and relative genetic distances of linked DNA markers on individual chromosomes and the whole genome of an organism are determined genetically and represented graphically. Since the first rice RFLP linkage map reported by Chen et al in 1988, high-density rice RFLP linkage maps have been constructed at Cornell University, in Japan, and in Korea (Causse et al 1994, Kurata et al 1994, Cho et al 1998). The current RFLP map developed by the Japanese Rice Genome Research Program has more than 2,300 RFLP markers (Shomura et al 1997). In addition, rice molecular linkage maps with 208 AFLP and 306 microsatellite markers were constructed at IRRI and Cornel1 University, respectively (Maheswaran et al 1996, Chen et al 1997). Construction of a complete rice RAPD linkage map is in progress at IRRI (data not shown). Establishment of these molecular linkage maps has greatly facilitated efforts in gene/QTL mapping, physical mapping, and map-based cloning of important genes/QTLs in rice.

QTL mapping in rice

One of the most important applications of DNA markers and molecular linkage maps is to dissect genetic variation of quantitative traits into individual Mendelian factors through the design, execution, and analysis of QTL mapping experiments. The primary objective of a QTL mapping experiment is to understand the genetic basis of specific quantitative traits by determining the number, locations, gene effects, and actions of loci involved; gene interactions (epistasis); and QTL × E interactions. Another purpose of QTL mapping is to identify DNA diagnostic markers for particular phenotypes of interest so that MAS can be used to efficiently select progenies that carry alleles for target traits grown under nontarget environments. One of the long-term objectives of QTL mapping experiments is the molecular cloning of genes that underly complex quantitative traits through map-based cloning approaches.

The principle of mapping QTLs with DNA markers is to detect marker-trait associations based on linkage disequilibrium using appropriate experimental designs and statistical methods. Typically, a QTL mapping experiment uses a mapping population that may consist of different types of progeny (F_2, backcross, doubled haploid, or

recombinant inbred) from a cross between two inbred lines, which segregate for target quantitative traits and many markers. The population is evaluated both phenotypically for target traits and genetically for a complete linkage map with well-distributed markers. This type of data allows detailed resolution of genomic locations and effects of individual QTLs responsible for target trait variation by interval mapping and linear models. In the following sections, several factors that may have significant impacts on the accuracy, precision, and reliability of QTL mapping experiments will be explained.

Although undoubtedly QTLs can be identified in any mapping population segregating for target quantitative traits using marker-trait associations, several questions arise concerning QTLs identified. For instance, are QTLs real? In what aspects do QTLs differ from major genes? How precise were QTL parameters (locations and effects) in previous QTL mapping studies? Most important, how can QTL mapping results be used to apply MAS to the genetic improvement of quantitative traits? Results from recent QTL mapping studies in rice have shed some light on these issues.

To date, QTLs that affect a wide range of agronomic traits in several reference mapping populations have been mapped on individual chromosomes in rice. These QTLs are main-effect (additive and/or dominance) QTLs because they were detected by main-effect QTL models (Lander and Botstein 1989, Zeng 1994, Li 1997). The average number of the detected main-effect QTLs per trait/population/environment is 4.5 ± 4.8 and varies considerably, ranging from 0 to 10 depending largely on traits and mapping populations. In few cases did closely linked main-effect QTLs affect the same trait reported in a single mapping population. Several important points concerning the main-effect QTLs that affect quantitative traits in rice can be summarized in the following sections.

First, major genes that influence highly heritable traits are typically detected as large-effect QTLs (with large LOD scores) and are identifiable across populations and environments. Very often, only 1–2 such large-effect QTLs, each explaining a significant proportion of trait variation in a mapping population, are identified. For instance, several already mapped rice genes have been mapped to the same genomic locations by QTL analysis (Fig. 1). These include *sd-1* for plant height (Huang et al 1996), *Xa4* for bacterial blight resistance (Li et al 1999), *Rf3* for cytoplasmic male sterility fertility restoration (Zhang et al 1997, Yao et a1 1997), *S-5* for "wide compatibility" (Zheng et al 1992, Liu et a1 1997), *Ta9* for tiller and leaf angle (Li et al 1998b), and *QHd3* and *QHd8* for heading date (Li et al 1995, Xiao et al 1995, 1996). These results clearly indicate that QTLs are real.

Second, typical QTLs have relatively small main effects and represent more than 80% of the loci identified in rice. Because main-effect QTL models (Lander and Botstein 1989, Zeng 1994) were used in all previous QTL analyses, the reported phenotypic effects of these QTLs may have been over- or underestimated. Evidence indicates that a significant portion of these small main-effect QTLs may have been involved in epistasis (Li et al 1997a, b, Yu et al 1997, Li et al 1998a). We also expect that small-effect QTLs may show a greater degree of QTL × environment interactions

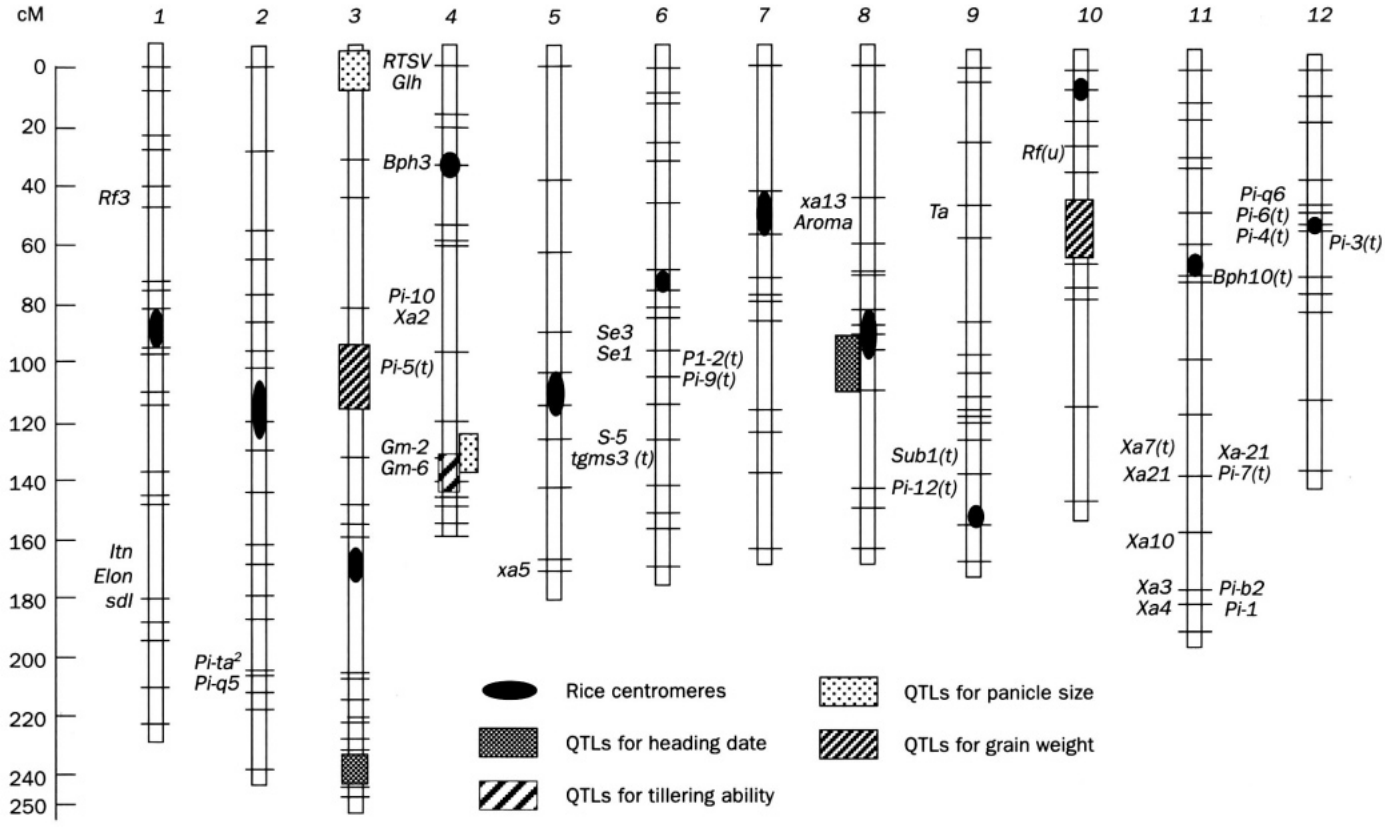

Fig. 1. A rice RFLP linkage map with 182 RFLP markers constructed from 282 recombinant inbred lines from the Lemont/Teqing cross showing genomic locations of some mapped genes and important quantitiative trait loci (QTLs).

because most of these are only detectable in specific populations and/or under specific environments. Thus, epistasis and QTL × E interactions appear to be two important but largely uncharacterized properties associated with the majority of the minor QTLs identified so far. Several recent studies indicate that complex phenotypes such as grain yield and its components may be influenced largely by epistatic QTLs that do not appear to have detectable main effects in a segregating population (Li et al 1997a,b, Yu et al 1997).

Finally, MAS experiments for the transfer of desirable QTLs to improve quantitative traits in rice should be carried out with caution. On the one hand, MAS should be efficient to transfer large-effect QTLs (major genes) and can be practiced based on previous mapping results to modify specific phenotypes, even though it is advisable to use flanking markers that embrace the target genomic region of 10 ~ 15 cM (Visscher et al 1996). But for complex traits such as drought tolerance and grain yield, because of the potential epistasis, QTL × E interactions, and relatively small QTL effects, current information on the identified QTLs is insufficient for designing a successful MAS experiment.

New developments in QTL mapping methodology

The statistical methodology for previous QTL mapping studies was developed based on the assumption that there is no epistasis between QTLs. A serious deviation from the assumption in real situations may have a significant impact on the accuracy, precision, and reliability of QTL mapping results (Li et al 1997a). In addition, most quantitative traits show varied degrees of genotype (G) × E interactions, which could not be adequately addressed using the current methodology. In the following sections, properties of several new genetic and statistical models, including epistasis and QTL × E interactions, were developed and explored using model comparison and computer simulation.

Epistasis and background genetic variation (BGV) control

In principle, phenotypic data and a complete linkage map with well-distributed markers constructed from a mapping population should allow the dissection of quantitative trait variation into individual QTL effects. In practice, because of many technical and economic reasons, only a limited (often small) number of individuals can be genotyped and phenotyped, and many segregating QTLs are responsible for target trait variation in a mapping population. Thus, background genetic variation, defined as noises arising from nonrandom sampling (due to small population size), linkage, and epistasis (interactions between nonallelic QTLs) can have a significant impact on the power, accuracy, precision, and reliability of identified QTLs (Zeng 1994, Li 1997). To illustrate the importance of BGV control, computer simulation was performed to compare the following three statistical models commonly used in QTL analyses:

$$y_k = \mu + a_i x_{ik} + \varepsilon_k \quad (1)$$

$$y_k = \mu + a_i x_{ik} + \Sigma_j a_j x_{jk} + \varepsilon_k \text{ for } j \neq i \quad (2)$$

$$y_k = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + \sum_f \mu_{M_{fk}} e_{M_f} + \sum_l \mu_{MM_{lk}} e_{MM_l} + \varepsilon_k \quad (3)$$

In model 1, y_k is the trait value of the k-th individual, μ is the population mean, a_i is the effect associated with QTL i, x_{ik} is the coefficient of the QTL effect derived from observed genotypes of markers flanking the QTL, and ε_k is the residual effect. Model 1 represents the simplest and most commonly used type of model, which identifies individual main-effect (additive or dominance) QTLs by detecting individual marker-trait associations based on F and t statistics (LOD or LR for interval mapping), and thus is also called the MT model (cf. Beavis 1997). Model 2 is based on the concept of composite interval mapping theorized by Zeng (1994). This model includes an additional term, $\Sigma_j a_j x_{jk}$, which allows the detection of multiple main-effect QTLs and control of the background genetic effects caused by main-effect QTLs that segregate in the population.

Model 3 is the most recently developed model aiming at identifying QTLs involved in digenic epistasis. In addition, this model provides the most comprehensive BGV control by including multiple main-effect and epistatic QTLs. In the model, a_i and a_j are the additive effects of the two putative QTLs (Q_i and Q_j), respectively; aa_{ij} is the additive × additive epistatic effect between Q_i and Q_j; $x_{A_{ik}}$, $x_{A_{jk}}$, and $x_{AA_{ijk}}$ are coefficients of QTL effects derived from the observed genotypes of the markers (M_i, M_{i+} and M_{j-}, M_{j+}) and the testing points (r_{M_i}-Q_i and r_{M_i}-Q_i); $e_{M_f} \sim N(0, \sigma^2_M)$ is the effect of marker f with coefficient $\mu_{M_{fk}}$ (1 for $M_f M_f$ and -1 for $m_f m_f$); $e_{MM_i} \sim N(0, \sigma^2_{MM})$ is the effect of the l-th marker interaction (between marker A_l and marker B_l) with coefficient $\mu_{MM_{lk}}$ (1 for $M_A M_A M_B M_B$ or $m_A m_A m_B m_B$, and -1 for $M_A M_A m_B m_B$ or $m_A m_A M_B M_B$); and $\varepsilon_k \sim N(0, \sigma^2_\varepsilon)$ is the random residual effect. Inclusion of e_{M_f} and e_{MM_l} in the model is intended to absorb the main and epistatic effects of background QTLs.

Computer simulation based on a DH population with 200 individuals has demonstrated a few important properties of the three models described above (Tables 1 and 2). First, BGV control is required for obtaining unbiased estimates of QTL parameters when there is linkage between QTLs. Under no linkage, all three models produce unbiased estimates of the main and epistatic effects of QTLs. In the presence of linkage, however, model 1 tended to give seriously biased estimates for both main and epistatic QTL effects, whereas models 2 and 3 provided largely unbiased estimates. In particular, model 3 is much better than models 1 and 2 when all four QTLs are linked (Tables 1 and 2). The markers or marker pairs selected for BGV control in models 2 and 3 were the ones that were significant at $P = 0.005$ (selected threshold) in their respective multiple regression models.

Second, BGV control significantly increases the power in QTL detection. For example, under no linkage, the average LR (test statistics, 1 LOD = 4.61 LR) values for QTL main and epistatic effects were 32.7 and 6.8 for model 1, 35.8 and 9.2 for

Table 1. Impact of background genetic variation control and linkage on estimated QTL main effects.

Linkage	QTL	QTL effect[a]	Model 1			Model 2			Model 3		
			Estimated[b]	SD[c]	LR[d]	Estimated	SD	LR	Estimated	SD	LR
No	1	0.60	0.59	0.41	9.2	0.58	0.35	11.2	0.59	0.32	13.3
	2	–1.21	–1.23	0.37	21.3	–1.23	0.34	26.5	–1.23	0.32	31.5
	3	4.07	4.04	0.37	96.1	4.04	0.36	100.6	4.03	0.33	119.6
	4	0.25	0.26	0.39	4.2	0.27	0.34	4.8	0.27	0.32	5.3
Partial	1	0.60	0.58	0.40	9.0	0.58	0.35	11.2	0.58	0.32	13.1
	2	–1.21	–0.66	0.40	11.5	–1.16	0.37	21.6	–1.16	0.35	26.2
	3	4.07	3.78	0.34	88.5	4.00	0.35	95.2	4.00	0.32	113.6
	4	0.25	0.27	0.37	4.1	0.28	0.33	4.6	0.27	0.30	5.1
Complete	1	0.60	0.50	0.38	8.3	0.58	0.38	9.4	0.57	0.35	10.2
	2	–1.21	–0.08	0.40	12.0	–1.15	0.44	16.3	–1.15	0.42	19.2
	3	4.07	3.55	0.39	69.2	3.95	0.48	70.7	3.97	0.41	82.0
	4	0.25	1.26	0.39	38.1	0.46	0.40	8.3	0.46	0.35	9.0

[a] Parameters of preset QTL additive effects. [b] Estimated QTL main effects. [c] SD = standard deviation. [d] LR = likelihood ratio statistics averaged from 300 simulations. Sample size = 200, heritability = 0.50. Testing points were set exactly at the preset QTL positions. In all simulations, a single linkage map with four chromosomes and a total of 64 evenly distributed markers (10 cM between adjacent markers) was employed. Four QTLs have preset additive effects from –1.21 to 4.07. There were three different linkage relationships between the QTLs: (1) no linkage between the QTLs; (2) partial linkage QTLs 1 and 2 are linked, while QTLs 3 and 4 are independent; (3) all four QTLs are linked on a single chromosome.

Table 2. Impact of different background genetic variation control and linkage on estimated QTL epistatic effects.

Linkage	Interacting QTLs			Model 1			Model 2			Model 3		
	QTL1	QTL2	Effect[a]	Estimated	SD	LR	Estimated	SD	LR	Estimated	SD	LR
	1	2	1.31	1.36	0.48	9.7	1.34	0.36	14.7	1.34	0.33	17.2
	1	3	1.08	1.08	0.40	9.5	1.09	0.39	10.1	1.09	0.35	12.0
	1	4	–0.38	–0.41	0.48	1.8	–0.38	0.39	2.2	–0.37	0.34	2.4
No	2	3	0.21	0.26	0.36	1.5	0.25	0.37	1.5	0.24	0.32	1.6
	2	4	–1.71	–1.71	0.45	14.9	–1.71	0.35	24.6	–1.71	0.33	27.5
	3	4	–0.41	–0.38	0.34	1.8	–0.37	0.34	1.9	–0.37	0.30	2.2
	1	2	1.31	1.59	0.50	12.7	1.58	0.38	19.4	1.35	0.35	16.2
	1	3	1.08	1.42	0.39	15.9	1.43	0.38	16.7	1.16	0.36	12.8
	1	4	–0.38	–0.45	0.48	2.0	–0.45	0.39	2.6	–0.43	0.34	2.8
Partial	2	3	0.21	0.24	0.38	1.3	0.24	0.38	1.3	0.22	0.34	1.4
	2	4	–1.71	–1.80	0.45	15.8	–1.80	0.33	25.2	–1.72	0.32	25.5
	3	4	–0.41	–0.83	0.34	5.8	4.83	0.34	6.1	–0.47	0.31	2.9
	1	2	1.31	1.77	0.47	14.1	1.78	0.43	20.6	1.48	0.39	11.4
	1	3	1.08	1.27	0.36	12.3	1.27	0.44	12.6	1.26	0.35	8.8
	1	4	–0.38	–0.47	0.47	2.3	–0.46	0.40	2.6	–0.41	0.40	2.1
Complete	2	3	0.21	–0.18	0.38	0.9	–0.18	0.42	0.9	0.08	0.37	1.1
	2	4	–1.71	–1.77	0.42	17.8	–1.76	0.41	23.8	–1.81	0.36	16.3
	3	4	–0.41	–1.39	0.43	12.1	–1.41	0.44	12.7	–0.72	0.43	3.4

[a] The epistatic parameters were preset to be present between individual QTL pairs located at positions *pi* and *pj*. The estimated epistatic effects (estimated) and their test statistics (LR values) were the averages obtained by 300 simulations at the exact positions of each of the QTL pairs with a sample size of 200 and trait heritability = 0.50.

model 2, and 42.4 and 10.5 for model 3. In the presence of linkages between all QTLs, model 1 significantly underestimated or overestimated QTL main and epistatic effects, so its power (LR values) was falsely decreased or increased. Applying BGV control (models 2 and 3) greatly improved the situation. Overall, model 3 was superior to models 1 and 2 in detecting both QTLs and epistasis, whereas model 2 had a higher power than model 1.

Third, BGV control increases the precision of the estimated genetic parameters of the detected QTLs, particularly in the presence of epistasis (Table 2). Measured as the mean standard deviation (SD) of the estimated main and epistatic QTL effects, the precision of model 3 was 9.9% higher than that of model 1 and 17.1% higher than that of model 2, and that of model 2 was 6.6% higher than that of model 1. Results in Tables 1 and 2 also indicate that QTLs with small main and epistatic effects (< 2% in R^2) are largely unidentifiable, whereas those with relatively large main and/or epistatic effects (> 5% in R^2) can always be detected with model 3.

QTL × environment interaction

Quantitative traits are influenced by environments and tend to show varied degrees of G × E interactions. To dissect G × E interactions into individual QTL × E interactions, an extension of model 2 to include QTL × E effects with unconditional and conditional effects in mixed linear models was developed by Zhu and Weir (1998):

$$Y_{hk} = \mu_{h(t)} + a_{ih(GE(t))}x_{hk} + E_{h(GE(t))} + \Sigma_j a_{jh(GE(t))}x_{jhk} + \varepsilon_{hk(GE(t))} \text{ for } i \neq j, \quad (4\text{-}1)$$

and

$$Y_{hk} = \mu_{h(GE(t/t-1))} + a_{ih(GE(t/t-1))}x_{hk} + E_{h(GE(t/t-1))} + \Sigma_j a_{hj(GE(t/t-1))}X_{hjk} + \varepsilon_{hk(GE(t/t-1))} \quad (4\text{-}2)$$

where $\mu_{h(t)}$ is the population mean in h-th environment at time t, $a_{ih(GE(t))}x_{hk}$ is the genetic effect of the i-th QTL in the h-th environment at time t, $E_{h(GE(t))}$ is the effect of the h-th environment at time t, and so on. Model 4-1 is unconditional whereas model 4-2 is conditional, giving the extra effect during the period of t - 1. These models can quantify main-effect QTL × E interactions and allow us to examine main-effect QTL expression during different developmental stages (Yan et a1 1998).

Wang et a1 (1999) extended model 3 to include QTL × E interactions aiming at mapping QTLs involved in digenic epistasis under several environments. The phenotypic value of the k-th DH line in environment h can be expressed as the following mixed linear model (h = 1, 2, Λ, s; k = 1, 2, Λ, n_h),

$$\begin{aligned} y_{hk} = {} & \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} \\ & + \mu_{E_{hk}} e_{E_h} + \mu_{A_iE_{hk}} e_{A_iE_h} + \mu_{A_jE_{hk}} e_{A_jE_h} + \mu_{AA_{ij}E_{hk}} e_{AA_{ij}E_h} \\ & + \sum_{f(h)} \mu_{MM_{fk(h)}} e_{MM_{f(h)}} + \sum_{l(h)} \mu_{MM_{lk(h)}} e_{MM_{l(h)}} + \varepsilon_{hk} \end{aligned} \quad (5)$$

where μ is the population mean; a_i and a_j are the additive effects of loci Q_i and Q_j, respectively; aa_{ij} is the additive × additive epistatic effect of loci Qi and Qj; $x_{A_{ik}}$, $x_{A_{jk}}$, and $x_{AA_{ijk}}$ are coefficients of these QTL effects; e_{E_h} is the random effect of environment h with coefficient $\mu_{E_{hk}}$; $e_{A_iE_h}$ (or CAE) is the additive × environment interaction effect with coefficient $\mu_{A_iE_{hk}}$ (or $\mu_{A_jE_{hk}}$) for Q_i (or Q_j); e_{AAijEh} is the epistasis × environment interaction effect with coefficient $\mu_{AA_{ij}E_{hk}}$; $e_{M_{f(h)}}$ is the effect of marker f nested within the *h-th* environment with coefficient $m_{M_{fk(h)}}$; $e_{MM_{f(h)}}$ is the effect of marker × marker interaction nested within the h-th environment with coefficient $m_{MM_{fk(h)}}$; and e_{hk} is the residual effect.

For model 3 without QTL × E (QE) interactions, we can test QTL effects by setting H_o: $a_i = a_j = aa_{ij} = 0$. In this case, LR has an approximate x^2 distribution with $df = 3$. The alternative hypothesis (H_1) is that not all of the QTL effects are equal to zero. For model 5 with QE interactions, we can test both QTL effects and QE interaction effects by setting H_o: $a_i = a_j = aa_{ij} = 0$ and $s^2_{A_iE} = s^2_{AA_{ij}E} = 0$. This hypothesis is equivalent to testing no QTLs in the two intervals across all the environments involved. The alternative hypothesis (H_1) is that not all of the QTL main effects and QE interaction effects are equal to zero. Therefore, the LR has an approximate x^2 distribution with $df = 6$. Additional hypothesis tests for other combinations of the genetic parameters can also be conducted.

Rejection of H_0 indicates that at least one of the QTL effects is not equal to zero. The hypothesis test for deviation of QTL effects from zero can be conducted by a *t*-test (Wang et al 1999).

Estimates of putative QTL positions can be obtained based on the above significance tests at peak points of the statistics (LR and/or t) along chromosomes. For significant aa_{ij} and/or $s^2_{AA_{ij}E}$, the two prior testing points ($r_{M_f\text{-}Q_i}$ and $r_{M_f\text{-}Q_j}$) are taken as the estimated positions of Q_i and Q_j. When a_i (or a_j) and/or $s^2_{A_iE}$ (or $s^2_{A_jE}$) are significant, $r_{M_i\text{-}Q_i}$ (or $r_{M_f\text{-}Qj}$) is taken as the estimated position of Q_i (or Q_j).

In summary, the mixed linear model 3 provides a basic framework that can easily be extended to cover more complex experimental designs in QTL mapping experiments by inclusion of additional factors (such as environments, QE interactions, and/or randomized complete blocks). Model 5 represented a direct extension of model 3 to include QE interactions. Monte Carlo simulations showed that model 5 maintained the most important property of model 3, the unbiasedness of estimated QTL effects (additive and epistatic effects) and predicted QE interaction effects. Environmental effects (e.g., soil types, daylength, and general temperature regimes, etc.) could also be taken as fixed effects in model 5. Environments and QE interactions are treated as random in the model. Otherwise, QTL effects (additive and epistatic effects) and fixed environmental and QE interaction effects would be confounded and unestimable due to the singularity of the estimation matrix. The random effects in models 3 and 5 follow normal distributions, which may often be more or less violated in practice. Nevertheless, violation of this assumption is unlikely to cause serious problems because current mapping populations usually have more than 200 individuals, which are considered to be large samples statistically. Thus, our simulation showed that the

mixed model approach had many favorable properties for mapping QTLs with epistatic effects and QE interactions.

Computer simulation and real data analyses demonstrated that models 3 and 5 were far superior to models 1, 2, and 4 in power, precision, and accuracy for QTL mapping (Wang et al 1999). But because of their complexity (more genetic parameters included), we expect models 3 and 5 to be more sensitive to missing data. A software based on models 3 and 5 suitable for QTL mapping of recombinant inbred (RI), doubled-haploid (DH), and backcross (BC) populations, QTLMAPPER v. 1.0, is available to any researcher (Wang et al 1999).

Experimental design parameters

Besides the statistical models, the design and execution of phenotyping experiments are important for the success of QTL mapping. Several factors, such as progeny type, mapping population size, and precision in phenotypic measurements, all influence the power, precision, and accuracy of QTL mapping results. Determining the optimum combination of these parameters for a QTL mapping experiment depends largely on research objectives and trait heritability to obtain reliable QTL results at minimum efforts and costs. Computer simulation indicates that for DH, RI, or BC progenies with two genotypes at individual marker loci, an effective population size of 200 individuals is sufficient to detect QTLs with main and/or epistatic effects $\geq$ 5% in R^2 of target trait variation (data not shown). Generally, for a given population size, DH and RI populations are the most powerful for estimating the additive genetic effects of QTLs, but they are unable to estimate the nonadditive genetic effects. BC progenies from the North Carolina Design III are the least powerful for estimating additive genetic effects but are powerful for nonadditive genetic effects. F_2 populations are the most efficient for studying different gene actions but are the least accurate in estimated QTL parameters because they have the maximum number of genotypes. A design using a DH or RI population and its two BC populations (backcrossed to their parents) is powerful for resolving and estimating different gene actions of QTLs that affect complex traits, but this requires significantly increased efforts in making crosses and phenotyping (Xiao et al 1995).

AB-QTL analysis and near-isogenic introgression lines (NIILs)

QTLs can also be identified using advanced backcross QTL analysis (AB-QTL) (Tanksley and Nelson 1996). This strategy uses BC_2- or BC_3-derived progenies for QTL analyses and is particularly useful in detecting desirable QTLs of additive and or dominant gene actions from exotic germplasm. It is conceivable that in advanced BC generations, the genetic background of a mapping population is biased toward the recurrent parent, so that introgressed donor genomic segment(s) and associated phenotypic effect(s) or QTL(s) can be easily identified and accurately resolved. An additional advantage is that the AB-QTL method may result in direct improvement of the recurrent parents by simultaneous introgression and identification of desirable QTLs from exotic germplasm. A modification of this strategy involves using multiple re-

cipients (elite genotypes) and multiple diverse donors for massive introgression of target traits using BC breeding procedures to develop large numbers of NIIL sets for genetic improvement of elite cultivars. This should allow the simultaneous identification and introgression of large numbers of desirable alleles at many QTLs for genetic enhancement of the elite gene pool, which may have great potential for genetic improvement of crops. In addition, large numbers of near-isogenic lines (NILs) for QTLs can be easily generated from NIILs. These QTL NIL sets, coupled with large insert libraries such as bacterial artificial libraries, physical maps of the rice genome, and new DNA chip technology (Ruan et a1 1998), make it possible to clone and assign functions to important QTLs.

Future perspectives

QTL mapping of rice has two prospective and challenging areas. Theoretically, many questions concerning epistasis and QTL × E interactions remain largely unresolved. These include how epistasis (detected as interactions between QTLs) arises from gene interactions at the genic level (gene expression and regulation), at the physiological level (biochemical feedback and physiological homeostasis), and at the phenotypic level (competition between individuals and interactions with environments). Because epistatic loci are often involved in the same biochemical pathway, mapping, cloning, and characterizing epistatic loci are expected to result in a more complete understanding of important biochemical pathways and their connection with physiological aspects of complex phenotypes. New developments needed in this area may include the development of more powerful genetic and statistical models providing better resolution and parameterization of epistatic loci that affect complex traits for various types of mapping populations. Furthermore, fine and physical mapping and characterization of individual epistatic QTLs leading to the final cloning of QTLs require specifically constructed genetic materials such as NIL sets for individual complex phenotypes (Li 1997). With all these developments and anticipated complete physical maps and DNA sequence data of the rice genome in the near future, coupled with a map-based DNA hunting strategy and DNA chip technology (Ruan et a1 1998), cloning and function assignment of large numbers of QTLs will eventually come true.

In applications, as most DNA marker technologies become cheaper and automated, comprehensive integration of DNA marker technology, functional genomics, and bioinformatics with conventional breeding techniques is imminent. This should result in a new era of molecular breeding. In this era, we anticipate that the availability of high-throughput DNA marker technologies and bioinformatics on allelic diversity for genes of known functions will be used intensively in almost every step of breeding, including parental selection, population development, selection strategy, and progeny management. Also, the identification of desirable genes/QTLs and multilocus genotypes, and germplasm characterization and enhancement, will merge in the breeding process for the development of the "best" rice cultivars with greatly increased productivity, stability, and quality in all target environments.

References

Beavis WD. 1997. QTL analyses: power, precision, and accuracy. In: Molecular dissection of complex traits. Boca Raton, Fla. (USA): CRC Press. p 145-162.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Genet. 32:314.

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, et al. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251-1274.

Chen X, Temnkh S, Xu Y, Cho YG, McCouch SR. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet. 95:553-567.

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM, Eun MY. 1998. Integrated map of AFLP, SSLP, and RFLP markers using a recombinant inbred population of rice (*Oryza sativa L.*). Theor. Appl. Genet. 97:370-380.

Huang N, Courtois B, Khush GS, Lin H, Wang G, Wu P, Zheng KL. 1996. Association of quantitative trait loci for plant height with major dwarf genes in rice. Heredity 77:130-137.

Hauser MT, Adhami F, Dorner M, Fuchs E, Glossi J. 1998. Generation of co-dominant PCR-based markers by duplex analysis on high resolution gels. Plant J. 16(1):117-125.

Khush GS, Bennett J, Datta SK, Brar DS, Li Z. 1998. Advances in rice genetics and biotechnology. Proceedings of the 19th Session of the International Committee Meeting, Cairo, Egypt.

Kurata N, Nagamura Y, Tamamoto K, Miyamoto Y, Kirihara T. et al. 1994. A 300 kb interval genetic map of rice including 883 expressed sequences. Nature Genet. 8:365-372.

Lander ES, Botstein D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Li ZK. 1997. Molecular analysis of epistasis. In: Molecular dissection of complex traits. Boca Raton, Fla. (USA): CRC Press. p 119-130.

Li ZK, Pinson SRM, Paterson AH, Stansel JW. 1998a. Genetic dissection of source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Mol. Breed. 4:419-426.

Li ZK, Luo LJ, Mei HW, et al. 1999. A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae.* Mol. Gen. Genet. 261:58-63.

Li ZK, Paterson AH, Stansel JW. 1998b. RFLP facilitated analysis of tiller and leaf angles in rice (*Oryza sativa L.*). Euphytica. (In press.)

Li ZK, Pinson SRM, Paterson AH, Park WD, Stansel JW. 1997a. Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453-465.

Li ZK, Pinson SRM, Paterson AH, Park WD, Stansel JW. 1997b. Genetics of hybrid sterility and hybrid breakdown in an inter-subspecific rice (*Oryza sativa* L.) population. Genetics 145:1139-1148.

Li ZK, Pinson SRM, Stansel JW, Park WD. 1995. Identification of quantitative trait loci (QTL) for heading date and plant height in rice using RFLP markers. Theor. Appl. Genet. 91:374-381.

Li ZK, Luo LJ, Mei HW, Paterson AH, Shu QY, Zhong DB. Wang DL, Ying CS, Stansel JW, Khush GS. 1998c. Overdominant epistatic loci are the primary genetic basis of heterosis in rice. In: Abstracts of 18th International Congress of Genetics, Beijing. p 133 (8325).

Liu BH. 1997. Computational tools for study of complex traits. In: Molecular dissection of complex traits. Boca Raton, Fla. (USA): CRC Press. p 43-80.

Liu KD, Wang J, Li HB, Xu CG, Liu AM, Li XH, Zhang QF. 1997. Agenome-wide analysis of wide compatibility in rice and the precise location of the S-5 locus in the molecular map. Theor. Appl. Genet. 95:809-814.

Lizuka M, Mashiyama S, SekiyaT, Hiyashi K. 1992. Cloning and polymerase chain reaction-single-strand confirmation analysis of anonymous Alu repeats on chromosome 11. Genomics 12:139.

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, Huang N. 1996. Polymorphism, distribution, and segregation of AFLP markers in a double haploid rice population. Theor. Appl. Genet. 94:39-45.

Olsen M, Hood L, Cantor C, Botsein D. 1989. A common language for physical mapping of the human genome. Science 245:1434.

Paterson AH ZK. 1997. Of blending, beans, and bristles: the foundations of QTL mapping. In: Molecular dissection of complex traits. Boca Raton, Fla. (USA): CRC Press. p 1-12.

Robeniol JA, Constantino SV, Resurreccion AP, et al. 1996. Sequence-tagged sites and low-cost DNA markers for rice. In: Rice genetics. Manila (Philippines): International Rice Research Institute. p 293-306.

Ruan Y, Gilmore J, Conner T. 1998. Towards *Arabidopsis* genome analysis: monitoring expression profiles of 1400 genes using cDNA microarrays. Plant J. 15(6):821-833.

Shomura A, Yano M, HarushimaY, et al. 1997. The current molecular map of rice. Proceedings of the International Conference on Plant &Animal Genome V. San Diego, Calif. (USA). p 87.

Tanksley SD, Nelson JC. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Visscher PM, Haley CS, Thompson R. 1996. Marker-assisted introgression in backcross breeding programs. Genetics 144:1923-1932.

Vos PR, Hogers M, Bleecker M, et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407-4414.

Wang DL, Li ZK, Paterson AH, Zhu J. 1999. Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. Theor. Appl. Genet. (In press.)

Williams JGK, Livak AR, Rafalsky KJ, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:5631.

Xiao JH, Li J, Yuan L, Tanksley SD. 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor. Appl. Genet. 92:230-244.

Xiao JH, Li J, Yuan L, Tanksley SD. 1995. Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140:745-754.

Yan J, Zhu J, He C, Benmoussa M, Wu P. 1998. Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). Genetics 150:1257-1265.

Yao FY, Xu CG, Yu SB, et al. 1997. Mapping and genetic analysis of two fertility restoration loci in the wild-abortive cytoplasmic male sterility system of rice (*Oryza sativa* L.). Euphytica 98:183-187.

Yu SB, Li JX, Xu CG, et al. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc. Natl. Acad. Sci. USA 94:9226-9231.

Zhang G, Bharaj TS, Lu Y, Virmani SS, Huang N. 1997. Mapping of the Rf-3 nuclear fertility-restorer gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. Theor. Appl. Genet. 94:27-33.

Zeng ZB. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

Zheng K, Shen P, Qian H, Wang J. 1992. Tagging genes for wide compatibility in rice via linkage to RFLP markers. Chinese J. Rice Sci. 6:145-150.

Zhu J, Weir BS. 1998. Mixed model approaches for genetic analysis of quantitative traits. In: Chen LS, Ruan SG, Zhu J, editors. Advanced topics in biomathematics: Proceedings of the International Conference on Mathematical Biology, World Scientific Publishing Co., Singapore. p 321-330.

Notes

Authors' address: Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute.

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Improving drought, salt, and freezing stress tolerance using a single gene for a stress-inducible transcription factor in transgenic plants

K. Yamaguchi-Shinozaki and K. Shinozaki

Many genes have been shown to be induced by environmental stresses such as drought, salt loading, and freezing and function in the stress tolerance of plants. We precisely analyzed the promoter regions of a drought-inducible *Arabidopsis* gene, *rd29A,* in transgenic plants, and identified a novel cis acting element containing 9-bp TACCGACAT (DRE, dehydration responsive element). DRE plays an important role in dehydration and high-salt- and low-temperature-induced gene expression in *Arabidopsis.* Two cDNA clones that encoded DRE-binding proteins, DREB1A and DREB2A, were isolated by yeast one-hybrid screening. Both the DREB proteins specifically bind to the DRE sequence and activate the transcription of genes driven by the DRE sequence in *Arabidopsis.* Expression of the DREB1A gene and its two homologs was induced by low-temperature stress, whereas expression of the DREB2A gene and its single homolog was induced by dehydration. These results indicate that two independent families of DREB proteins, DREB1 and DREB2, function as transacting factors in two separate signal transduction pathways under low-temperature and dehydration conditions, respectively. Overexpression of the DREB1A cDNA, driven by the constitutive 35S CaMV promoter in transgenic plants, activated strong expression of the target stress-inducible genes under unstressed conditions, which, in turn, increased tolerance of freezing and drought. But the overexpression of stress-inducible genes controlled by the DREB1A protein caused severe growth retardation under normal growth conditions. The stress-inducible *rd29A* promoter minimized negative effects on plant growth.

Plant productivity is affected markedly by environmental stresses such as drought, salt loading, and freezing. Plants respond to these stresses at the molecular and cellular levels as well as at the physiological level. These stresses induce the expression of a variety of genes (Shinozaki and Yamaguchi-Shinozaki 1997). The products of these genes are thought to function not only in stress tolerance but also in the regulation of gene expression and in signal transduction in the stress response. These gene products can be classified into two groups. The first group includes proteins that probably

function in protecting cells from dehydration. The second group contains protein factors that are involved in further regulation of gene expression and signal transduction and that function in stress response (Shinozaki and Yamaguchi-Shinozaki 1997). The second group of genes seems to be useful in improving the tolerance of *Arabidopsis* to stresses by gene transfer, as they can regulate many of the stress-inducible genes involved in stress tolerance,

Drought is one of the most severe environmental stresses; it affects almost all plant functions. Abscisic acid (ABA) is produced under water-deficit conditions and plays an important role in drought tolerance. Most drought-inducible genes that have been studied to date are also induced by ABA. It appears that dehydration triggers the production of ABA, which, in turn, induces various genes. Several reports have described genes that are induced by dehydration but that are not responsive to exogenous ABA treatments. These findings suggest the existence of ABA-independent and ABA-dependent signal-transduction cascades between the initial signal of drought or cold stress and the expression of specific genes (Shinozaki and Yamaguchi-Shinozaki 1997). To understand the molecular mechanisms of gene expression in response to drought stress, *cis*- and *trans*-acting elements that function in ABA-independent and ABA-responsive gene expression by drought stress have been precisely analyzed. In this chapter, we summarize recent progress in our research on *cis*- and *trans*-acting factors involved in ABA-independent gene expression in drought stress response. We also describe the production of transgenic plants that overexpressed multiple stress-inducible genes by incorporating a single gene for a stress-inducible transcription factor using *Arabidopsis* as a model.

Function of water-stress-inducible genes

Drought stress induces a variety of genes, and functions of their gene products have been predicted from sequence homology with known proteins. Genes induced during drought stress are thought to function not only in protecting cells from dehydration by producing important metabolic proteins but also in regulating genes for signal transduction in the drought stress response (Shinozaki and Yamaguchi-Shinozaki 1997). Thus, these gene products are classified into two groups (Fig. 1).

The first group includes proteins that probably function in stress tolerance: water channel proteins involved in the movement of water through membranes, the enzymes required for the biosynthesis of various osmoprotectants (sugars, proline, and betaine), proteins that may protect macromolecules and membranes (LEA protein, osmotin, antifreeze protein, chaperon, and mRNA-binding proteins), proteases for protein turnover (thiol proteases, Clp protease, and ubiquitin), and the detoxification enzymes (glutathione S-transferase, soluble epoxide hydrolase, catalase, superoxide dismutase, and ascorbate peroxidase).

The second group contains protein factors involved in further regulation of signal transduction and gene expression that probably function in stress response: protein kinases, transcription factors, PLC, and 14-3-3 proteins. The existence of a vari-

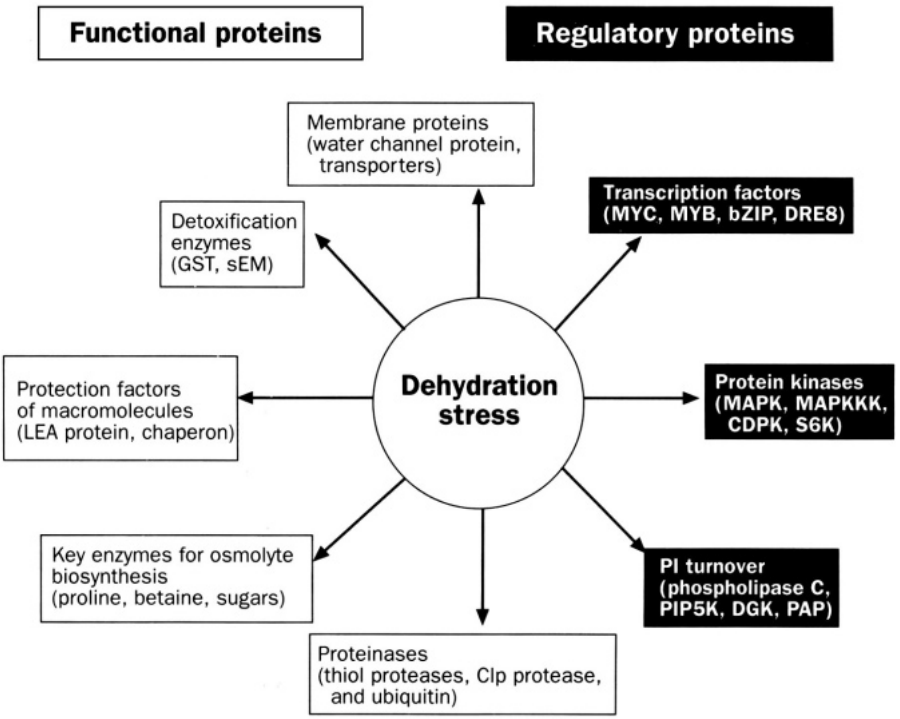

Fig. 1. Drought-stress inducible genes and their possible functions in stress tolerance and response. Gene products are classified into two groups. The first group includes proteins that probably function in stress tolerance (functional proteins), and the second group contains protein factors involved in the further regulation of signal transduction and gene expression that probably function in stress response (regulatory proteins).

ety of drought-inducible genes suggests complex responses of plants to drought stress. Their gene products are involved in drought stress tolerance and stress responses.

Expression of dehydration-induced genes in response to environmental stresses and ABA

The expression patterns of genes induced by drought were analyzed by RNA gel-blot analysis. Results indicated broad variations in the timing of induction of these genes under drought conditions. Most of the drought-inducible genes respond to treatment with exogenous ABA, whereas some do not. Therefore, there are ABA-dependent and ABA-independent regulatory systems of gene expression under drought stress. Analysis of the expression of ABA-inducible genes revealed that several genes require protein biosynthesis for their induction by ABA, suggesting that at least two

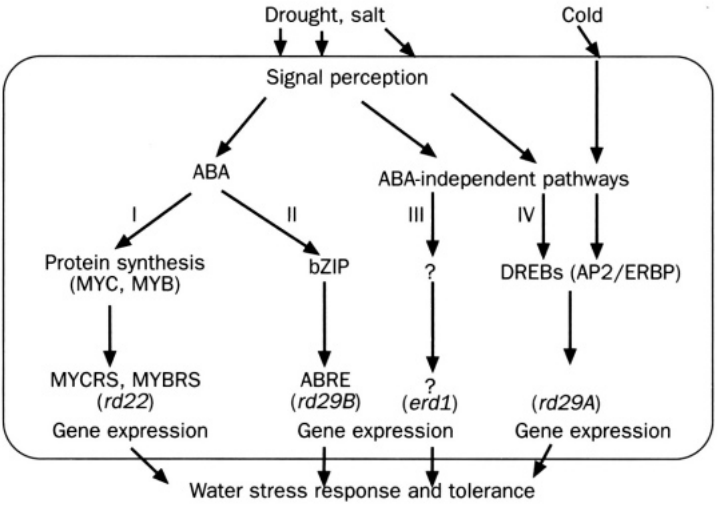

Fig. 2. Signal transduction pathways between the initial dehydration stress signal and gene expression. There are at least four signal transduction pathways: two are ABA-dependent (I and II) and two are ABA-independent (III and IV). Protein synthesis is necessary for one of the ABA-dependent signal pathways (I). ABRE is involved in one of the ABA-dependent pathways (II). In one of the ABA-independent pathways, DRE is involved in the regulation of genes not only by drought and salt but also by cold stress (IV). Another ABA-independent pathway is controlled by drought and salt, but not by cold (III).

independent pathways exist between the production of endogenous ABA and gene expression during stress.

We identified at least four independent signal pathways that function under drought conditions (Fig. 2): two are ABA-dependent (pathways I and II) and two are ABA-independent (pathways III and IV). One of the ABA-independent pathways overlaps with that of the cold response (pathway IV). One of the ABA-dependent pathways requires protein biosynthesis (pathway II, Shinozaki and Yamaguchi-Shinozaki 1997). The complex signal transduction pathways in drought response provide a molecular basis for the complex physiological responses of plants to drought stress.

Identification of a novel cisacting element involved in drought-responsive expression

Several genes are induced by drought, salt, and cold in aba (ABA-deficient) or abi (ABA-insensitive) *Arabidopsis* mutants. This suggests that these genes do not require ABA for their expression under cold or drought conditions. Among these genes, the expression of a drought-inducible gene for *rd29A/lti78/cor78* was extensively analyzed (Yamaguchi-Shinozaki and Shinozaki 1994). At least two separate regulatory systems function in gene expression during drought and cold stress; one is ABA-independent (Fig. 2, pathway IV) and the other is ABA-dependent (pathway II).

To analyze the *cis*-acting elements involved in the ABA-independent gene expression of *rd29A,* we constructed chimeric genes with the *rd29A* promoter fused to the b-glucuronidase (*GUS*) reporter gene and transformed *Arabidopsis* and tobacco plants with these constructs. The *GUS* reporter gene driven by the *rd29A* promoter was induced at significant levels in transgenic plants by conditions of dehydration, low temperature, or high salt or by treatment with ABA (Yamaguchi-Shinozaki and Shinozaki 1993). The deletion, gain-of-function, and base substitution analysis of the promoter region of the *rd29A* gene revealed that a 9-bp conserved sequence, TACCGACAT (DRE, dehydration responsive element), is essential for regulating the expression of *rd29A* under drought conditions. Moreover, DRE has been demonstrated to function as a *cis*-acting element involved in the induction of rd29A by either low-temperature or high-salt stress (Yamaguchi-Shinozaki and Shinozaki 1994). Therefore, DRE seems to be a *cis*-acting element involved in gene induction by dehydration, high salt, or low temperature, but does not function as an ABA-responsive element in the induction of *rd29A*.

Important roles of DRE-binding proteins during drought and cold stress

Two cDNA clones that encode the DRE-binding proteins DREB1A and DREB2A were isolated by using the yeast one-hybrid screening technique. The deduced amino acid sequences of DREB1A and DREB2A showed no significant sequence similarity, except in the conserved DNA-binding domains found in the EREBP and APETALA2 proteins that function in ethylene-responsive expression and floral morphogenesis, respectively. But each DREB protein contained a basic region in its N-terminal region that might function as a nuclear localization signal and an acidic C-terminal region that might act as an activation domain for transcription. These data suggest that each DREB cDNA encodes a DNA-binding protein that might function as a transcriptional activator in plants.

The ability of the DREB1A and DREB2A proteins expressed in *Escherichia coli* to bind the wild-type or mutated DRE sequences was examined using the gel retardation method. The results indicate that the binding of these two proteins to the DRE sequence is highly specific. To determine whether the DREB1A and DREB2A proteins are capable of transactivating DRE-dependent transcription in plant cells, we performed transactivation experiments using protoplasts prepared from *Arabidopsis* leaves. Coexpression of the DREB1A or DREB2A proteins in protoplasts transactivated the expression of the *GUS* reporter gene. These results suggest that DREB1A and DREB2A proteins function as transcription activators in the cold- and dehydration-responsive expression, respectively, of the *rd29A* gene (Liu et al 1998, Fig. 3).

We isolated cDNA clones encoding two DREB1A homologs (named DREB1B and DREB1C). The DREBlB clone was identical to CBFl (Stockinger et a1 1997). We also isolated cDNA clones encoding a DREB2A homolog and named it DREB2B. Expression of the DREB1A gene and its two homologs was induced by low-temperature stress, whereas expression of the DREB2A gene and its single homolog was in-

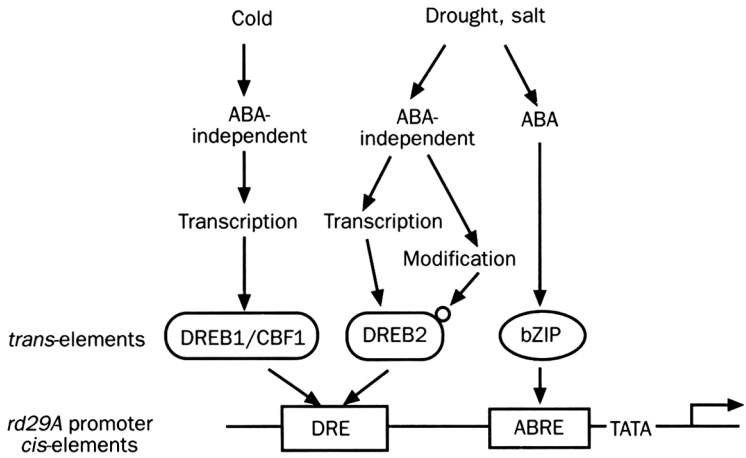

Fig. 3. A model of the induction of the *rd29A* gene and cis and transacting elements involved in stress-responsive gene expression. Two *cis*-acting elements, DRE and ABRE, are involved in the ABA-independent and ABA-responsive induction of *rd29A,* respectively. Two types of different DRE-binding proteins, DREB1 and DREB2, separate two different signal transduction pathways in response to cold and drought stresses, respectively. DREB1s/CBF1 are transcriptionally regulated whereas DREB2s are controlled post-translationally as well as transcriptionally. ABRE-binding proteins encode bZIP transcription factors.

duced by dehydration (Liu et al 1998, Shinwari et al 1998). Overexpression of the DREB1A cDNA in transgenic *Arabidopsis* plants not only induced strong expression of the target genes under unstressed conditions but also caused dwarfed phenotypes in the transgenic plants. These transgenic plants also revealed freezing and dehydration tolerance, which was also shown in the CBF1 transgenics (Liu et al 1998, Jaglo-Ottosen et al 1998). In contrast, overexpression of the DREB2A cDNA induced weak expression of the target genes under unstressed conditions and caused growth retardation of the transgenic plants (Liu et al 1998). These results indicate that two independent families of DREB proteins, DREB1 and DREB2, function as transacting factors in two separate signal transduction pathways under low-temperature and dehydration conditions, respectively (Liu et al 1998).

Overproduction of the DREBlA and CBF1/DREB1B cDNA driven by the 35S CaMV promoter in transgenic plants significantly improved stress tolerance of drought and freezing. But the DREB1A transgenic plants revealed severe growth retardation under normal growth conditions. The DREBlA cDNA driven by the stress-inducible rd29A promoter was expressed at a low level under unstressed control conditions and was strongly induced by dehydration, salt, and cold stresses. The *rd29A* promoter minimized negative effects on plant growth, whereas the 35S CaMV promoter caused severe growth retardation under normal growth conditions (Kasuga, Liu, Miura, Yamaguchi-Shinozaki, and Shinozaki, submitted). Moreover, this stress-inducible

promoter enhanced tolerance of drought, salt, and freezing at a higher level than that of the 35S CaMV promoter. Because DRE also functions in other crop plants, both the DREB1A cDNA and the *rd29A* promoter can probably be used to improve the dehydration and freezing tolerance of crops by gene transfer.

References

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF. 1998. *Arabidopsis* CBFl overexpression induces *cor* genes and enhances freezing tolerance. Science 280: 104-106.

Liu Q, Sakuma Y, Abe H, Kasuga M, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1998. Two transcription factors, DREBl and DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in *Arabidopsis.* Plant Cell 10: 1391-1406.

Shinozaki K, Yamaguchi-Shinozaki K. 1997. Gene expression and signal transduction in water-stress response. Plant Physiol. 115:327-334.

Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M. Yamaguchi-Shinozaki K, Shinozaki K. 1998. An *Arabidopsis* gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression Biochem. Biophys. Res. Commun. 250:161-170.

Stockinger EJ, Gilmour SJ, Thomashow ME 1997. *Arabidopsis thaliana* CBFl encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. USA 94:1035-1040.

Yamaguchi-Shinozaki K, Shinozaki K. 1994. A novel *cis*-acting element in an *Arubidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6:251-264.

Yamaguchi-Shinozaki K, Shinozaki K. 1993. Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. Mol. Gen. Genet. 236:331-340.

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Perspectives on marker-aided selection

Mapping and marker-assisted selection to improve the stay-green trait for drought tolerance in sorghum

P.K. Subudhi, G.B. Magpantay, D.T. Rosenow, and H.T. Nguyen

Drought is a major constraint to crop productivity. In sorghum, drought stress during and after the flowering stage causes premature leaf senescence that, in turn, leads to stalk lodging, charcoal rot, and significant yield loss. More than 80% of commercial sorghum hybrids in the United States are grown under nonirrigated conditions and most of them have preflowering drought resistance but do not have any significant postflowering drought resistance, typically called the stay-green trait. Stay-green is a drought-resistance mechanism that gives sorghum plants resistance to premature senescence under severe soil moisture stress during the postflowering stage. We have identified four quantitative trait loci (QTLs) for this trait using a recombinant inbred line population developed from B35 x Tx7000. Three major stay-green QTLs were consistently identified in all field trials, accounting for 46% of the phenotypic variance. Physical mapping of these QTL regions and, ultimately, the isolation of the stay-green genes will lead to a better understanding of drought resistance in sorghum. Research is under way to introgress major stay-green QTLs into elite breeding lines through marker-assisted selection.

Water constrains agriculture in the United States and around the world. Soils subjected to continuous drought together with shallow soils subjected to frequent water deficit make up 45% of the U.S. land surface (Boyer 1982). The drought-tolerance trait of sorghum makes it one of the most important feed and food crops in the arid and semiarid regions of the U.S. and the world. More than 80% of the sorghum in the U.S. is grown under nonirrigated conditions. The development and use of crop cultivars adapted to drought-prone conditions is a long-term solution for improving and stabilizing crop productivity. Molecular mapping of drought-tolerance traits will enhance breeding efficiency through marker-assisted selection and lay a foundation for understanding the genetic basis of drought tolerance in plants.

Characteristics of drought tolerance in sorghum

Drought tolerance in sorghum is a complex trait affected by several interacting plant and environmental factors. Over the past 20 years, Dr. Darrell Rosenow and his co-

workers have focused considerable effort on drought tolerance as a primary breeding objective. Their research is part of an overall sorghum improvement program supported by the Texas Agricultural Experiment Station, USAID, and seed industry. They have found that the stage of growth (GS) at which moisture stress occurs is very important in determining the response or reaction of sorghum to water stress. Using field-screening nurseries in West Texas, they have determined and described two distinct responses to drought stress in sorghum (Rosenow and Clark 1981, Rosenow et al 1983). One type (preflowering) is expressed when plants are stressed during panicle formation before flowering (GS-2), whereas the other (postflowering) is expressed when moisture stress occurs during the grain development stage (GS-3).

Postflowering tolerance is especially important because the negative effect of drought on yield is significant at this stage of development. The symptoms of susceptibility to postflowering drought stress include premature plant (leaf and stem) death, stalk collapse, lodging, stalk rot, and sometimes a significant reduction in seed size, particularly at the base of the panicle. Stay-green cultivars retain their leaves in the active photosynthetic state and fill grain normally when subjected to water-stress conditions during the grain-filling period. Such green stalks have good resistance to stalk lodging and stalk rot. Although preflowering drought directly affects grain yield, the yield reduction under postflowering moisture stress is primarily through lodging associated with premature plant death and stalk rot of postflowering drought-susceptible cultivars, but sometimes also through reduced seed size. Preflowering and postflowering drought responses are repeatable across years and locations and resistance or susceptibility at each stage can be described in terms of visual plant symptoms. The term "stay-green'' has been used to describe the postflowering drought-tolerance response (Rosenow and Clark 1981).

Stay-green genes confer resistance to drought-induced senescence in sorghum

Stay-green in sorghum is an important component of postflowering drought tolerance. It delays the onset of senescence in sorghum, resulting in greater functional leaf area during grain filling and even after physiological maturity. Increased accumulation of soluble sugars in stay-green types thereby reduces the dependence on stored assimilates from the stem to fill the grains (McBee et al 1983). The higher concentration of stem sugars improves the digestible energy content of the stover or, if translated into growth of axial branches (Vietor et al 1989), increases the amount of total harvestable fodder.

Thomas and Smart (1993) reported that, in many crop species, stay-green plants have increased resistance to disease and drought and possess leaves with higher nutritional quality and attractiveness to grazing animals. Sorghum genotypes with postflowering drought resistance expressed by the stay-green trait exhibit increased resistance to charcoal rot (Rosenow 1984) and lodging (Henzell et al 1984, Woodfin et al 1988). Stay-green genotypes also contain more cytokinins (McBee 1984) than

senescent genotypes, which may reduce the rate of drought-induced senescence (Thomas and Smart 1993).

In sorghum, stay-green genes confer resistance to postflowering drought stress by reducing premature death of leaves and plants, stalk lodging, and charcoal rot disease when the plants are exposed to moisture stress during the late stages of grain development (Rosenow 1987).

Results from our research showed that B35 (a stay-green line) retained much more of the chlorophyll than non-stay-green lines Tx7000 and Tx430 under postflowering stress environments (Table 1). The reduction in grain yield is relatively less in stay-green genotypes than in non-stay-green genotypes. Under severe postflowering drought conditions, the hybrids from non-stay-green parents could have more than 50% lodging compared with less than 10% lodging in the hybrids with one stay-green parent (Rosenow, unpublished data).

Using stay-green genes in sorghum

Specific drought responses have been observed to be heritable and can be transferred through conventional breeding methods. In some lines (e.g., B35), the stay-green trait appears to be dominant in F_1 hybrids, whereas in others (e.g., R9188) it appears to be recessive (Wanous 1989). Experiments conducted using the cross B35 (stay-green inbred line) by Tx7000 (non-stay-green inbred line) revealed dominant action of major genes for this trait (Walulu et al 1994). Broad-sense and narrow-sense heritability estimates were 0.80 and 0.60, respectively, indicating that the stay-green trait is heritable and that progress from selection can be attained. Field screening techniques have been developed for drought-tolerance selection. Progress in improving sorghum drought tolerance with field screening methods has been very slow, however, because

Table 1. Preflowering rating, stay-green rating, and chlorophyll content of 635 (stay-green line) and Tx7000 and Tx430 (non-stay-green lines) under control and drought-stress conditions.

Cultivar	Preflowering score[a]	Stay-green score[b]	Chlorophyll content[c] (%)		
			Control	Stress	Reduction
B35	3.38	1.9	57.7	44.5	22.9
Tx7000	2.23	4.9	56.0	13.9	75.1
Tx430	2.10	3.4	57.5	28.6	50.3

[a]The preflowering drought-tolerance rating was done on a 1–5 scale, where 1 = excellent and 5 = very poor response during panicle development stage under severe water deficit prior to flowering. This is a composite preflowering drought-resistance score of several affected traits such as delay in flowering, panicle and floret abortion, poor panicle exsertion, reduced panicle size, leaf rolling, leaf bleaching, and leaf firing, which can also be scored Individually. [b]Stay-green was visually rated on a 1–5 scale at physiological maturity on a plot basis under postflowering drought stress. A rating of 1 indicates essentially no leaf death, while 5 indicates 100% plant (leaves and stem) death. [c]Chlorophyll content was measured at the basal leaf blade of the second and fourth leaves from the top of three plants per plot with a Minolta Chlorophyll Meter SPAD-502.

of the lack of control on the timing and intensity of moisture stress and interactions between the plant (especially growth stage) and other climatic factors. Hence, identifying molecular markers linked to the stay-green trait will facilitate breeding for drought tolerance in sorghum.

Grain yield and lodging are critically important to producers and are often related. The stay-green trait should have a major direct benefit to sorghum producers by reducing losses from lodging associated with premature leaf and stalk death under drought. The stay-green trait does not appear to reduce grain yield of hybrids, although it does improve lodging resistance.

Mapping of QTLs controlling the stay-green trait in sorghum

Two RIL (recombinant inbred line) populations, developed from the crosses B35 × Tx7000 and B35 × Tx430, were used to map the stay-green trait. Inbred line B35, a derivative of IS12555, a Durra from Ethiopia, is susceptible to preflowering drought stress but has outstanding postflowering drought tolerance, whereas Tx7000 and Tx430 have good preflowering drought tolerance but are susceptible to postflowering drought stress (Table 1). For the B35 × Tx7000 population, the parental lines and 98 F_7 RILs were evaluated under postflowering stress conditions at two locations, Lubbock and Halfway, during 1993 and 1994. All these trials were irrigated till the flowering stage, but irrigation was withdrawn just before anthesis to allow moisture stress to develop during the grain-filling stage. The stay-green expression of individual RILs along with parental lines was rated visually on a plot basis on a scale of 1 to 5, based on the degree of leaf and plant death. A score of 1 indicates essentially no leaf death, whereas 5 corresponds to complete plant (leaf and stem) death. Visual scoring in field conditions has been demonstrated to be a reliable indicator of stay-green response (Wanous et al 1991). Four stay-green QTLs, located on three linkage groups, were identified in this population. Of those, three stay-green QTLs, Stg1, Stg2, and Stg3, individually accounted for 20%, 30%, and 16% of phenotypic variance and were consistently identified in both years. The fourth stay-green QTL, Stg4, was identified only during 1994 and it explained 13% of phenotypic variance. For all the QTLs, the stay-green alleles were contributed by the stay-green parent B35.

For the B35 × Tx430 population, 96 F_6 lines along with their parents were evaluated under postflowering stress environments at the same two locations during 1993 and 1994. Plots were evaluated for stay-green expression at the end of the linear grain-fill period. Seven stay-green QTLs were identified in this population (Crasta et al 1999), of which three were major QTLs (StgA, StgD, and StgG). These were located on three linkage groups together, and accounted for 42% of the phenotypic variance. Four minor QTLs (StgB, StgI.1, StgI.2, and StgJ) contributed an additional 25% of the phenotypic variance in stay-green ratings. In all three major QTLs and two minor QTLs, alleles from B35 contributed to an improved stay-green expression, whereas alleles from Tx430 improved stay-green rating in the other two minor QTLs. This observation confirmed that Tx430 contains some stay-green genes because it is

slightly better than Tx7000 for stay-green response. The three major QTLs and two minor QTLs were consistently identified across most, if not all, environments, while the remaining two minor QTLs had high QTL × environment interaction. In general, the QTL study indicated that genetic control of the stay-green trait in grain sorghum is more complex than the earlier finding by Walulu et a1 (1994).

Consistency of stay-green QTLs

From the above QTL analysis, we observed that two stay-green QTLs were consistent between both the RIL populations. The QTLs Stg2 and Stg4 of the B35 × Tx7000 RIL population overlapped with QTLs StgA and StgJ of the B35 × Tx430 population, respectively. Another QTL (Stg3 and StgD of B35 × Tx7000 and B35 × Tx430, respectively) was located on the same linkage group but in different locations in both populations. Single marker analysis in the B35 × Tx430 population (Crasta 1995) indicated that markers corresponding to Stgl of the B35 × Tx7000 population were highly correlated with the stay-green ratings. From this analysis, we concluded that Stg2 or the corresponding QTL StgA of the B35 × Tx430 population is the most important stay-green QTL, which explained the highest percentage of phenotypic variance and was consistent in both populations in all the environments. In order of importance, the stay-green QTL Stg2 is followed by Stgl and Stg3. A close look at the stay-green QTL profile of the best and poorest stay-green RIL lines of the B35 × Tx7000 population indicated that Stgl, Stg2, and Stg3 are consistently present in most of the best stay-green lines and absent in the poorest stay-green lines. This is reinforced by the fact that the QTLs for chlorophyll content also overlapped with these three major stay-green QTLs. Some functionally important genes such as genes encoding key photosynthetic enzymes, heat shock proteins, cell membrane ATPase, and an abscisic acid (ABA) responsive gene were observed near the Stg1 and Stg2 QTL regions. ABA is an essential mediator between drought and plant responses (Gosti et al 1995). The maize cDNA clone CSU71 is a CAB gene that encodes chlorophyll a/b binding proteins of the photosystem II complex located near Stg2. We need to investigate whether these genes have any role in stay-green expression. We have indications from field testing of advanced backcross lines on a limited scale that the QTL 1 and 2 combination always gives better resistance to postflowering stress. The interaction among QTLs, however, is also expected to play an important role in stay-green expression. To study interactions among stay-green QTLs, near-isogenic lines (NILs) for all possible QTL combinations are being developed for field evaluation.

Marker-assisted selection for stay-green QTLs in sorghum

The objective of the ongoing stay-green project at Texas Tech University is to transfer the stay-green QTLs from B35 (stay-green line) to Tx7000, a non-stay-green line, by marker-assisted backcross breeding (Fig. 1). All four QTLs—Stgl, Stg2, Stg3, and Stg4—have been targeted for introgression. In every backcross generation, about 150 to 200 individuals were screened with QTL-linked restriction fragment length poly-

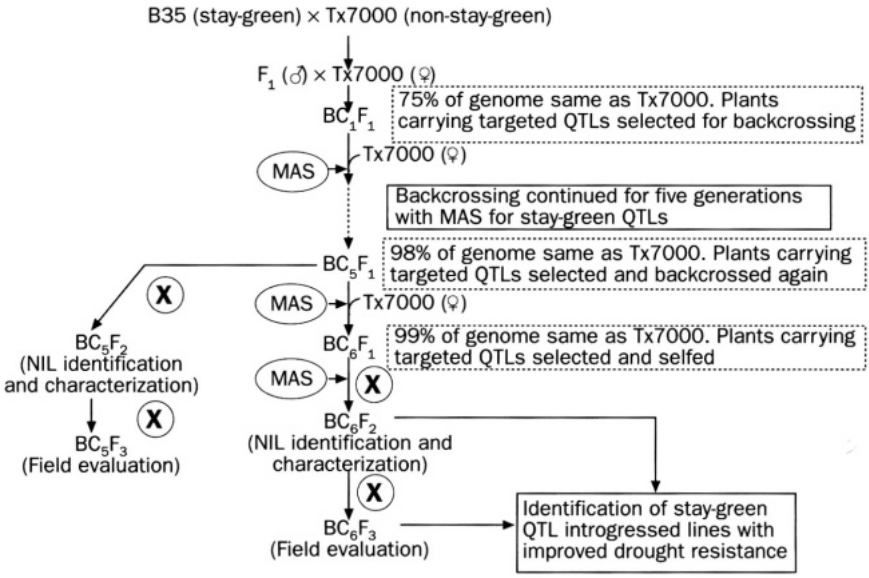

Fig. 1. Scheme for developing near-isogenic lines (NILs) for stay-green QTLs using marker-assisted selection (MAS).

morphism markers and individuals with all four stay-green QTLs were selected for further backcrossing. The selected individuals were used as male parents for backcrossing with the Tx7000 parent. After repeating marker-assisted backcrossing for several generations, homozygous NILs for individual stay-green QTLs have been identified in the BC_5F_2 generation. This activity also led to the development of NILs for different QTL combinations. The developed homozygous NILs for individual QTLs, specifically the major QTLs Stg1 and Stg2, were crossed to Tx7000 to develop a large BC_6F_2 mapping population for fine-mapping purposes. Because it is not possible to evaluate the stay-green response on an individual plant basis, the BC_6F_2 plants will be selfed further to develop BC_6F_3 families for field-testing. We are selfing the selected individuals from advanced backcross generations that are still heterozygous for some stay-green QTLs to generate stable and homozygous introgression lines in a Tx7000 background. By the end of 1999, the NILs introgressed with all the QTLs in the Tx7000 background will be available for field-testing. The NILs with individual QTLs and all possible combinations of stay-green QTLs will be evaluated in the field to study interactions among the QTLs.

Currently, marker-assisted introgression of stay-green QTLs to several elite sorghum lines used for commercial hybrid development is under way in collaboration with the seed industry. Field evaluation of the introgressed lines and their hybrids will ultimately determine the value of the stay-green trait in a stress environment.

Conclusions and future prospects

The identification of stay-green QTLs in two RIL populations not only facilitates marker-assisted breeding for improved drought tolerance but also forms a strong basis for elucidating the genetic basis of the senescence phenomenon in general and the stay-green trait in particular. Three major QTLs and a few minor QTLs were identified in each population, of which the QTL explaining the largest percentage of phenotypic variance and a minor QTL were consistent in both populations. Marker-assisted backcross breeding resulted in the development of a series of near-isogenic lines that are being used to dissect the genetic and physiological basis of the stay-green trait in sorghum.

The candidate gene approach (Rothschild and Soller 1997), which helps to link the genes of the relevant biochemical pathway to the trait of interest, is gradually becoming a powerful tool in QTL studies. With the development of functional genomic tools such as microarray technology (Schena et al 1995) and the growing EST (expressed sequence tags) database, it is now possible to identify the candidate genes that underly drought tolerance by studying the expression of many genes simultaneously. To achieve this objective, the NILs and the population derived from them will be valuable material in correlating the expression of those identified genes to the stay-green response under drought stress.

Research on comparative mapping has led us to believe that all the grasses probably have a single genetic system (Bennetzen and Freeling 1993, Moore et al 1995). The close correspondence of QTLs for similar phenotypes has also been reported in different taxa (Paterson et al 1995). In maize and rice, breeding for drought tolerance is also an important objective and the stay-green phenomenon has been noticed in these crops. After the stay-green QTLs are delimited to smaller genomic regions using NILs through a substitution-mapping strategy (Paterson et al 1990), the markers located in these regions can be used to screen the rice and maize BAC library to determine the orthologous regions in rice and maize for this trait. It is interesting to know the gene content of the corresponding regions in rice and maize. Thus, sequencing of the orthologous BACs from rice and maize along with the corresponding sorghum BACs will help in determining the micro-synteny and study of genome organization among these grass relatives. Because sorghum has superior tolerance for water and heat stress, we can visualize improving drought tolerance in maize and rice through the use of sorghum stay-green genes, once they are isolated.

References

Bennetzen JL, Freeling M. 1993. Grasses as a single genetic system: genome composition, colinearity and compatibility. Trends Genet. 9:259-261.

Boyer JS. 1982. Plant productivity and environment. Science 218:444-448.

Crasta OR, Xu W, Rosenow DT, Mullet JE, Nguyen HT. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: association of QTLs influencing premature senescence and maturity. Mol. Gen. Genet. (In press.)

Crasta OR. 1995. Molecular genetic analysis of stay green, a post-flowering drought resistance trait in grain sorghum (*Sorghum bicolor* L. Moench). Ph.D. thesis. Texas Tech University, Lubbock, Texas, USA.

Gosti F, Bertauche N, Vartanian N, Giraudat J. 1995. Abscisic acid-dependent and independent regulation of gene expression by progressive drought in *Arabidopsis thaliana.* Mol. Gen. Genet. 246: 10-18.

Henzell RG, Dodman RL, Done AA, Brengman RL, Mayers PE. 1984. Lodging, stalk rot, and root rot in sorghum in Australia. In: Mughogho LK, editor. Sorghum root and stalk diseases, a critical review. Proceedings of the Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases, Bellagio, Italy, 27 Nov-2 Dec 1983. Patancheru (India): International Crops Research Institute for the Semi-Arid Tropics. p 225-235.

McBee GG, Waskom RM, Miller FR, Creelman RA. 1983. Effect of senescence and nonsenescence on carbohydrates in sorghum during late kernel maturity states. Crop Sci. 23:372-376.

McBee GG. 1984. Relation of senescence, nonsenescence, and kernel maturity to carbohydrate metabolism in sorghum. In: Mughogho LK, editor. Sorghum root and stalk diseases, a critical review. Proceedings of the Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases. Bellagio, Italy, 27 Nov-2 Dec 1983. Patancheru (India): International Crops Research Institute for the Semi-Arid Tropics. p 119-129.

Moore G, Devos KM, Wang Z, Gale MD. 1995. Grasses, line up and form a circle. Curr. Biol. 5:737-739.

Paterson AH, DeVema JW, Lanini B, Tanksley SD. 1990. Fine mapping of quantitative trait loci using selective overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124:735-742.

Paterson AH, Lin YR, Li Z, Schertz KF, Doebly JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE. 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714-1718.

Rosenow DT. 1984. Breeding for resistance to root and stalk rot in Texas. In: Mughogho LK, editor. Sorghum root and stalk diseases, a critical review. Proceedings of the Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases, Bellagio, Italy, 27 Nov-2 Dec 1983. Patancheru (India): International Crops Research Institute for the Semi-Arid Tropics. p. 209-217.

Rosenow DT. 1987. Breeding sorghum for drought resistance. In: Menyonga JM, Bezuneh T, Youdeowei A, editors. Proceedings of the International Drought Symposium. OAU/STRC-SAFGRAD Coordination Office, Ouagadougou, Burkina Faso. p 19-23.

Rosenow DT, Clark LE. 1981. Drought tolerance in sorghum. In: Loden HD, Wilkinson D, editors. Proceedings of the 36th Annual Corn and Sorghum Research Conference, 9-11 Dec 1981, Chicago, Ill., USA. p 18-31.

Rosenow DT, Quisenberry JE, Wendt CW, Clark LE. 1983. Drought tolerant sorghum and cotton germplasm. Agric. Water Manage. 7:207-222.

Rothschild MF, Soller M. 1997. Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe (Newsl. Agric. Genom.) 8:13-20.

Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467-470.

Thomas H, Smart CM. 1993. Crops that stay green. Ann. Appl. Biol. 123:193-219.
Vietor DM, Cralle HT, Miller FR. 1989. Partitioning of ^{14}C-photosynthate and biomass in relation to senescence characteristics of sorghum. Crop Sci. 29:1049-1053.
Walulu RS, Rosenow DT, Wester DB, Nguyen HT. 1994. Inheritance of the stay green trait in sorghum. Crop Sci. 34:970-972.
Wanous MK, Miller FR, Rosenow DT. 1991. Evaluation of visual rating scales for green leaf retention in sorghum. Crop Sci. 31:1691-1694.
Wanous MK. 1989. Inheritance of stay green, a post-flowering drought resistance response in *Sorghum bicolor* (L.) Moench. M.S. thesis, Texas A & M University, College Station, Texas, USA.
Woodfin CA, Rosenow DT, Clark LE. 1988. Association between the stay green trait and lodging resistance in sorghum. In: Agronomy abstracts, American Society of Agronomy, Madison, Wis., USA. p 102.

Notes

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Marker-assisted selection for improving drought tolerance in tropical maize

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Drought is thought to cause maize grain losses of >20 million tons annually in the tropics. Recurrent selection for improved drought tolerance has resulted in yield gains under midseason drought of 5% (about 75–100 kg ha^{-1}) yr^{-1}. Grain yield under drought is negatively correlated (r = –0.3 to –0.6) with the anthesis-silking interval (ASI), a secondary trait observed at flowering. DNA molecular markers (MMs) were used in a segregating population from the cross of lines P_1 (short ASI) × P_2 (long ASI), evaluated under several levels of drought, to identify five QTLs that were stable over stressed environments. A backcross marker-assisted selection (MAS) scheme to improve the drought tolerance of an elite but drought-susceptible inbred line, CML 247, has been successfully completed, using PCR-based markers as a preselection tool. A second MAS experiment used MMs to detect changes in the frequency of alleles at loci having known association with drought tolerance as a result of recurrent selection in an open-pollinated population. Plant selection based on the presence/absence of those alleles whose frequency changed could give increased drought tolerance in less time than recurrent selection, and preliminary results are encouraging. New breeding schemes involving optimal combinations of MAS and conventional selection to improve drought tolerance in maize hold considerable promise for the future.

Drought is a major cause of maize yield loss in the tropics, and for this reason breeding for drought tolerance has become a major focus of the International Maize and Wheat Improvement Center's (CIMMYT) Maize and Applied Biotechnology Programs. Loss to drought alone in the tropics is thought to exceed 20 million tons of grain per year (worth around US$2 billion in 1998), or around 17% of well-watered production (Edmeades et al 1992), but has been as high as 60% in severely affected regions such as southern Africa in 1991-92. Drought is a pervasive cause of yield instability in maize-based cropping systems in most years and environments. Probabilities of moisture deficit are highest at the start and end of the rains, but dry spells occur randomly throughout the season. Because the incidence and intensity of drought cannot be predicted, a drought-tolerant variety must also perform well under well-watered conditions and under drought: one cannot be at the cost of the other.

Uncertainty relating to the Occurrence of drought also affects the ease with which selection can be carried out for drought tolerance—too often rain interrupts this process. Selection for specific genomic segments associated with drought tolerance, us-

ing molecular markers (MMs), may therefore significantly improve the efficiency of selection. Molecular markers have been extensively mapped in the genome of temperate maize to obtain genetic linkage maps and to identify quantitative trait loci (QTL) (Lee 1995). But few studies on quantitative trait inheritance have been conducted under abiotic stresses, probably because of problems encountered under stress conditions, such as the reduced heritability of the trait due to increased environmental effects (Blum 1988) or the difficulty of obtaining the level of stress needed to provide optimal expression of the trait.

Most traits of economic importance are complex and quantitative, being regulated by families of genes. Yield is probably the most polygenic and complex of these. The improvement of polygenic traits through marker-assisted selection (MAS) is also complex, as demonstrated by the abundance of studies based on computer simulations (Lande and Thompson 1990, Hospital and Charcosset 1997) and the paucity of published data on this topic (Stuber 1995). Few papers, if any, have demonstrated a clear superiority of using MMs for quantitative trait improvement in a practical plant breeding program (Mohan et al 1997). The difficulty is due to the number of genes involved in their expression, each with a small effect on the plant phenotype, and their interactions (epistasis) (Ribaut and Hoisington 1998).

In this chapter, we describe the process of identifying appropriate secondary traits and CIMMYT's experience in selecting for drought tolerance in tropical maize. Two different MAS experiments for drought improvement have been conducted at CIMMYT and preliminary results are presented here. Finally, issues that directly affect the efficiency of MAS for quantitative traits are discussed in the context of a practical maize breeding program for drought tolerance.

Drought and the maize crop

A first step in obtaining yield is to have sufficient plant stand, but a limited attempt at improving seedling establishment has resulted in only modest increases in survival under water deficit (Bänziger et al 1997). Maize yield is reduced two to three times more when water deficits coincide with flowering compared with other growth stages (Shaw 1976, Grant et al 1989). Maize is thought to be more susceptible than other rainfed crops because of its physical separation of male and female flowers and its near-synchronous development of spikelets, borne usually on a single ear and single stem. One indicator of a high growth rate per female spikelet at flowering in maize is rapid silk extrusion. This is reflected in a short anthesis-silking interval (ASI), i.e., the difference in days between pollen shedding and silk emergence, because time to anthesis is little affected by drought. It appears that ASI is inversely proportional to partitioning of dry matter to the growing ear, female spikelet growth rate, and perhaps plant water potential, and is a trait that is easily observed by breeders. Plants with a large ASI under drought are often barren or have few grains per ear. Grain yield of maize grown under severe water stress at flowering and during grain filling is highly correlated with kernel *number* per plant ($r = 0.90$, $P<0.01$; Bolaños and Edmeades

1996). Grain number per plant in water-deficient maize appears to depend directly on the flux of current photosynthate to the ear during the 2 wk bracketing flowering (Schussler and Westgate 1995), implying that stem reserves of preflowering assimilate have little role in spikelet fertility. Sink strength of the ear may also be impaired because acid invertase activity in ovaries of water-stressed plants is much reduced (Zinselmeier et al 1995, Saini and Lalonde 1998). Pollen viability is a less important factor determining grain set, unless very hot conditions (>38 °C) are encountered at flowering (Schoper et al 1986). Besides, when silks whose appearance has been substantially delayed because of drought are pollinated with fresh pollen, pollination can be shown to have occurred, but kernel abortion is frequently observed (Westgate and Boyer 1986).

Because of the central importance of the flowering and grain-filling periods in determining grain number and grain weight, CIMMYT physiologists and breeders have concentrated their efforts on increasing tolerance for water deficits coinciding with these growth stages.

Identification and use of suitable secondary traits

Putative drought-tolerance traits have been reviewed extensively (Blum 1988, Ludlow and Muchow 1990, Boyer 1996), though few such traits have proven useful in plant breeding programs. Given the plethora of secondary traits mentioned in the literature, it is essential that drought researchers establish their relative value before incorporating them into their selection scheme. Ideally, a secondary trait should be genetically associated with grain yield under drought, highly heritable, cheap and fast to measure, nondestructive, stable over the measurement period, observed at or before flowering so that undesirable parents are not crossed, and not associated with yield loss under unstressed conditions (Edmeades et al 1998). The adaptive value of a secondary trait under field conditions can be assessed by the following methods: (1) analyses of correlation and heritability among progenies of a single population; (2) direct and correlated changes in traits due to selection; (3) divergent selection for that trait, or groups of traits, to create isopopulations; (4) simulation modeling; and (5) statistical procedures based on selection index theory.

CIMMYT physiologists have used the first three approaches extensively. Heritabilities and correlations among putative secondary traits from a large number of progeny trials clearly indicate that grain yield under severe drought stress at flowering is closely associated with variation in ears per plant, and that among the true secondary traits ASI has the closest association with grain yield (Table 1). Traits that are indicative of plant water status individually explained <5% of the variation in grain yield under field conditions. Changes that have occurred during selection for drought tolerance have also confirmed this relative ranking of traits (Bolaños and Edmeades 1993a,b, Bolaños et al 1993, Chapman and Edmeades 1999). Other studies in tropical maize involving correlations and divergent selection have shown that variation in osmotic concentration in leaf tissue, and hence osmotic adjustment, explains little or none of

Table 1. Broad-sense heritabilities observed under severe drought stress and genotypic correlations between grain yield and selected traits under severe drought stress for S_1 progenies drawn from several maize populations. Heritability of grain yield under severe stress was 0.43 ± 0.10 and yields averaged 14% of well-watered plots. For details, see Bolaños and Edmeades (1996).

	Trials (no.)	Heritability under stress	Genotypic correlation
Ears per plant	9	0.54 ± 0.08	0.90 ± 0.14
Grains per ear	8	0.39 ± 0.13	0.71 ± 0.22
Grains per plant	8	0.47 ± 0.08	0.86 ± 0.15
Weight per grain	9	0.43 ± 0.14	0.14 ± 0.17
Days from sowing to anthesis	9	0.72 ± 0.08	–0.58 ± 0.12
Anthesis-silking interval	8	0.51 ± 0.12	–0.60 ± 0.24
Leaf roiling score	9	0.52 ± 0.09	–0.03 ± 0.15
Leaf erectness score	1	0.74 ± 0.07[a]	–0.28 ± 0.19[b]
Leaf senescence score	9	0.54 ± 0.08	0.14 ± 0.15
Canopy temperature	4	0.25 ± 0.05	–0.20 ± 0.15
Tassel branch number	1	0.82 ± 0.04[a]	0.15[b]

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

the variation in grain yield under drought (Bolaños and Edmeades 1991). In temperate maize, however, the contribution of osmotic adjustment to yield stability may be more substantial (Lemcoff et a1 1998). Bänziger and Lafitte (1997), using approach 5 above, determined that the use of secondary traits plus yield during selection for tolerance of maize for low soil N was about 20% more efficient than selection for yield alone, and there is reasonable evidence that this is true under drought as well. We emphasize, however, that very few putative secondary traits have passed these tests for true usefulness in the selection process.

Conventional selection for drought tolerance in tropical maize

This has been described in detail elsewhere (Bolaños and Edmeades 1993a, b, Byrne et al 1995, Beck et al 1996, Edmeades et al 1999a). In summary, selection began in 1975 in the elite lowland tropical white dent population, Tuxpeño Sequía, at Tlaltizapán, Mexico, where timing and intensity of stress can be managed by irrigation. This population underwent eight cycles of recurrent full-sib selection[1] in the rain-free winter

[1]Full-sib selection refers to a selection scheme based on "families" where every plant (or seed) shares the same male and female parent. Typically, a full-sib family is made up of seeds from a single ear that has been fertilized by pollen from one other plant. Some of these seeds are grown in a plot that is evaluated under the desired environment, and the best fraction of these families is then sown out again from unused (remnant) seed and intercrossed, to make up a new version of the population. In the process of intercrossing, new full-sib families are created and they provide the materials for evaluation during the next cycle of selection. In an S_1 recurrent selection scheme, ears of families are formed by pollinating the plant with its own pollen rather than the pollen of another plant. Selected families are intercrossed, and a new set of self-pollinations is made from plants derived from these crosses and it comprises the next selection cycle.

season at this site. Each of 250 families was grown in single-row plots under three regimes of increasing drought intensity—namely, well-watered (WW), intermediate stress (IS; water withdrawn during late flowering and throughout grain filling), and severe stress (SS; no water applied from 3 wk before silking onward). Selection of the 50–80 best families for recombination was based on an index (or ideotype) aimed at high grain yield under SS and IS, delayed foliar senescence under SS and IS, and reduced canopy temperatures and ASI under IS and SS. Improvement began in a new and diverse group of five elite maize populations in 1985-86 using an S_1 recurrent selection scheme. Within each selection cycle, 500–600 S_1 families were prescreened under drought and heat in the Sonoran Desert at Obregon, northwest Mexico. The superior 200 families thus identified were grown in Tlaltizapan during winter from remnant seed under the three water regimes described, and the best 50 S_1 families recombined to complete the cycle. Selection was principally for increased shelled grain yield, ears per plant, and stay-green under stress, and for decreased ASI and tassel sterility. Less important traits were upright unrolled leaves, small tassels, and lodging resistance.

Evaluations of progress in these populations have generally been conducted in large plots over at least two seasons in environments that varied in available water (grouped as WW or drought) and, in recent years, soil N supply. Evaluations in 1987-

Table 2. Effects of selection for drought tolerance on changes per selection cycle in four maize populations when evaluated at 3-6 water-stressed (SS) sites, at 5-8 well-watered (WW) sites, or at 2-3 low-N sites in evaluations conducted at different stages of population improvement. The number in parentheses following the population name indicates the number of cycles of selection being evaluated. Unless specified, all locations were in Mexico. ASI = anthesis-silking interval. *,, ns: significant rate of change per selection cycle at P<0.01, P<0.05, or P>0.05 (Beck et al 1996, Bänziger et al 1999, Edmeades et al 1999a,b).**

Population	Grain yield (kg ha^{-1})			Anthesis WW	ASI SS	Ears plant^{-1} SS
	SS	WW	Low N	(d)	(d)	
Evaluation 1987-89						
Tuxpeño Seq. (8)	100**	125**		–0.40**		
Tuxpeño Seq. (8)[a]	52 ns	101**		–0.24**		
Evaluation 1992-94						
La Posta Seq. (3)	229**	53 ns	233	–0.52**	–1.18**	0.07**
Pool 26 Seq. (3)	288**	177*	207	–0.93**	–1.50**	0.08**
Tuxpeño Seq. (8)	80**	38**	86	–0.32**	–0.44**	0.02**
Pool 18 Seq. (3)	146**	126**	190		–2.13**	0.05**
Evaluation 1997-98						
La Posta Seq. (5)	154**	110 ns	134*	0.33**	–1.01**	0.03*
Pool 26 Seq. (3)	163**	142*	27 ns	–0.30 ns	–1.84**	0.04**
Tuxpeño Seq. (10)	104*	67 ns	21 ns	–0.35 ns	–0.58 ns	0.02 ns

[a] These estimates of gain are from 11 trials grown at international sites, most of which were outside Mexico (Byrne et al 1995).

89 were only of the Tuxpeño Sequía population. In 1992-94, these were extended to include three more populations undergoing S_1 recurrent selection. Gains in grain yield from selection (Table 2) were usually smaller for full-sib selection than for S_1 selection, partly because of differences in selection intensity between the two schemes. In general, gains averaged about 100 kg ha^{-1} yr^{-1} , or 5% yr^{-1} at a yield level of around 2 t ha^{-1} , where such a yield resulted from drought stress occurring during flowering and grain filling. Yield improvements under drought were paralleled by increases in ears per plant and declines in ASI. Populations generally became earlier to flower by 0.2 to 0.7 d $cycle^{-1}$ despite our objective of maintaining the time from planting to anthesis constant. Yield gains from selection under drought observed under low N in the 1992-94 evaluations were similar to those observed under drought (Table 2; Banziger et al 1999).

Evaluations of progress during 1997-98 were in three very low N environments (mean yield 1.8 t ha^{-1} , or <35% of potential), five environments with severe drought (1.3 t ha^{-1} , or about 20% of potential), and two unstressed environments (6.4 t ha^{-1}). Increases in grain yield were less than in earlier evaluations (Table 2), probably because the mean yield of the trials under stress was lower than before. Yield improvements under low N averaged 43% of those observed under drought, suggesting that under severe N stress the gains in grain yield observed under drought are not transferred as completely as those reported by Banziger et al (1999) under more moderate levels of N stress.

What changed with selection? Yield improvements were largely the result of reduced barrenness (i.e., increased ears per plant) under drought and an associated increase in harvest index because total biomass production was unaffected by selection (Bolaños and Edmeades 1993a, Edmeades et al 1999a). Tassel size was reduced in Tuxpeño Sequía and root biomass in the top 50 cm of soil declined by 35%. There was no change in any trait indicative of plant water status (e.g., predawn or noon water potential, osmotic adjustment, canopy temperature, water extraction profiles) (Bolaños et al 1993). In all populations, ASI became shorter under drought (Table 2). Eight cycles of selection in Tuxpeño Sequía led to significantly faster spikelet and ear growth, but also to a 21% reduction in final spikelet number (Edmeades et al 1993). Thus, fewer spikelets were formed, grew more rapidly, and were ultimately more successful in forming grain, especially under conditions of drought at flowering. The anthesis-silking interval is apparently a reflection of partitioning of dry matter to the ear under drought and under low N. Gains under water deficits were at no cost to yield in unstressed environments because partitioning to the ear increased in all environments. Partitioning, generally considered to be a constitutive trait, required a carefully managed drought stress to expose symptoms of its genetic variation (ASI; barrenness) in these diverse maize populations.

MAS under drought: Is there a need?

Drought screening in the field is often difficult because in the tropics screening is confined to the one dry season encountered each year, whereas in temperate areas

breeders must rely on screening in dry locations within the normal growing season. In both cases, unexpected rainfall can render the test useless for drought screening. Moreover, for the best expression of tolerance at flowering, a suitable level of water stress must occur at that growth stage, or the effectiveness of screening again declines. Second, selection efficiency diminishes because of a decrease in the heritability of grain yield caused by a decline in genetic variance relative to error variance (Bolaños and Edmeades 1996). Third, the collection of secondary trait data such as ASI is time-consuming, and the need to collect such data during the one dry season of the year may slow down a normal breeding program. Marker-assisted selection for drought tolerance, in the light of these practical constraints to conventional screening, appears to have a role as a complementary breeding tool.

Given the importance of the effect of water deficits on grain production in tropical maize, a project began at CIMMYT in 1991 to dissect out genetic components of maize drought tolerance. Key QTLs were identified for several traits based on their stability over water regimes and their capacity to account for phenotypic variance. Specific attention was given to ASI. Using this information, MAS experiments were developed for both lines and population improvement.

Genetic dissection of target traits observed under drought

From a cross between two tropical inbred lines, P_1 (short ASI, good level of drought tolerance) and P_2 (long ASI, drought-susceptible), an F_2 population of 260 individuals was genotyped. Based on the polymorphisms identified at different loci (150) with restriction fragment length polymorphism (RFLP) markers, a linkage map was constructed using MAPMAKER software (Lander et a1 1987). In 1992 and 1994, F_3 families derived from the F_2 plants were evaluated for ASI, several morphological and physiological traits, yield, and yield components in the field under several water regimes.

Quantitative trait loci involved in the expression of the target traits were identified using two complementary statistical approaches: simple interval mapping (SIM, MAPMAKER/QTL; Lander and Botstein 1989) and composite interval mapping (CIM; Zeng 1994). Six QTLs (LOD score >2.5) for ASI on chromosomes 1, 2, 5, 6, 8, and 10 were identified with SIM (Fig. 1; Ribaut et a1 1996). These QTLs together accounted for 47% of the phenotypic variability and were responsible for a change in AS1 of about 11 d. Using CIM, an additional QTL was identified on the short arm of chromosome 1. Transgressive segregation was observed for AS1 when alleles associated with a shortened ASI were also identified in the susceptible parental line, P_2. The five QTL segments contributed by P_1 were responsible for a 7.5-d reduction in ASI and were stable over years and stress levels. In contrast, QTLs involved in the expression of yield and its components, such as weight per grain, ears per plant, and grain number, were not consistently identified across stress levels and explained only a small percentage of the phenotypic variance for these traits. From these first QTL analyses, we concluded that (1) the identification of MMs of interest at QTL peaks associated with ASI and yield components has to be achieved using field data ob-

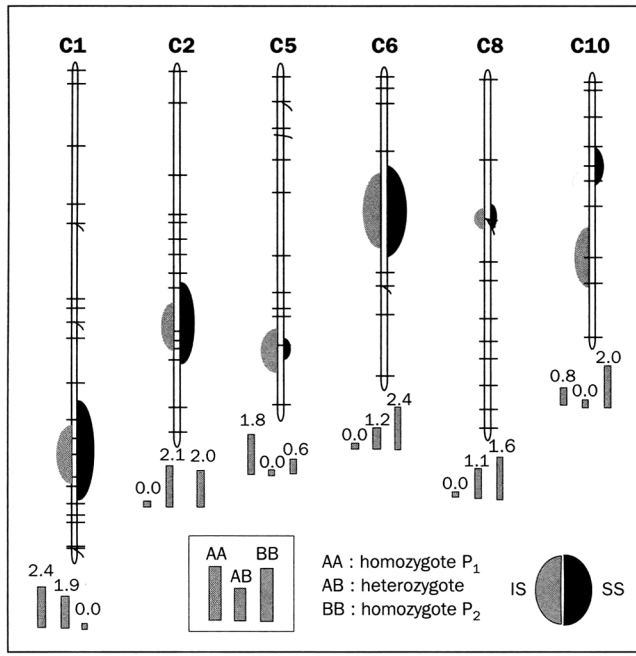

Fig. 1. Location on maize chromosomes of QTLs for anthesis-silking interval (ASI) detected using MAPMAKER/QTL with simple interval mapping. Genomic regions responsible for the expression of ASI under intermediate stress (IS) (left) and severe stress (SS) (right) are represented by ellipses for LOD scores higher than 2.5. The width of the ellipses is proportional to the percentage of phenotypic variance explained by that QTL. The bars at the bottom of the chromosomes indicate the allelic contribution (days) of the two parental lines at the QTL position (Ribaut et al 1996).

tained under drought, (2) MAS using only QTLs involved in the expression of yield components under drought would not be efficient, and (3) MAS using only QTLs involved in the expression of secondary traits of interest, such as ASI, would not be the most efficient either because at one important genomic position the allele contributing to a reduction in ASI also contributed to a grain yield decrease (Fig. 2). The most successful MAS strategy should therefore be to use the "best QTLs" from different traits in the form of an index. These QTLs should be stable across environments, account for a large percentage of the phenotypic variance, and, if not involved directly in the expression of yield, be involved in the expression of traits significantly correlated with yield.

MAS for improving drought tolerance in elite lines

Based on the QTL and mapping information described above, a backcross marker-assisted selection (BC-MAS) project began in 1994. The line P_1 was used as the

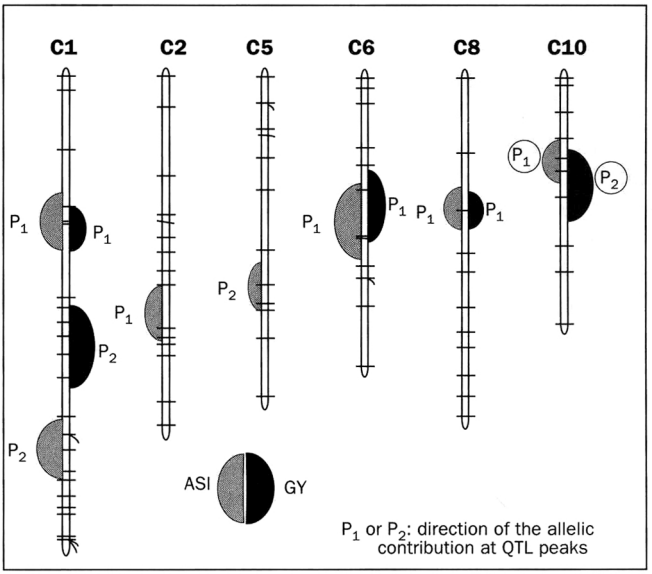

Fig. 2. Location on maize chromosomes ot QILs tor anthesis-silking interval (ASI) ana grain yield (GY) detected using composite interval mapping based on the combined data sets from both intermediate and severe stress field conditions. The parental line that was the source of alleles contributing to a short ASI or a greater yield is indicated for each QTL (Ribaut et al 1997a). The circles on chromosome 10 refer to an "opposite" allelic contribution, a short ASI inducing a reduction in GY.

drought-tolerant donor and CML247 was used as the recurrent parent. CML247 is an elite tropical inbred line developed by CIMMYT, with outstanding combining ability and good yield per se under well-watered conditions. It is susceptible to drought, in part because its ASI is large under drought. Genetic data from a segregating F_2 population derived from the $P_1 \times$ CML247 cross were combined with F_3 evaluations in the field under different water regimes to identify QTLs for traits of interest. The QTLs for ASI identified in this cross were quite consistent with those in the original $P_1 \times P_2$ cross. Of the five QTLs originally identified from P_1 that conferred a short ASI, only the QTL on chromosome 6 was not detected in the second cross. The QTL on the short arm of chromosome 1 was shifted by 40 cM in the new cross, and the three other QTLs on chromosomes 2, 8, and 10 were in similar positions in both. A new QTL for ASI was detected on the short arm of chromosome 3. These results demonstrate the need to make a new genetic map when the recurrent line is changed in BC-MAS schemes. A single good-quality trial under drought conditions, however, might be enough to identify QTLs of interest, providing QTL identification has been previously carried out in another cross involving the donor line.

Five genomic regions involved in the expression of a short ASI were selected to be transferred from P_1 into CML247. The screening of large populations (about 2,000 plants) at each selection cycle during backcrossing has been possible because of the development of reliable PCR-based markers, used here as preselection tools (Ribaut et al 1997b). After two BCs and two self-pollinations, the best genotype was fixed from the donor line for the five target regions (12% of the genome), as well as for 7% of the genome lying outside the QTL regions (Fig. 3). The 70 best BC_2F_3 (i.e., S_2 lines) were identified and crossed with two CIMMYT tester inbreds, CML254 and CML274. These hybrids, as well as the BC_2F_4 families (S_3 lines) derived from the selected BC_2F_3 plants, were evaluated in 1997-98 under several water regimes. Preliminary results show that the mean of the 70 selected genotypes performed better than the control crossed with CML254 and CML274, and the best genotype among the 70 selected (BC_2F_3 × testers) performed two (× CML274) to four times (× CML254) better than the control (Table 3). No yield reduction was observed under well-watered conditions. Nevertheless, this trial must be repeated in 1998-99 before final conclusions can be drawn from lines and hybrids because of the unusually severe El Niño-induced drought stress encountered in 1997-98.

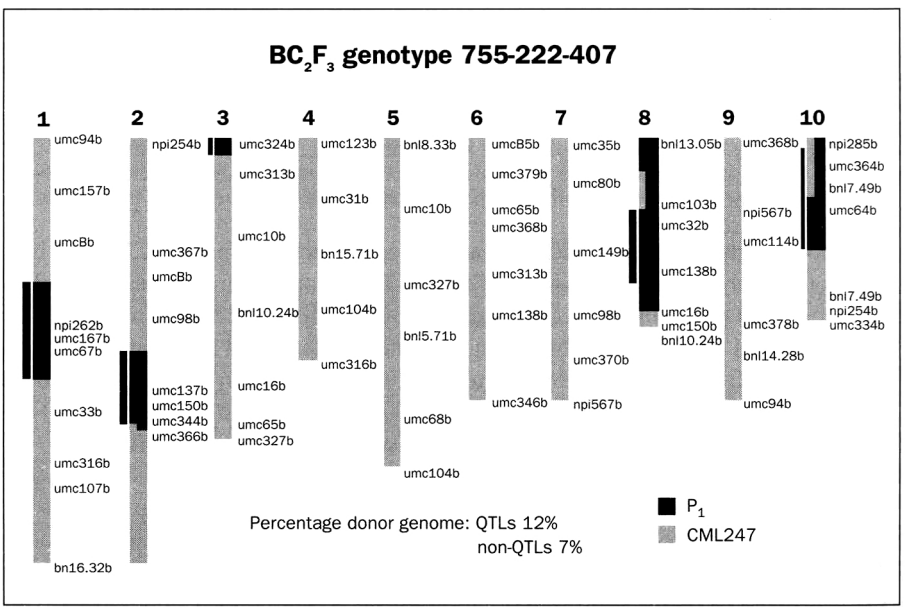

Fig. 3. Genomic composition of the best genotype obtained after four cycles of marker-assisted selection (two BCs plus two self-pollinations). Seventy RFLP markers, provided by the University of Missouri-Columbia (UMC), Brookhaven National Laboratory (BNL), and Native Plants Incorporated (NPI), were used to conduct the genetic dissection of the different genotypes. The five target genomic regions are represented by black bars to the left of the chromosomes.

Table 3. Grain yields per plot (kg ha^{-1} ± SD) under different water regimes of MAS-improved versions of CML247 versus the original CML247 when crossed with two CIMMYT tester inbred lines (CML254 and CML274). The mean of the control was calculated based on 10 identical entries, while the mean of the improved genotypes represents 70 different entries selected after MAS. "Best" is the yield of the highest-yielding individual cross out of the 70.

Genotype	Drought conditions		Well-watered conditions	
	CML254	CML274	CML254	CML274
CML247 (control)				
Mean	798 ± 478	1,995 ± 898	6,185 ± 804	7,648 ± 688
CML247 (MAS)				
Mean	1,995 ± 957	2,195 ± 878	7,182 ± 862	7,648 ± 918
Best	4,522	4,057	8,715	9,909

Table 4. Allele frequency changes at two loci segregating in C_0, C_4, and C_8 of Tuxpeño Sequía, a lowland tropical maize population subjected to full-sib recurrent selection for improved drought tolerance.

Chromosome 2	*umc371*	C_0	C_4	C_8
	Allele 1	0.18 ± 0.03	0.18 ± 0.03	0.06 ± 0.02
	Allele 2	0.11 ± 0.02	0.09 ± 0.02	0.01 ± 0.01
	Allele 3	0.04 ± 0.01	0.08 ± 0.02	0.10 ± 0.02
	Allele 4	0.32 ± 0.03	0.31 ± 0.03	0.56 ± 0.03
	Allele 5	0.36 ± 0.03	0.32 ± 0.03	0.25 ± 0.03
Chromosome 10	*bnl 7.49*			
	Allele 1	0.01 ± 0.01	0.05 ± 0.01	0.00
	Allele 2	0.37 ± 0.03	0.35 ± 0.03	0.22 ± 0.03
	Allele 3	0.22 ± 0.03	0.41 ± 0.03	0.44 ± 0.03
	Allele 4	0.17 ± 0.03	0.05 ± 0.01	0.14 ± 0.03
	Allele 5	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.02
	Allele 6	0.22 ± 0.03	0.12 ± 0.02	0.10 ± 0.02

MAS in open-pollinated populations

The second MAS project involves the improvement of drought tolerance in the open-pollinated population Tuxpeño Sequia (Bolaños and Edmeades 1993a). Varieties derived from such populations remain one of CIMMYT's major products from its maize breeding program. Changes in frequencies of drought-adaptive alleles over cycles of recurrent selection at loci of known map position were quantified using MMs and 116 individuals randomly chosen from each of C_0, C_4, and C_8 of Tuxpeño Sequía (Table 4). Loci scattered over the maize genome were assayed using RFLPs. Special attention was given to genomic regions responsible for the expression of ASI and grain yield identified in the $P_1 \times P_2$ study because the two inbreds are derived partially from Tuxpeño germplasm. There-is strong evidence that the alleles associated with short ASI in P_1 and P_2 are also present in this population. Allelic frequencies that increased, decreased, or remained stable were recorded. Genomic regions were classified into

those presenting marked, moderate, or no changes in allelic frequency due to recurrent selection. Some major frequency changes were detected at loci previously identified as regions responsible for expression of ASI. These alleles could provide a rapid diagnostic tool for screening lines or individuals with potential drought tolerance in Tuxpeño germplasm, and selection based on the presence of these alleles should provide a significant improvement in the drought tolerance of this population.

To test this hypothesis, 21 DNA markers were used to screen 400 plants from C_0 and C_4. Based on their allelic composition, the 50 "best" and "worst" genotypes from each of the two cycles were selected and were evaluated in 1997-98 under several water regimes. Preliminary analysis of those results in this extremely dry season showed a significant difference in yield performance under drought conditions between the two groups of genotypes selected using markers. If those results are confirmed, they will show that MAS is of real value for improving the performance of open-pollinated varieties under drought.

Limitations to MAS and its prospects for polygenic trait improvement

The difficulty of manipulating quantitative traits is related to the number of genes involved with relatively small individual effects and their epistatic interactions. This implies that several regions (or QTLs) must be manipulated at the same time to have a significant impact and that the effect of individual regions cannot be identified easily. For this reason, accurate and repeatable field tests are required to precisely identify and quantify QTL effects and their stability across environments (Beavis and Keim 1996). Progress in field designs, such as the use of incomplete blocks, row and column designs, and spatial analyses, has increased the precision of field trials that are so essential to high-quality phenotyping. The development of new statistical approaches, such as composite interval mapping, has also improved the accuracy by which QTLs can be identified. When these are combined with multienvironment field data in a joint analysis, QTL by environment interactions can now be quantified (Jiang and Zeng 1995). Limitations remain, however, and are principally related to the limited number of samples that can be analyzed, the small number of lines that can be improved within a given time, and the belief that QTL identification is required whenever a new germplasm source is used.

With the development of new polymerase chain reaction (PCR)-based markers, large sample sizes can be handled more efficiently, as demonstrated by our first MAS experiment. The impact on the efficiency of the selection process by varying the initial screened population size (N) during the transfer of one to five genomic regions was evaluated through simulation at CIMMYT. Here, the maize genome was considered as the model, and the BC-MAS approach was chosen to test the efficiency of selection using MMs. An increase in the size of the screened population always reduces the total number of selection cycles required. The desirability of screening a large versus a small population increases in proportion to the number of genomic

regions and their nature (single gene vs QTL). The effective population size (n), defined here as the number of genotypes having the donor allele at the target loci, was calculated as screened population size and the number of target regions for transfer was varied from one to five (Table 5). Efficiency was defined as the product of resources required and time for completion of MAS. Results of simulation show that the most efficient selection scheme was obtained from screening an initial population size that resulted, after selection at target loci, in an effective population size ranging from 50 to 100 genotypes.

Although an increase in the screened population size and the use of PCR-based markers represent significant steps forward in MAS, clear limitations in terms of outputs (versus inputs) that fit breeders' requirements when several QTLs have to be manipulated are still present. As shown in Table 5, the most efficient strategy for a BC-MAS scheme involving several QTLs, with an effective population size of 50–100 genotypes, requires the screening of a large population of thousands of plants. Moreover, the number of crosses that can be manipulated at the same time and the QTL identification needed for each cross remain barriers to the widespread use of MAS and have raised questions related to its cost-effectiveness.

To overcome these constraints, new MAS strategies must be developed that focus on the use of MMs at one or a few key steps in the selection process. As an example, a new approach that interactively combines the use of MMs and conventional breeding has been developed recently at CIMMYT (Ribaut and Betran 1999). The novel aspect of this strategy involves selecting plants at early generations with a fixed, favorable genetic background at specific loci using a single large-scale MAS (SLS-MAS) step, while maintaining allelic segregation in the rest of the genome as much as possible. The first step is to choose elite lines with a high level of allelic complementarity and excellent expression of traits of interest, followed by the identi-

Table 5. Expected number (n) of genotypes heterozygous at target genomic regions as affected by size of screened population (N) and number of genomic regions to be transferred (1, 3, or 5). Calculations were conducted for the transfer of genes (a single DNA marker) and QTLs, for which the two flanking DNA markers of the target region are expected to be heterozygotes. Two different levels of recombination among QTLs (15% and 30%) are also considered.

Screened population size	n for gene transfer (no recombination)			n for QTL transfer (15% recombination)			n for QTL transfer (30% recombination)		
N	1	3	5	1	3	5	1	3	5
50	25	6.3	1.6	21.3	3.8	0	17.5	2.1	0
100	50	12.5	3.1	42.6	7.7	1.4	35.0	4.3	0
200	100	25.0	6.3	85.1	15.3	2.8	69.9	8.6	1.1
400	200	50.0	12.5	170.2	30.7	5.5	139.9	17.2	2.1
800	400	100	25.0	340.4	61.4	11.1	279.7	34.3	4.2
1,600	800	200	50.0	680.9	122.8	22.2	559.4	68.7	8.4
3,200	1,600	400	100	1,362	245.6	44.4	1,119	137.3	16.8
6,400	3,200	800	200	2,723	491.2	88.8	2,238	274.7	33.6

fication of desirable genomic regions for each of these lines so that favorable alleles can be captured from both parents. The lines are then intercrossed to develop segregating populations from which individuals homozygous for favorable alleles at target loci are selected in an SLS-MAS step. The goal of the scheme is to conduct MAS only once at an early stage of recombination, and it requires the selection of a minimum number of plants to maintain sufficient allelic variability at nonselected loci. This combination of MAS and conventional breeding steps provides many options and has been adopted to improve drought tolerance in germplasm suited to eastern and southern Africa. The project is ongoing, and the evaluation of its success will be possible through field evaluation of the improved germplasm in 2001.

Conclusions

Conventional selection for tolerance of drought imposed during reproductive development in maize has resulted in increases in grain yield of around 100 kg $ha^{-1} yr^{-1}$, with concomitant decreases in barrenness and ASI. Success in selection has depended on the capability to create a reliable level of water deficit near flowering so that genotypes with a short ASI and capacity to form an ear under stress can be identified in each selection cycle. Rain and a lack of suitable irrigation facilities can reduce this capability, and MAS could therefore increase the overall efficiency of conventional selection for drought tolerance.

Marker-assisted selection for drought tolerance in an elite inbred line and in an open-pollinated variety has been successfully conducted at CIMMYT, although some results await confirmation during the coming dry cycle (1998-99). The use of PCR-based markers has considerably increased the efficiency of line conversion because it allows thousands of plants to be screened at each BC selection step. This in turn has significantly reduced the number of selection cycles required. Large-scale selection in MAS, although always more rapid, is not always justified when cost versus time is taken into account. Before any experiment, the most efficient strategy has to be defined considering principally the effective population size expected at each selection cycle.

With the development of new technologies, such as reverse genetics and DNA chips, gene characterization of the complete maize genome is becoming a real possibility. Information from these technologies will allow us to manipulate several identified genes simultaneously and germplasm improvement for drought tolerance should be greatly facilitated. This, combined with the development and use of breeding schemes that optimize conventional approaches and MAS at key selection steps in the best germplasm available, will greatly increase future prospects for improved drought tolerance in maize on a relatively large scale.

References

Banziger M, Lafitte HR. 1997. Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci. 37:1110-1117.

Banziger M, Edmeades GO, Quarrie S. 1997. Drought stress at seedling stage—are there genetic solutions? In: Edmeades GO, Banziger M, Mickelson HR, Peña-Valdivia CB, editors. Developing drought and low-N tolerant maize. El Batán (Mexico): Centro Internacional de Mejoramiento de Maiz y Trigo. p 348-354.

Banziger M, Edmeades GO, Lafitte HR. 1999. Selection for drought tolerance increases maize yields over a range of N levels. Crop Sci. 39. (In press.)

Beavis WD, Keim P. 1996. Identification of quantitative trait loci that are affected by environment. In: Kang MS, Gauch HG, editors. Genotype-by-environment interaction. Boca Raton, Fla. (USA): CRC Press. p 123-149.

Beck D, Betrán J, Bänziger M, Edmeades GO, Ribaut JM, Willcox M, Vasal SK, Ortega A. 1996. Progress in developing drought and low soil nitrogen tolerance in maize. In: Wilkinson D, editor. Proceedings 51st Annual Corn and Sorghum Research Conference, Chicago, 10-11 Dec. 1996. Washington, D.C. (USA): ASTA. p 85-111.

Blum A. 1988. Plant breeding for stress environments. Boca Raton, Fla. (USA): CRC Press.

Bolaños J, Edmeades GO. 1991. Value of selection for osmotic potential in tropical maize. Agron. J. 83:948-956.

Bolaños J, Edmeades GO. 1993a. Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. Field Crops Res. 31:233-252.

Bolaños J, Edmeades GO. 1993b. Eight cycles of selection for drought tolerance in lowland tropical maize. II. Responses in reproductive behavior. Field Crops Res. 31:253-268.

Bolaños J, Edmeades GO. 1996. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Res. 48:65-80.

Bolaños J, Edmeades GO, Martinez L. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. Field Crops Res. 31:269-286.

Boyer JS. 1996. Advances in drought tolerance in plants. Adv. Agron. 56:187-218.

Byrne PF, Bolaños J, Edmeades GO, Eaton DL. 1995. Gains from selection under drought versus multilocation testing in related tropical maize populations. Crop Sci. 35:63-69.

Chapman SC, Edmeades GO. 1999. Selection improves tolerance to mid/late season drought in tropical maize populations. II. Direct and correlated responses among secondary traits. Crop Sci. (Accepted.)

Edmeades GO, Bolaños J, Lafitte HR. 1992. Progress in breeding for drought tolerance in maize. In: Wilkinson D, editor. Proceedings 47th Annual Corn and Sorghum Research Conference 1992, Chicago, 8-10 Dec. 1992. Washington, D.C. (USA): ASTA. p 93-111.

Edmeades GO, Bolaños J, Hernandez M, Bello S. 1993. Causes for silk delay in a lowland tropical maize population. Crop Sci. 33:1029-1035.

Edmeades GO, Bolaños J, Bänziger M, Ribaut JM, White JW, Reynolds MP, Lafitte HR. 1998. Improving crop yields under water deficits in the tropics. In: Chopra VL, Singh RP, Varma A, editors. Crop productivity and sustainability: shaping the future. Proceedings 2nd International Crop Science Congress, New Delhi, 10-17 Nov. 1996. New Delhi: Oxford and IBH. p 437-451.

Edmeades GO, Bolaños J, Chapman SC, Lafitte HR, Bänziger M. 1999a. Selection improves drought tolerance in tropical maize populations. I. Gains in biomass, grain yield and harvest index. Crop Sci. (Accepted.)

Edmeades GO, Bolaños J, Elings A, Ribaut JM, Bänziger M, Westgate ME. 1999b. The role and regulation of the anthesis-silking interval in maize. Paper presented at ASA-CSSA-SSSA Conference, 17-22 Oct. 1998. (In press.)

Grant RF, Jackson BS, Kiniry JR, Arkin GF. 1989. Water deficit timing effects on yield components in maize. Agron. J. 81:61-65.

Hospital F, Charcosset A. 1997. Marker-assisted introgression of quantitative trait loci. Genetics 147:1469-1485.

Jiang C, Zeng Z-B. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111-1127.

Lande R, Thompson R. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743-756.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181,

Lander ES, Botstein D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLPlinkage maps. Genetics 121:185-199.

Lee M. 1995. DNA markers and plant breeding programs. Adv. Agron. 55:265-344.

Lemcoff JH, Chimenti CA, Davezac TAE. 1998. Osmotic adjustment in maize (*Zea mays* L.): changes with ontogeny and its relationship with phenotypic stability. J. Agron. Crop Sci. 180:241-247.

Ludlow MM, Muchow RC. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Mohan M, Nair S, Bhagwat A. Krishna TG, Yano M, Bhatia CR, Sasaki T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed. 3:87-103.

Ribaut JM, Hoisington DA, Deutsch JA. Jiang C, González-de-León D. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor. Appl. Genet. 92:905-914.

Ribaut JM, Jiang C, González-de-Leon D, Edmeades GO, Hoisington DA. 1997a. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor. Appl. Genet. 94:887-896.

Ribaut JM, Hu X, Hoisington DA, González-de-Leon D. 1997b. Use of STSs and SSRs as rapid and reliable preselection tools in a marker-assisted selection-backcross scheme. Plant Mol. Biol. Rep. 15:154-162.

Ribaut JM, Hoisington DA. 1998. Marker-assisted selection: new tools and strategies. Trends Plant Sci. 3:236-239.

Ribaut JM, Betrán FJ. 1999. Single large-scale marker-assisted selection (SLS-MAS): a new approach. Mol. Breed. (Accepted.)

Saini HS, Lalonde S. 1998. Injuries to reproductive development under water stress, and their consequences for crop productivity. J. Crop Prod. 1:223-248.

Schoper JB, Lambert RJ, Vasilas BL. 1986. Maize pollen viability and ear receptivity under water and high temperature stress. Crop Sci. 26:1029-1033.

Schussler JR, Westgate ME. 1995. Assimilate flux determines kernel set at low water potential in maize. Crop Sci. 35:1074-1080.

Shaw RH. 1976. Water use and requirements of maize: a review. In: Agrometeorology of the maize (corn) crop. World Meteorol. Org. No. 481. p 119-134.
Stuber CW. 1995. Mapping and manipulating quantitative traits in maize. Trends Genet. 11:477-481.
Westgate ME, Boyer JS. 1986. Reproduction at low silk and pollen water potentials in maize. Crop Sci. 26:951-956.
Zeng ZB. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.
Zinselmeier C, Schussler JR, Westgate ME, Jones RJ. 1995. Low water potential disrupts carbohydrate metabolism in maize ovaries. Plant Physiol. 107:385-391.

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QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet

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Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal grain and fodder crop in the driest areas of the semiarid tropics. Drought at the crop's reproductive stage is one of the most important environmental factors limiting pearl millet productivity; improved adaptation to such drought stress is an important breeding objective. We used molecular marker tools to better understand the inheritance and expression of grain yield and grain yield-component traits in three terminal drought-stress environments differing in the intensity and duration of late reproductive stage stress. Test-crosses of mapping population progenies derived from a cross between two inbred lines differing in their response to drought were evaluated under paired stress and nonstress environments to understand the biological basis of drought tolerance, and to identify quantitative trait loci (QTLs) associated with drought tolerance. Detailed analysis led to the identification of flanking markers for QTLs associated with increased harvest index, improved grain filling, and maintenance of grain yield in terminal drought-stress environments. We discuss the significance of the putative QTLs identified, the steps to be followed in their marker-assisted backcross transfer to the elite parent of this mapping population, and the potential for their further use in the improvement of pearl millet productivity in water-limited environments.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal grain and fodder crop in the driest areas of South Asia and sub-Saharan Africa. It is grown almost entirely as a rainfed crop in areas where inter- and intraseasonal variation in rainfall is the single most important environmental factor that limits its productivity (Mahalakshmi et al 1987, van Oosterom et al 1996). Although both intermittent and terminal drought stress are common in pearl millet-growing areas, terminal drought stress is more damaging to the yield stability and productivity of the crop (Bidinger et al 1987a,b). In general, the early stages of plant development of pearl millet are less sensitive to drought because the crop has the ability to recover quickly and fully when water becomes available (Bidinger et al 1987a, Mahalakshmi et al 1987). Improving the adaptation of pearl millet to terminal drought-stress environments is therefore a major objective in breeding programs aimed at improving the crop's productivity and yield stability.

Plant breeders and crop physiologists (Blum 1988, Ludlow and Muchow 1990, Fussel et al 1991) have proposed that better-adapted and higher-yielding genotypes

of crop plants can be bred more efficiently and effectively if attributes that help maintain yield under water-limited conditions could be identified and used as selection criteria. But progress in breeding genotypes with improved drought tolerance has been slow, mainly because natural drought-stress environments are highly variable in the timing, duration, and severity of this stress. In addition, the whole-plant response to drought is complex and is conditioned by a number of component responses that both interact and differ in their individual responses to the intensity and duration of water deficits.

Performance of pearl millet and other cereals under terminal drought-stress conditions is influenced not only by genetic differences in drought tolerance but also by differences in time to flowering (drought escape) and differences in yield potential (Fischer and Maurer 1978, Bidinger et al 1987a). In terminal drought-stress environments, the combined effects of phenology and yield potential can account for as much as 50% of the variation in pearl millet grain yield (Bidinger et al 1987a). It has been proposed that this crop's grain yield in water-limited environments can be improved if specific traits and responses for drought tolerance can be identified and incorporated into otherwise high-yielding genotypes of appropriate crop duration (Bidinger et al 1987b, Fussel et al 1991).

Susceptibility during postflowering drought stress in pearl millet is often characterized by reduced grain size, a reduced number of effective panicles, and a reduced number of grains per panicle, all of which lead to reduced grain production per unit area. Though genetic variation in expression of these traits in drought-stress environments exists in the available pearl millet germplasm (Bidinger et al 1987b), the inheritance and biological basis of drought tolerance are not well understood.

In recent years, the development of molecular marker technologies and the use of these markers in quantitative trait locus (QTL) analysis have opened up new avenues for the study of complex traits and responses such as drought tolerance (Quarrie 1996, Prioul et al 1997, Ribaut et al 1997, Tuinstra et al 1997). Genome analysis based on DNA polymorphism can reveal the genetic determinants of complex phenotypes and provide tools for manipulating these to maximize selection response (Paterson et al 1991, Tanksley 1993). By QTL analysis, individual genetic components associated with a complex trait can be identified; these can then be manipulated independent of the target environment without any confounding effects of the other segregating loci.

The objectives of this study were to

1. Characterize a set of mapping population test-crosses for the expression of postflowering drought tolerance in a range of terminal drought-stress environments,
2. Identify plant traits that contribute to increased grain yield in these stress environments,
3. Identify QTLs associated with drought tolerance of grain yield and its component traits in these stress environments,

4. Better understand the biological basis of morphological and physiological traits in determining drought tolerance in pearl millet, and
5. Design a marker-assisted breeding program to improve pearl millet grain yield in terminal drought-stress environments.

Materials and methods

Plant material and genetic map construction

Two early maturing inbred lines, H 77/833-2 and PRLT 2/89-33, whose hybrids differ in performance under terminal drought stress, were used as parents to produce a segregating population for restriction fragment length polymorphism (RFLP) map construction and for QTL analysis. H 77/833-2, referred to as the H 77 parent, is the male parent of a number of early, high-tillering, thermo-tolerant pearl millet hybrids, including HHB 67 (843A × H 77/833-2), that are widely cultivated in northwestern India. PRLT 2/89-33, referred to as the PRLT parent, is an inbred derived from ICRISAT's Bold-Seeded Early Composite, an elite breeding population based predominantly on Iniari landrace germplasm from West Africa, which has a superior grain-filling ability under terminal drought stress. The Iniari landrace materials differ from Rajasthani-type materials such as H 77/833-2 in many plant characters: they have fewer nodal and basal tillers, larger seeds, thicker panicles, and broader leaves. An F1 hybrid was produced between the two inbred lines and a single F_1 plant was selfed to produce the seed from which 150 F_2 plants were grown. Leaf tissues were collected from each of 150 individual F_2 plants for DNA isolation and RFLP genotyping necessary for genetic map construction.

Pearl millet DNA isolation, restriction enzyme digestion, gel electrophoresis, Southern transfer, probe labeling, and filter hybridization were essentially as described in Liu et a1 (1994). Linkage analysis was carried out using Mapmaker (Lander and Botstein 1989). The RFLP map of this cross currently comprises 50 markers reasonably well distributed over the seven linkage groups of pearl millet, with an average spacing of approximately 7 cM. The genetic map length of this cross (approximately 350 cM) obtained using these 50 markers was comparable to the consensus map of pearl millet (Liu et al 1994).

For phenotyping grain yield and its component traits in terminal drought-stress environments, a subset of 104 F_3 progenies derived from the mapped individual F_2 plants was test-crossed to a common female parent (843A) to produce 104 test-cross hybrids. Test-cross hybrids were produced on the parental inbred lines as well. Traits were phenotyped using test-crosses rather than the mapped progenies for several reasons:

1. To restore plant vigor in inbred progenies that are otherwise too weak for screening under stress conditions;
2. To employ a more realistic representation of pearl millet cultivars that farmers use, which are either a homogeneous set of highly heterozygous single-cross hybrid plants or a heterogeneous mixture of heterozygous open-pollinated plants;

3. To follow the normal selection procedure of superior hybrid parent lines, based on their general and specific combining ability assessed in test-crosses on (an) unrelated inbred tester(s); and, finally,
4. To use the dominantly inherited early flowering of the tester to reduce variation in flowering time within the test-cross population and to focus the mapping on specific traits for drought tolerance rather than traits or responses associated with drought escape.

Crop management and data recorded

The performance of test-crosses under terminal drought stress was characterized using managed terminal-stress environments created with controlled irrigation. The test-crossed progenies of 104 F_4 lines (experiment 1) and 92 F_4 lines (experiments 2 and 3) and two parents were evaluated using a randomized complete block design in three field experiments at the ICRISAT-Patancheru, India, research farm during the dry seasons (January to May) of 1997 (experiment 1) and 1998 (experiments 2 and 3). The dry season at ICRISAT-Patancheru is usually rain-free, with a high mean air temperature and large water vapor pressure deficit, which provide an excellent opportunity to expose plants to controlled but severe drought stress by adjusting the timing of irrigation (Bidinger et al 1987a). Drought stress in experiment 1 began at the early grain-filling stage by withholding irrigation at approximately the flowering stage. Irrigation in experiments 2 and 3 was withheld a week before flowering time to initiate stress at the flowering stage. An unexpected rain and hail storm relieved drought stress during grain filling in experiment 1, resulting in a small reduction in grain yields. Experiments 2 and 3, however, were free from such interruption by rains, and yields were reduced by much greater amounts. In addition to the terminal stress environment, all the experiments had an irrigated control environment that received normal irrigation throughout the growing season. The irrigated control treatment applied 50 mm of irrigation water at weekly intervals so as to saturate the soil near its field capacity using either drip irrigation (experiments 2 and 3) or surface flooding (experiment 1).

All three experiments were machine-planted on ridges spaced 0.6 m apart, and thinned to a final plant population of approximately 10 plants m^{-2} at 2 wk after planting. Test-crosses in all three experiments were evaluated using a randomized complete block design with three replications. In experiment 1, they were evaluated in a plot size of 2 rows 4 m long; 3 m of these two rows were used to record pre- and postflowering data. In experiments 2 and 3, they were evaluated in a single-row plot 4 m long (because of space constraints under the shelter) and pre- and postflowering data were recorded for a 3-m row. Times to flowering were recorded as the days to stigma emergence in 50% of the main shoot panicles in a plot. At harvest, data were recorded on the number of plants and number of panicles per plot, dry stover yield, grain yield, and 100-seed weight. Data on grain yield, stover yield, total plant biomass yield at maturity, and panicle numbers were expressed on a per-m^2 basis. Panicle grain number [(100 × grain yield)/(panicle number × 100-seed weight)] was derived

from these primary data. Harvest index (grain yield/biomass yield) was calculated on a plot basis. In addition to agronomic traits, the mapping population test-crosses were also evaluated for their expression of physiological responses to drought stress such as leaf rolling, leaf senescence, and osmotic adjustment (data not reported).

Data analysis

Analysis of variance was performed to determine the significance of genetic variation between test-crosses for all the traits measured in the irrigated control and in stress environments. Drought tolerance (i.e., expression maintenance under stress conditions) of grain yield and grain yield-component traits was calculated as the ratio of trait expression in each stress environment to trait expression in the paired irrigated control environment.

QTL mapping was performed on the entry mean performance values for individual traits from control and stress environments and on their calculated drought-tolerance values using the method of interval mapping with the QTL mapping software package Mapmaker-QTL 1.1 (Lander and Botstein 1989). Genetic effects attributed to individual putative QTLs and the percent phenotypic variation explained by each QTL were also estimated by the software. An additive genetic model was used for the analysis of test-cross progenies. This is because test-cross progenies derived from a heterozygous F_2 plant are a sample of the two parental alleles in combination with the tester allele, and the average value of the heterozygote is the average of the homozygotes (Cowen 1988, Beavis et al 1994).

Results and discussion

Expression of grain yield and grain yield-component traits

As a result of terminal drought stress, the reduction in grain yields, compared with those of the irrigated controls, in three terminal-stress environments ranged from 28% in experiment 1 to 61% in experiment 3 (Fig. 1). The variation in drought-stress intensities in these three terminal-stress environments was due to the later time of initiation of the stress and the effects of the rainstorm that occurred during grain filling (experiment 1) and to different sowing dates and soil moisture holding capacities in the fields used for experiments 2 and 3. This variation in stress intensities observed in the three stress environments gave us an opportunity to determine whether it is possible to identify QTLs that consistently increase pearl millet grain yields in a range of terminal drought-stress environments.

In experiments 1 and 2, terminal drought stress reduced grain yield primarily by reducing 100-seed weight. In experiment 3, reductions in grain yield were attributable to reductions in 100-seed weight, panicle number m^{-2}, and panicle grain number, indicating an earlier onset of the stress (Fig. 2). Although 100-seed weight was the component most affected in all three stress environments, terminal drought stress also resulted in reduced stover and biomass yields, and harvest indices, in all three stress environments. Because of the effects of drought on component traits, the nor-

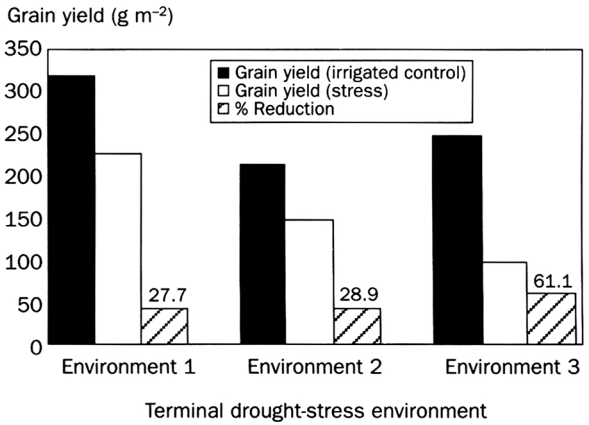

Fig. 1. Mean grain yield of mapping population test-crosses in the irrigated control and terminal-stress treatments in the three experiments.

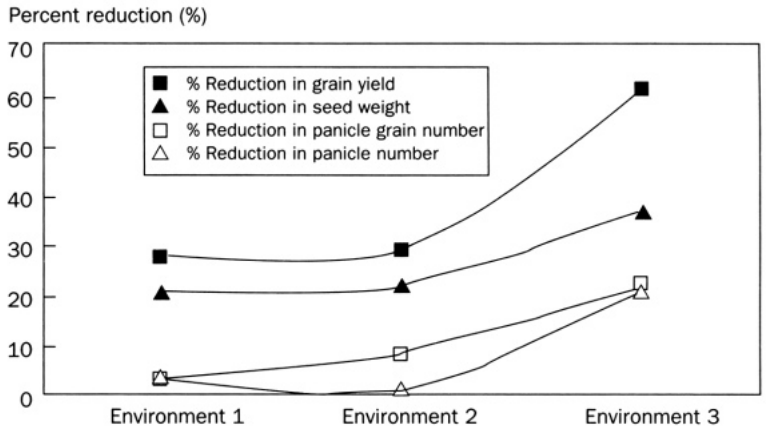

Fig. 2. Reductions in means of grain yields and grain yield-component traits for mapping population test-crosses in three terminal drought-stress experiments.

mal (i.e., control treatment) component-trait correlations of traits such as 100-seed weight and harvest index with grain yield were substantially higher in the stress environments than in the control environments (Table 1).

Table 1. Means and heritabilities of traits evaluated in three terminal drought-stress environments. The significance of differences of the means of individual test-crosses in each of the terminal drought-stress environments is given.

Trait	Experiment 1		Experiment 2		Experiment 3	
	Mean	h^2	Mean	h^2	Mean	h^2
Panicle number m^{-2}	23.6[a]	25.6	14.2[a]	75.8	13.g[a]	65.2
Panicle grain number	1,181[a]	48.8	1,447[a]	75.9	1,116[a]	64.4
100-seed weight (g)	0.80[a]	70.1	0.73[a]	68.9	0.61[a]	57.5
Harvest index (%)	38.7[a]	41.4	39.4[a]	56.1	35.0[a]	42.3
Grain yield (g m^{-2})	220 ns	21.0	146 ns	8.3	93.5[b]	27.8

[a]Significant at <0.001 level of probability. [b]Significant at <0.01 level of probability. ns = nonsignificant.

Genetic parameters of the test-cross population

Genotypic differences among test-crosses for all grain yield-component traits in all the stress environments were highly significant (P <0.001), as judged by F-ratios from the analyses of variance (Table 1). Genetic differences among the test-crosses for grain yield were significant, however, in experiment 1 only. Coefficients of variation were low for almost all traits in all three stress environments, which indicated that the field experiments were conducted with an adequate degree of precision. The low heritabilities (h^2) observed for some traits in stress environments were due to the lower genetic variation observed for these traits in stress environments compared with their counterpart irrigated control environments. A larger ratio of genotype by environment variance to genotypic variance and reduced population means in the stress environments both lead to lower trait heritabilities there than in nonstressed environments (Blum 1988).

Effect of flowering time and yield potential on expression of grain yield in stress environments

The range in flowering time (based on the main panicle) in the mapping population test-crosses was relatively small (Fig. 3A). In all three stress environments, grain yield was not correlated to either flowering time or grain yield in the irrigated controls (Fig. 3A,B). Similar effects of flowering time and yield potential on the grain yield of mapping population test-crosses in stress environments were also evident in other growing seasons (Yadav et al 1998). This suggests that the differences in grain yield of test-crosses in stress environments were not influenced by differences in either flowering time or yield potential. But there were significant grain yield differences in only one environment. This also meant that, by using this mapping population test-cross, mapping can focus on traits and responses that are directly associated with drought tolerance per se rather than on those that simply reflect indirect effects of drought escape and/or yield potential.

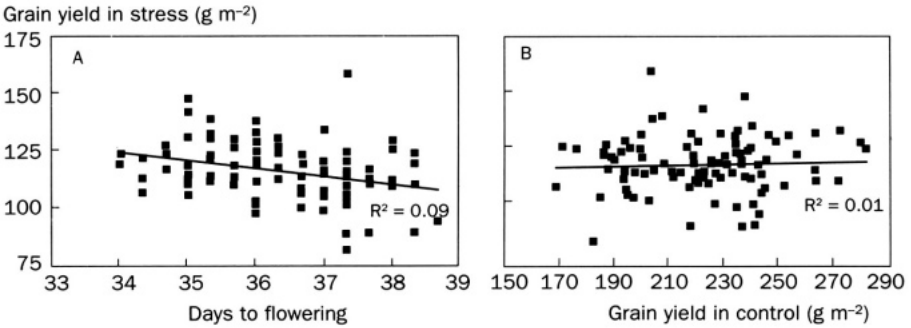

Fig. 3. Relationship of flowering time in the irrigated control to grain yield in the stress environment (A) and relationship of grain yield potential in the irrigated control to grain yield in the stress environment (B) for mapping population test-crosses in terminal drought-stress experiments (data shown for experiment 1 only).

QTL analysis

QTLs associated with the maintenance of harvest index, 100-seed weight, and grain yield in stress environments

QTLs for 100-seed weight and harvest index in all three terminal-stress environments mapped to linkage group 2 (Fig. 4A). The QTL for panicle grain number in experiment 3 also mapped to this genomic region on linkage group 2 (this component was reduced by terminal drought stress only in experiment 3). The PRLT allele for this putative QTL on linkage group 2 was associated with increased 100-seed weight, harvest index, and panicle grain number in these stress environments. Panicle number m^{-2} and stover yield in stress environments were also mapped to this position, but the effect of the PRLT allele on these traits was to reduce their performance in stress environments (Fig. 4A). A QTL for maintenance of biomass yield in the most severe stress environment (experiment 3) also mapped to this linkage group and the PRLT allele was associated with increased maintenance of biomass yield. Increased harvest index and 100-seed weight associated with this QTL, simultaneously with a reduction in stover yield, suggested that increased 100-seed weight in the stress environments may have been achieved by the effect of this QTL on more efficient translocation of stored assimilates in the stover. Maintenance of biomass yield associated with this QTL in experiment 3 further suggested that this QTL is associated with increased dry matter production also during the stress period. Delayed leaf rolling was also mapped to this position on linkage group 2 and it was associated with increased harvest index, 100-seed weight, and panicle grain number in the stress environments.

In this mapping population, a QTL for panicle number m^{-2} mapped to linkage group 2 in both the stress environments and irrigated control environments (data not shown). This QTL, however, was not associated with drought tolerance of panicle number m^{-2} (Fig. 4A). This indicated that this QTL was associated with inherent ge-

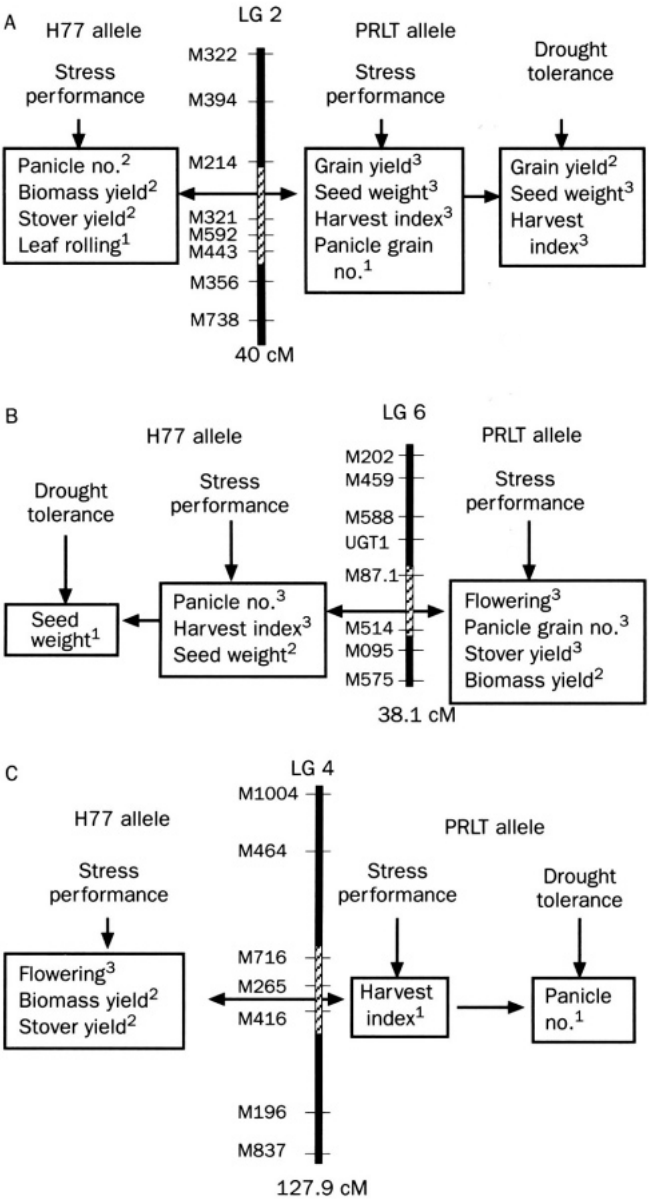

Fig. 4. Quantitative trait loci on linkage groups 2 (A), 4 (B), and 6 (C) associated with the expression of test-cross grain yield and grain yield-component traits in three stress environments. Traits shown on the left side of the linkage group are those whose performance at the QTL is increased by the H 77 allele and on the right side of the linkage group are those whose performance at the QTL is increased by the PRLT allele. Numbers written as superscripts on the trait names indicate the total number of stress environments in which the particular trait was mapped at that QTL.

Table 2. Grain yield-component trait correlations with grain yield in irrigated control and terminal drought-stress environments of three experiments.

Trait	Experiment 1		Experiment 2		Experiment 3	
	Control	Stress	Control	Stress	Control	Stress
Panicle number m^{-2}	0.46[a]	0.48[a]	0.52[a]	0.46[a]	0.50[a]	0.31[a]
Panicle grain number	0.39[a]	0.24[a]	0.27[a]	0.21[a]	–0.13 ns	0.61[a]
100-seed weight	0.05 ns	0.07 ns	0.01 ns	0.40[a]	0.35[a]	0.60[a]
Harvest index	0.38[a]	0.47[a]	0.28[a]	0.54[a]	0.24[a]	0.80[a]

[a]Significant at ≤0.01 level of probability, ns = nonsignificant.

netic differences in panicle number m^{-2} in these mapping population test-crosses. Though panicle number m^{-2} was positively correlated to grain yield in both the imgated control and stress environments (Table 2), it was negatively associated with 100-seed weight at the QTL on linkage group 2 (Fig. 4A). To ascertain whether increased 100-seed weight associated with the QTL on linkage group 2 was due to its effect on increased harvest index or due to inherent genetic differences in panicle number m^{-2}, 100-seed weight was predicted as a function of panicle numbers in the irrigated control environment using regression. Interestingly, the QTL for such predicted values of 100-seed weight mapped to this same region on linkage group 2 and the PRLT allele at this interval was associated with increases in both harvest index and 100-seed weight. Similarly, the predicted values of grain yield from the panicle number m^{-2} in all three stress environments also mapped to this QTL on linkage group 2 and the PRLT allele consistently increased grain yield in the three stress environments. This indicated that the effect of this QTL on increased harvest index was achieved due to its effect on increased 100-seed weight and grain yield in the stress environments. As expected, the drought tolerance of grain yield, 100-seed weight, harvest index, and panicle grain number in stress environments was also mapped to this genomic region on linkage group 2 (Fig. 4A). The PRLT allele for this QTL increased both drought tolerance and the performance of grain yield, 100-seed weight, harvest index, and panicle grain number in the stress environments. The effect of this QTL on harvest index, 100-seed weight, and grain yield was evident in all three terminal drought-stress environments and this explained up to 24.6% of the phenotypic variation in grain yield (Table 3).

QTL associated with maintenance of harvest index and panicle number m^{-2} in stress environments

Another interesting putative QTL for harvest index was obtained on linkage group 4 (Fig. 4B). The PRLT allele for this QTL was associated with increased harvest index in the stress environments of experiment 1. At this QTL, increased harvest index was again associated with decreased stover yield in the stress environments. As on linkage

Table 3. Effects (LOD score and the percentage of phenotypic variation explained) of the QTL identified on linkage group 2 on 100-seed weight, harvest index, and grain yield in terminal drought-stress environments of three experiments.

Trait	Experiment 1		Experiment 2		Experiment 3	
	LOD	r^2	LOD	r^2	LOD	r^2
100-seed weight	4.54	21.9	4.68	23.1	4.08	20.3
Harvest index	2.32	11.0	2.73	13.3	4.00	18.2
Grain yield m^{-2}	2.06	10.1	2.48	13.9	5.56	24.6

group 2, increased harvest index conferred by the PRLT allele for the QTL was associated with a simultaneous reduction in stover yield, again suggesting that increased harvest index was achieved by increased translocation of assimilates from the stover to the grains. Co-mapping of drought tolerance of panicle number m^{-2} at this QTL (Fig. 4B) suggests that increased harvest index in terminal drought-stress environments conferred by this allele led to the maintenance of effective tiller numbers in these environments.

QTL associated with maintenance of harvest index and seed weight in stress environments

Yet a third interesting putative QTL for harvest index in all three terminal drought-stress environments was obtained on linkage group 6 (Fig. 4C). The expression of harvest index, 100-seed weight, panicle number m^{-2}, panicle grain number, stover yield, and biomass yield in the stress environments was also mapped to this interval. In this case, the "H 77 allele" at this QTL increased harvest index, 100-seed weight, and panicle number m^{-2} in the stress environments but reduced panicle grain number, stover yield, and biomass yield (Fig. 4C). Again, as we observed earlier on linkage groups 2 and 4, increased harvest index mapping to this interval was associated with decreased stover yield and biomass yield. As for the QTL on linkage group 4, increased harvest index from this QTL on linkage group 6 was associated with earlier flowering and maintenance of effective tiller number (i.e., panicle number m^{-2}). As we observed on linkage group 2, increased harvest index from this QTL was also associated with maintenance of 100-seed weight (Fig. 4C).

Though panicle number m^{-2} in the stress environments was also mapped at this QTL on linkage group 6, it was again the inherent genetic differences in panicle number and not the drought-tolerance response of panicle number that were mapped at this QTL (Fig. 4C). The H 77 allele increased 100-seed weight and drought tolerance for this grain yield component, but was associated with reduced panicle grain number. This allele was not associated with increased grain yield in terminal drought-stress environments because of its antagonistic effects on the grain yield components of test-crosses.

Marker-assisted varietal improvement

In this study, three regions of the pearl millet genome specifically associated with terminal drought tolerance have been identified. One such region on linkage group 2 had its effect on grain yield whereas the other two had no effects on grain yield because of their antagonistic effects on other grain yield components. The putative QTLs identified have large effects (Table 3) and appear to correspond to possible tolerance mechanisms. These QTLs have been shown to be effective across a range of terminal drought-stress environments and should enhance drought tolerance over a range of conditions. By using test-crosses on 843A to identify these QTLs, we have demonstrated that they will be expressed in hybrid combination with one of the most economically important seed parents of early maturing pearl millet hybrids in India. The H 77/833-2 parent of the mapping population used in this study is the male parent of three single-cross hybrids released in India, one of which (HHB 67, on seed parent 843A) is rapidly becoming the most popular hybrid in that country. Improvement of the drought tolerance of this elite inbred should further improve the acceptability and yield stability of its hybrids in most drought-prone pearl millet-growing regions of South Asia.

The QTLs identified in this study can be used immediately in a marker-assisted backcrossing program for hybrid parental line improvement because their effects have already been assessed in the test-crosses. Marker-assisted backcrossing of the PRLT alleles for putative QTLs on linkage groups 2 and 4 into the H 77/833-2 background and of the H 77 allele for the putative QTL on linkage group 6 into the PRLT 2/89-33 background is already under way in our laboratory. The near-isogenic lines so developed will not only help us in confirming the value of these QTLs but will also provide genetic tools to further our understanding of the physiological and biochemical pathways involved in drought tolerance conditioned by the identified QTLs. In addition, they will provide a base for the map-based cloning of genes contributing to this drought tolerance. After initial testing, these versions of elite inbred pollinators with improved terminal drought tolerance will be made available for commercial hybrid seed production.

References

Beavis WD, Smith OS, Grant D, Fincher R. 1994. Identification of quantitative trait loci using a small sample of topcrossed and F_4 progeny from maize. Crop Sci. 34:882-896.

Bidinger FR, Mahalakshmi V, Rao GDP. 1987a. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. I. Factors affecting yield under stress. Aust. J. Agric. Res. 38:37-48.

Bidinger FR, Mahalakshmi V, Rao GDP. 1987b. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. II. Estimation of genotype response to stress. Aust. J. Agric. Res. 38:49-59.

Blum A. 1988. Plant breeding for stress environments. Orlando, Fla. (USA): CRC Press.

Cowen NM. 1988. The use of replicated progenies in marker-based mapping of QTLs. Theor. Appl. Genet. 75:857-862.

Fischer RA, Maurer R.1978. Drought resistance in spring wheat cultivars. 1. Grain yield response. Aust. J. Agric. Res. 29:897-912.

Fussell LK, Bidinger FR, Bieler P. 1991. Crop physiology and breeding for drought tolerance: research and development. Field Crops Res. 27:183-199.

Lander ES, Botstein D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Busso CS, Gale MD. 1994. An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor. Appl. Genet. 89:481-487.

Ludlow MM, Muchow RC. 1990. Critical evaluation of the traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Mahalakshmi V, Bidinger FR, Raju DS. 1987. Effect of timing of water deficit on pearl millet (*Pennisetum americanum*). Field Crops Res. 15:327-339.

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Linclon SE, Lander ES, Tanksley SD. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127:181- 197.

Prioul J-L, Quarrie S, Causse M, de Vienne D. 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. J. Exp. Bot. 48:1151-1163.

Quarrie SA. 1996. New molecular tools to improve the efficiency of breeding for increased drought resistance. Plant Growth Reg. 20: 167-178.

Ribaut JM, Jiang C, González de Leon D, Edmeades GO, Hoisington DA. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor. Appl. Genet. 94:887-896.

Tanksley SD. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233.

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in Sorghum bicolor (L.) Moench. Mol. Breed. 3:439-448.

van Oosterom EJ, Bidinger FR, Mahalakshmi V, Rao KP. 1996. Effect of water availability pattern on yield of pearl millet in semi-arid tropical environments. Euphytica 89: 165-173.

Yadav RS, Howarth CJ, Cavan GP, Skot KP, Bidinger FR, Mahalakshmi V, Hash CT. 1998. Drought tolerance mapping in pearl millet. Int. Sorghum Millet Newsl. (In press.)

Notes

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Perspectives on molecular marker research: (1) root system morphology traits

Analysis of two rice populations for constitutive root system morphology and preliminary QTL analysis

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Variation and quantitative trait loci (QTL) for a deep and thick root system were compared in anaerobic lowland soil 45 days after sowing. Two rice populations, 220 doubled-haploid lines from the cross of upland japonica and lowland indica (CT9993/IR62266) and 184 recombinant inbred lines from the cross of lowland indica (IR58821/IR52561), were compared. Thirteen traits, categorized into three groups (shoots, deep roots, and thick roots), were analyzed in two seasons of contrasting temperature and radiation. Large transgressive variations for deep root traits in CT9993/IR62266 and for thick root traits in IR58821/IR52561 were identified. Genotype by season interaction was always smaller than genotypic variation, but these were comparable for deep and thick root traits in IR58821/IR52561, with only three QTLs for deep and thick root traits identified in common across the two plantings. In CT9993/IR62266, seven common QTLs for deep or thick root traits were found, but four of them were the same for shoot dry weight or plant height. The results suggested potential genetic improvement of constitutive root traits of rainfed lowland rice by introgressing upland japonica and by crossing within lowland indica. Careful selection of the screening environment for phenotyping is required, however, and effects of shoot growth under saturated conditions need to be taken into account in the populations from upland and lowland crosses.

Rainfed lowland rice is grown in bunded fields, with soil conditions ranging from anaerobic to aerobic during crop growth (Wade et al 1998, Wade, this volume). Rice plants have to develop their root system under anaerobic flooded conditions, which results in different expressions of root anatomy and gross root morphology from those under an upland aerobic environment. Champoux et al (1995) compared selected lines in both aerobic and anaerobic conditions and found significant interaction for rooting depth. In rainfed lowlands, rice generally encounters a water deficit late in the growing season. Thus, two kinds of traits need to be studied. Constitutive traits are expressed under anaerobic, non-water-stressed conditions, do not require water stress for their expression, and may demonstrate variation that is subsequently modified by adaptive traits. Adaptive traits, such as root penetration index or osmotic adjustment

(Zhang et al, this volume, "Progress on the molecular mapping. . ."), are subsequently expressed in response to water deficit or soil physical/chemical barriers. Less research attention has been given to constitutive traits than to adaptive traits.

A deep and thick root system has been assumed advantageous for improved drought tolerance in the rainfed lowland ecosystem, based on evidence from upland rice (O'Toole 1982, Fukai and Cooper 1995). For a simulated rainfed lowland in a greenhouse, Azhiri-Sigari et al (1999) and Kamoshita et a1 (1999) observed a positive effect of root system development under anaerobic well-watered conditions on subsequent plant growth during progressive water stress. Genotypes differed in constitutive root traits and subsequent responses of adaptive root traits, especially in deeper soil layers, and this caused greater water extraction. In the field, the evidence is less clear. Roots are generally shallow in rainfed lowlands (Pantuwan et al 1997), but genotypes differ in root growth in deeper layers (Samson and Wade 1998). Despite the few roots in deeper layers, rainfed lowland rice can extract water from below 15-cm soil depth (Samson et al 1999).

Genetic improvement in the root system of rainfed lowland rice has been slow, however, partly because of the lack of a validated screening system for phenotyping. Attempts have begun to identify quantitative trait loci (QTLs) for root traits in order to screen lines for desired root traits by using DNA markers (Ray et al 1996, Champoux et al 1995, Yadav et al 1997, Price and Tomos 1997, Nguyen et al 1997). To achieve success, the choice of screening system for phenotyping in conditions appropriate to the target rainfed lowland environment is essential. This is because of sensitivity of QTL identification to errors in phenotyping related to difficulty in measurement, and trait manifestation affected by plant size and phenology that interact with the water stress imposed. Thus, genotype by environment interaction, even in the absence of water stress, may confound QTL identification, making some QTLs specific to the screening conditions imposed (Jansen et al 1995). Little attention has been given in QTL literature to the screening system for phenotyping the root system that is relevant to the target environment of rainfed lowland rice.

Only four rice populations have been tagged for QTLs associated with the expression of root traits under hydroponic (Price and Tomos 1997) or upland (Ray et al 1996, Champoux et a1 1995, Yadav et al 1997, Shashidhar et al, this volume, Zhang et al, this volume, "Progress on the molecular mapping. . .") conditions. The small number of populations may limit extrapolation of the results from characterization of experimental populations to screening of breeding lines. There were no reports for indica/indica populations, for anaerobic lowland conditions, or for rainfed lowland water stress in the field. Further phenotyping for QTL analysis is essential for constitutive and adaptive traits, with crosses from different genetic backgrounds, particularly for anaerobic lowland conditions for constitutive root traits.

This chapter examines phenotypic variation and QTLs for gross root morphology under anaerobic lowland conditions in contrasting growing seasons between two rice populations. The effect of screening environment on QTL identification and potential genetic improvement of rainfed lowland rice will also be discussed.

Materials and methods

Plant population

A population of 220 doubled-haploid lines (DHLs) from the cross between CT9993-5-10-1-M (CT9993; japonica, upland adapted) and IR62266-42-6-2 (IR62266; indica, lowland adapted) and a population of 184 recombinant inbred lines (RILs) from the cross between IR58821-2-3-B-1-2-1 (IR58821; indica, lowland adapted) and IR52561-UBN-1-1-2 (IR52561; indica, lowland adapted) were developed at the International Rice Research Institute (IRRI), Los Baños, Philippines (14°11'N, 121°15'E, 23 m altitude), to tag genes for root penetration capacity, gross root morphology, and osmotic adjustment. The DHLs were derived from anther culture (Shashidhar et a1 1994) and the RILs were developed by single-seed descent to the F_7 generation. The gross root morphology of the four parental lines was characterized under both stress and nonstress conditions in the greenhouse (Azhiri-Sigari et al 1999) and in the field (Sarkarung et al 1997, Samson and Wade 1998). Azhiri-Sigari et al (1999) showed that CT9993 had a slower initial growth rate under anaerobic flooded conditions, but had thicker roots and, in the later growth stage, deeper roots than IR62266. IR58821 had a larger deep root mass and greater root penetration capacity than IR52561 at Rajshahi, in northwest Bangladesh (Samson and Wade 1998).

Setup of pot experiments

Root morphology was evaluated in two different pot experiments at the IRRI greenhouse for each of the two populations (Table 1). For the 220 DHLs, the two experiments were conducted from November to December 1997 (DH1) and from May to July 1998 (DH2), respectively, using a 15 × 15 alpha design with three replicates. For the 184 RILs, the two experiments were conducted using a 14 × 14 row-column alpha design from August to September 1997 (RI1) and using a 12 × 16 alpha design from February to March 1998 (RI2), respectively, with three replicates. An evaporative

Table 1. Characteristics of the four screening environments.

Experiment code	Parents of lines	Number of lines[a]	Sowing date[b]	Heat sum[c] (°C d)	Radiation (MJ $m^{-2} d^{-1}$)	Soil temperature[d] (°C)
DH1	CT9993 IR62266	220 (36)	1 Nov 97	868	17.1	27–32
DH2	CT9993 IR62266	220 (1)	16 May 98	1,098	18.9	29–34
RI1	IR58821 IR52561	184 (0)	8 Aug 97	1,031	17.6	28–33
R12	IR58821 IR52561	184 (2)	3 Feb 98	987	21.5	27–34

[a]Number of lines missing in parentheses. [b]Harvest was at 45–47 days after sowing (DAS) for RI1 and R12, 42–44 DAS for DH1, and 48–50 DAS for DH2, respectively. [c]Base temperature was taken as 9 °C (Kropff et al 1994). Range of mean soil temperatures from 800 h to 1500 h.

gradient from the center to the side of the greenhouse was observed in the first experiment (RIl), so replicates were arranged perpendicular to this gradient in subsequent experiments.

Four to five pregerminated seeds of each DHL or RIL were sown on the wet soil and thinned to one healthy seedling per pot by about 10 d after sowing (DAS). The sowing dates were 1 November 1997, 16 May 1998, 8 August 1997, and 3 February 1998 in the DH1, DH2, RI1 , and RI2 experiments, respectively. Several lines were missing due to germination failure in each experiment (Table 1).

A cylindrical pot made of polyvinyl chloride (PVC), of 20-cm interval diameter and 55-cm depth with a plastic bag insert, was filled with 20 kg of air-dried Maahas clay soil (28% clay, 44% silt, and 28% sand, pH 5.2) (Wopereis 1993). At first, 17 kg of soil was carefully put into the plastic bag inside the pot to eliminate any gaps between the plastic bag and the inner wall of the pot, and about 6 kg of water was added. The whole soil layer was then mixed with a wooden stick until standing water remained. When the soil surface shrank, another 3 kg of air-dried soil was put into the pot, more water was added, and the soil was puddled again. The exterior of the pot was covered with aluminum foil at 23 DAS in RI1 and from sowing in the other three experiments to minimize any rise in soil temperature in pots in the greenhouse in order to minimize high-temperature effects on root growth (Nagai and Matsushita 1963).

Sufficient levels of N fertilizer (1.26 g of N as urea 46-0-0) were supplied based on the results of Regmi (1995). Solo-phos (0-18-0) equivalent of 0.33 g of P and muriate potash (0-0-60) equivalent of 0.62 g of K were also applied at puddling and mixed thoroughly into the puddled soil. The level of standing water was maintained at about 2 to 4 cm by watering daily. No disease or insect damage occurred.

Measurements

The daily minimum and maximum air temperature and soil temperature at 5-cm depth from the soil surface were recorded at 800 h and 1500 h in the greenhouse. Solar radiation data came from the IRRI wetland meteorological station about 500 m from the greenhouse. The heat sum with a base temperature of 9 °C and average daily solar radiation during the experimental period were calculated (Table 1). DH1 had a much smaller heat sum than DH2. RI1 had a lower solar radiation than RI2. Thus, each population was grown in two seasons of contrasting temperature and radiation. There was little difference in the range of measured soil temperatures across the four experiments.

The plants were sampled at 42-44 DAS in DH1 and at 48-50 DAS in DH2 and at 45-47 DAS in RI1 and RI2. A total of 13 traits were measured in three categories: shoot, deep root, and thick root traits (Table 2). Plant height and tiller number were measured 1 d before the sampling dates. Plants were cut at the soil surface. The soil mass inside the plastic sleeve was slowly pulled out of the PVC pots and the soil divided into layers of 0–10, 10–20, 20–25, 25–30, 30–35, 35-40, 40-45, and 45–50 cm from the soil surface. Roots were carefully separated from the soil on the 1 -mm

Table 2. The thirteen traits (grouped into shoot, deep root, and thick root traits) analyzed in the four screening experiments.

Abbreviation	Trait	Unit
Shoot traits		
SDW	Shoot dry weight	g plant^{-1}
PH	Plant height	cm
TN	Tiller number	plant^{-1}
Deep root traits		
RDW	Total root dry weight	g plant^{-1}
DRDW	Deep root dry weight below 30-cm depth	g plant^{-1}
DRDW/RDW	Deep root ratio	%
RDW/TN	Total root weight per tiller	mg tiller^{-1}
DRDW/TN	Deep root weight per tiller	mg tiller^{-1}
RDW/SDW	Root to shoot ratio	%
RD_{max}	Maximum rooting depth	cm
Thick root traits		
RT10	Root thickness in 0–10-cm depth	mm
RT25	Root thickness in 20–25-cm depth	mm
RTDEEP	Root thickness in deepest soil layer	mm

sieve screen (Schuurman and Goedewaagen 1965). The dry weight of each plant component was measured after drying at 70 °C for 4 to 5 d. Shoot dry weight was determined as the sum of aboveground biomass, including stem base. Total root dry weight and deep root dry weight below the 30-cm soil layer were obtained, and the deep root ratio, the proportion of the latter to the former, was calculated. Total root dry weight per tiller and deep root dry weight per tiller were examined by dividing total and deep root dry weight by the total number of tillers. Root to shoot ratio was determined by dividing total root dry weight by shoot dry weight. Maximum rooting depth was calculated from the deepest soil layer where roots were present and the longest root measured in the layer. Root thickness was measured by microcaliper at 0–10 cm, 20–25 cm, and the deepest soil layer for seven to ten randomly chosen primary roots.

Analysis of variance was conducted using the SAS package for an alpha design except for experiment DH1, in which a randomized block design was used because several lines were missing. Analysis of variance was also conducted for the combined data over two seasons for each population to estimate the size of the genotype by season interaction. Phenotypic correlation between traits was calculated for each experiment and for the combined data.

Map construction and QTL analysis

The two populations were genotyped with molecular markers of restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and microsatellites at Texas Tech University (Ali 1999, Zhang et al 1999). The two linkage maps were constructed with MapMaker Macintosh Version 2.0 and QTL analysis was conducted by employing interval mapping based on MapMaker/QTL (version 1.1) with the LOD value set to 2. For the CT9993/IR6226 DHL population, 154

lines were used to construct the map that consisted of 145 RFLPs, 153 AFLPs, and 17 microsatellites. For the IR58821/IR52561 RIL population, 166 lines were used for map construction and the map had 383 AFLPs and 106 RFLPs. To conduct QTL analysis, the lines used for phenotyping had to match those used for genotyping. Therefore, a subset of lines from each population (154 and 166, respectively) was selected for QTL analysis.

Results

Seasonal effect on growth

Though the sampling dates were similar, seasonal changes in temperature and solar radiation affected plant growth for each population (Table 3). Except for plant height, root to shoot ratio, root thickness in the 0–10-cm depth, and root thickness in the deepest soil layer, most traits had higher values in DH2 than in DH1. RI2 had higher values for shoot dry weight, tiller number, total root dry weight, and total root weight per tiller and lower values for plant height, deep root dry weight, deep root ratio, deep root weight per tiller, and maximum rooting depth than RI1. Root thickness in the 20–25-cm depth and root thickness in the deepest soil layer were higher in RIl than in RI2.

Genotype by season interaction

The parents differed in most of the traits in DH2, RI1, and RI2, but only shoot traits, total root weight per tiller, and root thickness in the 0–10-cm layer differed in DH1 (Table 3). Parental rankings were the same for most traits in both populations over the two seasons. CT9993 had consistently higher values for plant height, total root dry

Table 3. Mean values of 13 traits among each population in each season.

Trait	DH3	DH4	RI1	R12
Shoot traits				
Shoot dry weight (g plant^{-1})	21.9*	35.1***	29.5***	41.3*
Plant height (cm)	104***	108***	122***	113***
Tiller number (plant^{-1})	30***	35***	32	40
Deep root traits				
Total root dry weight (g plant^{-1})	2.86	3.98+	3.89***	5.41***
Deep root dry weight below 30-cm depth (g plant^{-1})	0.02	0.21	0.11***	0.06***
Deep root ratio (%)	0.7	5.1**	3.0**	1.0***
Total root weight per tiller (mg tiller^{-1})	99***	120***	125**	139***
Deep root weight per tiller (mg tiller^{-1})	0.8	6.6**	3.8***	1.4***
Root to shoot ratio (%)	13	12*	13*	13**
Maximum rooting depth (cm)	37	45	45*	40***
Thick root traits				
Root thickness in 0–10cm depth (mm)	1.3**	1.2***	1.3*	1.3**
Root thickness in 20–25cm depth (mm)	0.7	0.9*	1.1*	0.8**
Root thickness in deepest soil layer (mm)	0.4	0.4+	0.7	0.3

+, *, **, ***; the two parents differed significantly at P = 0.10, 0.05. 0.01, and 0.001, respectively.

weight per tiller, and root thickness in the 0–10-cm depth and lower values for shoot dry weight and tiller number than IR62266 in both DH1 and DH2. Maximum rooting depth was comparable. For deep root dry weight and deep root ratio, CT9993 had higher values (0.28 g, 6.4%) than IR62266 (0.18 g, 3.7%) in DH2, but their ranking changed in DHl (0.02 g and 0.5% in CT9993, 0.03 g and 0.8% in IR62266). IR58821 had consistently higher values for shoot dry weight, total root dry weight, deep root dry weight, deep root ratio, total root dry weight per tiller, deep root dry weight per tiller, root to shoot ratio, maximum rooting depth, root thickness in the 0–10-cm depth, and root thickness in the 20–25-cm depth and a lower value for plant height than IR52561 in both RI1 and RI2. For tiller number and root thickness in the deepest soil layer, IR58821 had higher values than IR52561 in RI1, but the values were comparable in RI2.

Genotype by season interaction (G × S) was significant, but its mean square was smaller than that of genotypic variation (G) for all traits in both populations. The ratio of G × S to G mean squares was in most cases less than 0.5 in the DHLs except for deep root dry weight, deep root ratio, deep root dry weight per tiller, and root thickness in the deepest soil layer. In RILs, the G × S to G mean square ratio was between 0.5 and 1.0 for shoot dry weight, total root dry weight, deep root ratio, deep root dry weight per tiller, root to shoot ratio, maximum rooting depth, root thickness in the 0–10-cm depth, and root thickness in the 20–25-cm depth, so the genotype by season interaction was larger in RILs than in DHLs.

Transgressive variation

In the combined analysis, the populations were comparable in the size of the genotypic variation for all 13 traits. Figure 1 shows examples of the distribution of deep root dry weight and root thickness in the 0–10-cm depth among parents and progeny. There was large transgressive variation for deep root dry weight in DHLs and for root thickness in the 0–10-cm depth in RILs.

Shoot-root correlation

There was a strong phenotypic correlation between shoot dry weight and total root dry weight in both populations, with coefficients higher in CT9993/IR62266 than in IR58821/IR52561 (Table 4). Deep root dry weight was also correlated with shoot dry weight, with the coefficient higher in CR9993/IR62266. Root thickness in the 0–10-cm depth and root thickness in the 20–25-cm depth were correlated with shoot dry weight and plant height in CR9993/IR62266, while in IR58821/IR52561 only root thickness in the 20–25-cm depth and shoot dry weight were correlated.

QTL analysis

Table 5 lists the numbers of QTLs for each of the 13 traits examined in each experiment. In general, there were more QTLs in common across the two seasons in CT9993/IR62266 than in IR58821/IR52561. For deep and thick root traits, seven and three QTLs were identified in common across the two seasons in CT9993/IR62266 and in

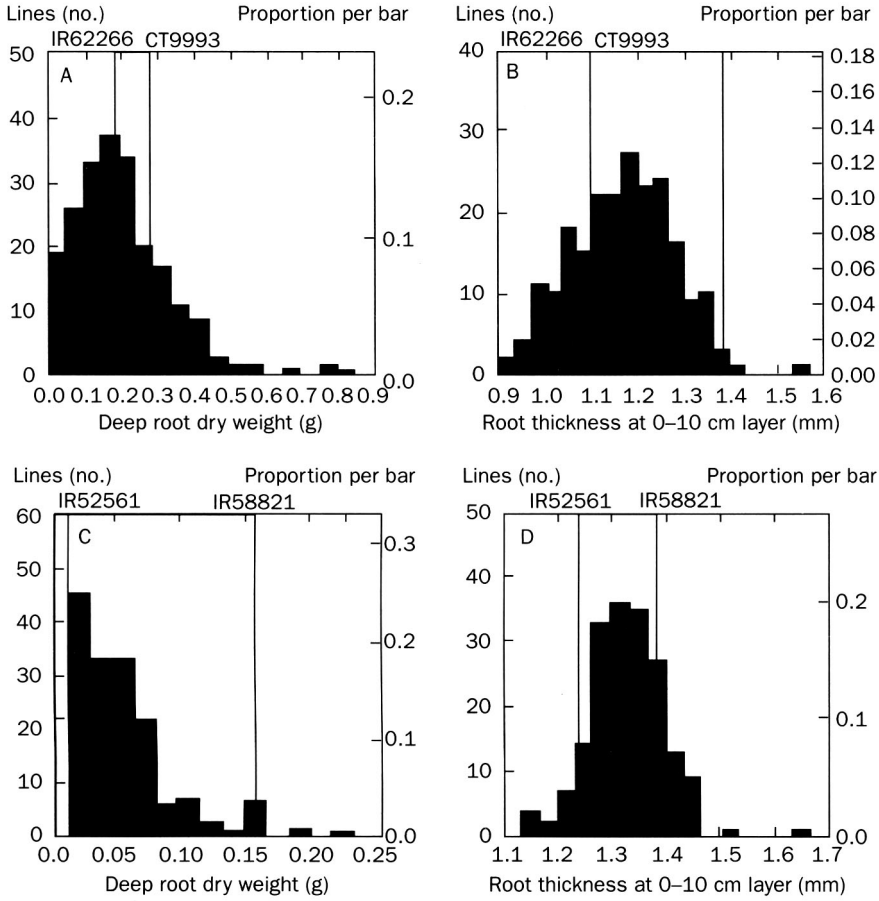

Fig. 1. Distribution of deep root dry weight (A,C) and root thickness at 0–10-cm soil depth (B,D) among lines of the population CT9993/IR62266 (A,B) and among lines of the population IR58821/IR52561 (C,D) in experiments DH2 and RI2.

Table 4. Phenotypic correlation between some of the root and shoot traits in CT9993/IR62266 and IR58821/IR52561.

Traits	CT9993/IR62266	IR58821/IR52561
Root dry weight and shoot dry weight	0.83**	0.73**
Deep root dry weight below 30-cm depth and shoot dry weight	0.54**	0.36**
Root thickness in 0–10-cm depth and shoot dry weight	0.34**	0.08 ns
Root thickness in 0–10-cm depth and plant height	0.50**	0.03 ns
Root thickness in 20–25-cm depth and shoot dry weight	0.48**	0.38**

** = significant at P= 0.01; ns = not significant.

Table 5. Number of QTLs for 13 traits in individual experiments and in common across both experiments for each population.

Trait	CT9993/IR62266			IR58821/IR52561		
	DH1	DH2	Both	RI1	RI2	Both
Shoot traits						
Shoot dry weight	4	3	1	4	2	0
Plant height	11	7	4	8	6	2
Tiller number	5	8	2	6	3	1
Deep root traits						
Total root dry weight	8	5	3	0	0	0
Deep root dry weight below 30-cm depth	0	6	0	6	6	1
Deep root ratio	2	5	2	7	5	0
Total root weight per tiller	7	7	3	6	7	1
Deep root weight per tiller	2	4	2	4	4	1
Root to shoot ratio	2	4	0	0	4	0
Maximum rooting depth	5	2	1	2	3	1
Thick root traits						
Root thickness in 0–10-cm depth	9	6	3	2	2	0
Root thickness in 20–25-cm depth	6	5	2	2	2	1
Root thickness in deepest soil layer	0	3	0	5	1	0

Table 6. Number of QTLs identified at the same marker locations for selected root and shoot traits in CT9993/IR62266 and IR58821/IR52561 populations.

Traits	CT9993/IR62266	IR58821/IR52561
Root dry weight and shoot dry weight	5	0
Deep root dry weight below 30cm depth and shoot dry weight	2	2
Root thickness in 0–10-cm depth and shoot dry weight	2	1
Root thickness in 0–10-cm depth and plant height	6	1
Root thickness in 20–25-cm depth and shoot dry weight	2	0

IR58821/IR52561, respectively. The range of phenotypic variation explained by a single QTL in common ranged from 8% to 34% in CT9993/IR62266, and from 8% to 22% in IR58821/IR52561. The QTLs for deep or thick roots traits, which accounted for more than 20% of phenotypic variation, were all mapped with QTLs for shoot traits in CT9993/IR62266. There were five QTLs for both shoot dry weight and total root dry weight and six QTLs for both plant height and root thickness in the 0–10-cm depth in CT9993/IR62266 (Table 6).

Discussion

For most traits, there was no crossover interaction between the parents of both populations in the two seasons. But a larger genotype by season interaction and more season-specific QTLs for deep and thick root traits were found in IR58821/IR52561 than in CT9993/IR62266. This contrasting response over populations emphasizes the plasticity of constitutive root traits, particularly in IR58821/IR52561. Consequently, selection of screening conditions for phenotyping that are relevant to the critical characteristics of the target environment in drought-prone rainfed lowland fields is essential.

At lower temperature in DH1, CT9993, which is characterized as having deep roots (Shashidhar et al 1994, Sarkarung et al 1997), did not express its deep root weight by the time of sampling (42-44 DAS). Wade et a1 (1999) and Azhiri-Sigari et al (1999) reported a slower shoot and root growth rate in CT9993 compared with other rainfed lowland genotypes under anaerobic lowland conditions. Considering the strong correlation between root and shoot biomass and many genetic linkages between root and shoot mass in CT9993/IR62266, QTLs for total root dry weight and deep root dry weight in CT9993/IR62266 need to be interpreted with care. Some may be pleiotropic, and some may simply be a result of differences in adaptation to anaerobic conditions. This requires further study.

In spite of large seasonal effects, even in anaerobic well-watered conditions, seven and three QTLs for deep and thick root traits were identified in common over seasons in CT9993/IR62266 and IR58821/IR52561, respectively, and transgressive variation was large for deep root dry weight in CT9993/IR62266 and for root thickness in the 0–10-cmdepth in IR58821/IR52561. Consequently, there is potential for genetic improvement of the root system in both of these populations. Though few lines had a larger deep root dry weight than IR58821 in the IR58821/IR52561 population, subsequent evaluation for adaptive root traits (such as osmotic adjustment or root penetration index) or for agronomic suitability (such as phenology) may reveal lines superior to IR58821 in the population.

This study confirmed the importance of the screening environment for phenotyping of constitutive root traits, and suggested a potential for genetic improvement of the root system of rainfed lowland rice by using the DNA marker approach. Further analysis of additional populations and of epistasis and comparison of constitutive and adaptive traits are needed.

References

Ali ML. 1999. Mapping quantitative trait loci for root traits related to drought resistance in rice (*Oryza sativa* L.) using AFLP markers. Ph.D. dissertation. Texas Tech University, USA.

Azhiri-Sigari T, Yamauchi A, Kamoshita A, Wade LJ. 1999. Genotypic variation in response of rainfed lowland rice to drought and rewatering. II. Root growth. Plant Prod. Sci. (Submitted.)

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. Field Crops Res. 40:67-86.

Jansen RC, Van Ooijen, JW, Stam P, Lister C, Dean C. 1995. Genotype by environment interaction in genetic mapping of multiple quantitative trait loci. Theor. Appl. Genet. 91:33-37.

Kamoshita A, Wade LJ, Yamauchi A. 1999. Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction. Plant Prod. Sci. (Submitted.)

Kropff MJ, van Laar HH, Natthews RB. 1994. ORYZA1: an ecophysiological model for irrigated rice production. SARP Research Proceedings. p 8.

Nagai T, Matsushita E. 1963. Physio-ecological characteristics in roots of rice plants grown under different soil temperature conditions. 1. Their ecological characteristics. Proc. Crop Sci. Soc. Jpn. 31:385-388.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37: 1426-1434.

O'Toole JC. 1982. Adaptation of rice to drought-prone environments: drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 195-213.

Pantuwan G, Fukai S, Cooper M, O'Toole JC, Sarkarung S. 1997. Root traits to increase drought resistance in rainfed lowland rice. Fukai S, Cooper M, Salisbury J, editors. In: Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held at Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings No. 77. p 170-179.

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95:143-152.

Ray JD, Yu LX, McCouch SR, Champoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor. Appl. Genet. 42:627-636.

Regmi KR. 1995. Influence of water deficit and nitrogen limitation on the phenotypic plasticity and root-shoot partitioning in rice cultivars. Ph.D. thesis. University of the Philippines Los Baños. 316 p.

Samson BK, Wade LJ. 1998. Soil physical constraints affecting root growth, water extraction, and nutrient uptake in rainfed lowland rice. Ladha JK, Wade LJ, Dobermann A, Reichardt W, Kirk GJD, Piggin C, editors. In: Rainfed lowland rice: advances in nutrient management research. Proceedings of the International Workshop on Nutrient Research in Rainfed Lowlands, 12-15 Oct. 1998, Ubon Ratchathani, Thailand. Los Baños (Philippines): International Rice Research Institute. p 231-244.

Samson BK, Ali A, Rashid MA, Mazid MA, Wade LJ. 1999. Water extraction by rainfed lowland rice in three topographic positions in Bangladesh. Plant Prod. Sci. (Submitted.)

Sarkarung S, Pantuwan G, Pushpavesa S, Tanupan P. 1997. Germplasm development for rainfed lowland ecosystems: breeding strategies for rice in drought-prone environments. Fukai S, Cooper M, Salisbury J, editors. In: Breeding strategies for rainfed lowland rice in drought-prone environments. Proceedings of an International Workshop held at Ubon Ratchathani, Thailand, 5-8 Nov. 1996. ACIAR Proceedings No. 77. p 43-49.

Schuurman JJ, Goedewaagen MAJ. 1965. Methods for the examination of root systems and roots. Wageningen (Netherlands): Centre for Agricultural Publications and Documentation. p 37-41.

Shashidhar HE, Ray JD, Nguyen HT, Sarkarung S, O'Toole JC. 1994. Genetic variation for root hardpan penetrability and restriction fragment length polymorphism survey in rice. Paper presented at the International Program on Rice Biotechnology, 7th annual meeting, 16-22 May 1994, Bali, Indonesia.

Wade LJ, George T, Ladha JK, Singh U, Bhuiyan SI, Pandey S. 1998. Opportunities to manipulate nutrient-by-water interactions in rainfed lowland rice systems. Field Crops Res. 56:93-112.

Wade LJ, Kamoshita A, Yamauchi A, Azhiri-Sigari T. 1999. Genotypic variation in response of rainfed lowland rice to drought and rewatering. I. Growth and water use. Plant Prod. Sci. (Submitted.)

Wopereis MCS. 1993. Quantifying the impact of soil and climate variability on rainfed rice production. PhD thesis. Wageningen Agricultural University, Wageningen, Netherlands. 188 p.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Zhang J, Zheng HG, Tripathy JN, Aarti A, Sarial AK, Robin S, Nguyen TT, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT. 1999. Locating genomic regions associated with osmotic adjustment and root penetration ability in rice. (In preparation.)

Notes

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Molecular marker-assisted tagging of morphological and physiological traits at the peak vegetative stage: two contrasting moisture regimes

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Root morphology under well-watered conditions sampled on two occasions and under moisture stress was studied in a randomly chosen subset of 56 doubled-haploid lines derived from a cross between IR64 and Azucena. A molecular map of the same population served as the basis for locating quantitative trait loci (QTLs) controlling root morphology and associated traits. Regions flanking the restriction fragment length polymorphism markers RZ730-RZ801 on chromosome 1 were associated with plant height in all three sampling environments. This position corresponds to *sd-1,* a semidwarfing gene. Fifteen QTLs were detected at the two developmental stages, of which only four were common. Twenty-one QTLs for different traits were detected under moisture stress. Although two QTLs for plant height on chromosomes 1 and 3 were common, none of the loci for root morphological traits was common between the two different moisture regimes. The absence of common QTLs for root traits between two developmental stages and two moisture regimes suggests the existence of parallel genetic pathways operating at different growth stages and under different moisture regimes. Root volume and total root number per plant decreased significantly under stress, whereas maximum root length and plant height exhibited nonsignificant increases under stress.

Rice (*Oryza sativa* L.) is a semiaquatic species adapted to a variety of climates. Selection under divergent ecological habitats, sometimes assisted by farmers or breeders, has enabled a vast array of genetic variation for several important survival and productivity traits to be produced and conserved. Intensive breeding efforts have resulted in enhanced productivity in irrigated rice habitats mainly because of fertilizer-responsive and genetically superior varieties developed particularly for this high-input situation. Rainfed rice research, in contrast, has received little attention from crop scientists. Traditional rice varieties that are nonresponsive to improved management practices are still cultivated in large areas of southern Karnataka, India. Such marginal lands receive comparatively few inputs because of the very nature of subsistence agriculture (IRRI 1995). There is an urgent need to develop rice varieties with

higher yield potential and drought-tolerance characteristics of traditional accessions. Improving productivity under water-limiting growth conditions by selecting high-yielding genotypes is not reliable because of the highly fluctuating nature of yield per se, which is the manifestation of complex interactions. For increased productivity, identifying the adaptive traits that determine yield under drought is essential.

Drought tolerance can augment rice productivity in rainfed rice lands affected by inadequate or erratic rainfall. Several physiological, morphological, and phenological traits have been reported to improve the performance of crops affected by drought. Adaptive mechanisms of plants in response to drought have been reported and reviewed by several scientists (Arraudeau 1989, Bidinger and Witcombe 1989, McWilliam 1989, Ludlow and Muchow 1990, Fukai and Cooper 1995, Nguyen et al 1997, Chopra and Sinha 1998). Root systems are an important component of drought resistance because they influence the amount of water available to the crop depending on their distribution in the soil. Among root morphological traits, maximum root length and root thickness are associated with drought tolerance in upland conditions. Increased root thickness improves drought tolerance because the roots are capable of increasing root length density and water uptake by producing more and larger root branches (Ingram et al 1994). It also allows roots to penetrate the hardpans characteristic of some lowlands. Under rainfed lowland conditions, greater root length density below 30 cm and a moisture-stress-induced dynamic response in the 10–30-cm soil layer were associated with drought tolerance (Ingram et al 1994).

Despite ample genetic variability for various parameters of root system architecture, its genetic improvement using conventional selection based on phenotype alone is difficult (O'Toole 1989). Screening genotypes for root traits has rarely been incorporated into breeding programs because of the time-consuming nature of the work and complex inheritance pattern (quantitative genetic control). O'Toole (1989) opined that molecular marker technology shows "near-term use for improving drought resistance breeding." Rapid advances in molecular marker technology have helped in developing highly saturated molecular maps in rice (McCouch et al 1988, Saito et al 1991, Causse et a1 1994, Kurata et al 1994) and have made it possible to study the inheritance pattern of complex traits such as grain yield (Stuber et al 1992) and fruit quality in tomato (Paterson et al 1991) and to study gene action governing complex triats (Stuber et al 1992, Basten et al 1996). Genes for any quantifiable trait can be tagged using high-density molecular maps. QTL mapping provides a powerful tool for conducting physiological and genetic research to understand and possibly improve drought tolerance. It facilitates screening for traits that are difficult to quantify and that are influenced by environmental "stimuli" (Hanson et al 1990).

In rice, reports on QTL mapping of component traits for drought tolerance are relatively few. Champoux et al (1995) pioneered tagging root morphology and leaf rolling in rice. Hardpan root-penetrating ability was studied by Ray et al (1996) and tagged to molecular markers. Recent reports of tagging root morphology and physiological traits include the ones by Yadav et al (1997), Price et al (1997), and Ali (1999). Lilley et al (1996) investigated osmotic adjustment in a mapping population

and found molecular markers associated with the expressed phenotype. Our study reports the tagging of QTLs for root and shoot morphology in two contrasting moisture regimes at the peak vegetative stage of crop growth (before flowering) to identify QTLs associated with "root genes" expressed under moisture stress and nonlimiting moisture conditions, and to study trait sensitivity of genotypes to low-moisture stress.

Materials and methods

Plant material

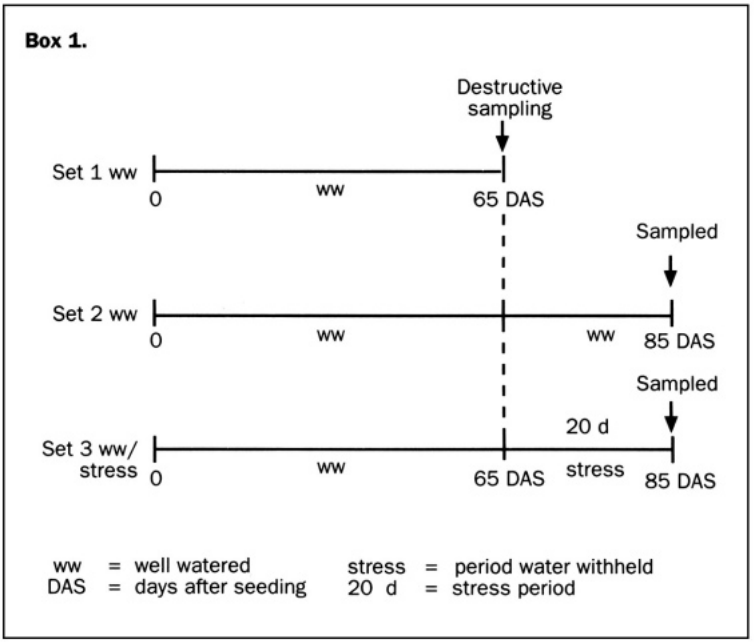
A doubled-haploid (DH) population consisting of 56 lines, a randomly chosen subset of a population of 135, originally developed from a cross between IR64, an indica variety, and Azucena, a traditional aromatic japonica variety developed by Guiderdoni et al (1992) at IRRI, was used. IR64 is a high-yielding improved semidwarf variety suitable for irrigated habitats, whereas Azucena is a more drought-tolerant variety.

RFLP map

Huang et a1 (1994) generated a preliminary restriction fragment length polymorphism (RFLP) map of this population. A total of 175 RFLP markers, which were polymorphic in a parental survey, were used to construct a linkage map using Mapmaker (Lander 1993). To date, the linkage map covers 2,005 cM, with an average distance of 11.5 cM between markers. Distribution of IR64 alleles for each marker and each line was roughly symmetrical, suggesting no overall bias toward either parent (Huang et al 1997).

Experimental details

The experiment was carried out at the Hebbal campus of the University of Agricultural Sciences, Bangalore, India, during the wet season of 1996. Fifty-six DH lines were grown in light gray polyvinyl chloride (PVC) cylinders (75 cm long, 18 cm diameter) in a randomized complete block design. In a field, PVC cylinders were placed in subsurface pits so that the tops of the cylinders were at the ground level. PVC cylinders were filled to 1.5 cm from the top with sandy loam soil and well-decomposed farmyard manure in the ratio of 4: 1. Three seeds per genotype were sown directly into the soil and were thinned after 10 d, taking care to maintain one plant per genotype in each replication for all the treatments. The experiment consisted of three sets of 56 genotypes, with three replications in each set (see Box 1). Moroberekan, Azucena, IR64, and IR20 were used as checks. The first set consisted of plants that were well watered (irrigated every other day) to 65 days after sowing (65 DAS, well-watered treatment). On the 65th day, plant height and tiller number plant^{-1} were recorded. Cylinders with soil and plant were thoroughly soaked in water and the soil column was carefully pushed onto an iron mesh for collecting root samples. The second set consisted of plants that were well watered (every other day) for 85 d (85 DAS, well-watered treatment). On the 85th day, data were recorded as in the 65 DAS treatment. The third set consisted of plants that were well watered for 65 d. From the 65th day onward, irrigation was withheld and rainfall was prevented using



a rainout shelter to develop moisture stress (85 DAS, moisture stress) slowly. On the 85th day (end of stress period), plant height and tiller number plant^{-1} were recorded and roots were sampled for further observations.

For the second and third sets of the experiment, soil moisture determinations were made by a gravimetric method (Brady 1985). Soil moisture was 22.0% and 3.5% under well-watered and moisture-stress conditions, respectively.

Plant height and tiller number plant^{-1} were measured on intact plants just before root sampling. After separating the soil column from the pipes, the root mass was washed thoroughly and root characters such as maximum root length (in cm), root volume (in cm^3 by the water displacement method), root number (by actually counting the roots after separating the crown portion while immersed in a trough of water), root thickness (at 1 cm from the crown of the plants measured in mm using a microscope and standardized ocular micrometer), and root and shoot dry weight (8) were recorded. Based on the dry-weight observations, root-to-shoot ratio by dry weight was computed. The observations were recorded as described above in all three sets/ treatments. In addition, in the third set (85 DAS, moisture-stress condition), leaf rolling using an index of 1 (no rolling) to 5 (completely rolled) (O'Toole and Moya 1978) and drought score devised by IRRI (IRRI 1980), based on a rating of dead leaves, were assessed, where 1 = green leaves and 5 = plants with dead leaves longer than 15 cm.

Statistical analysis and QTL mapping

The data of the three treatments were subjected to ANOVA to partition the variance. Interval analysis was performed to detect QTLs using the computer package Mapmaker/QTL (Lander 1993). A LOD threshold of 1.5 was adopted for analysis because the threshold value is a function of the sample size used (Doerge 1993). Mean values of all traits in the second and third sets of the experiment, which represented "85 DAS, well-watered treatment" and "85 DAS, moisture-stress treatment," were tested for significant differences using the paired t-test.

Results and discussion

The high level of genetic variation built into the DH population derived from the IR64/Azucena cross has encouraged its use by several groups around the world (Chen et a1 1997, Yadav et al 1997, Zheng et a1 1996, Hemamalini 1997). In this study, IR64 and Azucena contrasted significantly for most traits (Table 1). This contrasting behavior is responsible for the wide range of variability among the DH lines for the characters studied. Analysis of variance revealed highly significant differences among the DH lines for all the characters studied. Transgressants were observed for most traits.

Trait sensitivity to moisture stress

Mean values of "85 DAS, well-watered" and "85 DAS, moisture-stress" treatments were tested for significant differences using the paired t-test (Table 2). Although plant height and maximum root length were greater in the moisture stress, the increases were nonsignificant. Total number of tillers plant", total number of roots plant", root volume, root dry weight, and root thickness decreased significantly under stress. Other traits were not significantly affected by the magnitude of the drought treatment imposed in the present study. Trait sensitivity to stress was therefore evident.

Correlation between different traits is common and can arise both genetically and environmentally. Table 3 shows the comparison of correlation values under contrasting moisture regimes for root and shoot morphological traits. The different moisture regimes changed both the magnitude and the sign of the correlation coefficients. Total root number was significantly associated with root length and root volume under moisture stress, but no significant association was detected under the well-watered condition. The association of plant height with root volume and total root number was more dramatic. There was a complete reversal in the association, with the correlation being positive and significant under moisture stress and negative under the well-watered condition.

Different water regimes were at least partially responsible for the differential expression of most QTLs observed in this study, even though how the differential expression of QTLs is triggered by environmental factors remains unknown. Nevertheless, correlated traits have been mapped in similar positions, suggesting their common genetic control. When a single marker is associated with two or more traits, genetic drag may occur when selection is made for an allele at this locus. The effect of

Table 1. Descriptive statistics of 56 doubled-haploid (DH) lines derived from IR64 x Azucena and checks under well-watered and low-moisture-stress conditions.

Traits	Parents		Checks		DH			
	IR64	Azucena	IR20	Moroberekan	Mean	SD	Min.	Max.
65 DAS, well-watered								
Plant height (cm)	27.50 ± 4.50	50.47 ± 5.10	24.33 ± 1.96	58.17 ± 2.63	37.89 ± 1.14	8.51	21.53	54.17
Number of tillers	4.00 ± 1.31	3.00 ± 0.88	8.00 ± 1.42	4.00 ± 0.87	5.00 ± 0.21	1.58	2.00	9.00
Root length (cm)	16.33 ± 2.10	39.60 ± 1.50	14.00 ± 1.20	33.07 ± 3.25	20.48 ± 0.66	4.91	11.83	34.17
Root volume (mL)	13.50 ± 2.98	12.00 ± 1.68	14.33 ± 0.98	9.00 ± 0.81	8.63 ± 0.54	4.05	3.67	19.33
Root thickness (mm)	0.81 ± 0.14	1.14 ± 0.04	0.68 ± 0.03	1.30 ± 0.10	0.98 ± 0.01	0.09	0.81	1.20
Root dry weight (g)	1.60 ± 0.85	1.40 ± 0.48	3.20 ± 0.13	2.30 ± 0.68	1.14 ± 0.06	0.41	0.40	2.07
Shoot dry weight (g)	3.23 ± 0.55	2.47 ± 0.64	6.23 ± 0.18	4.67 ± 0.20	2.28 ± 0.11	0.82	0.70	4.13
Total dry weight (g)	4.80 ± 0.92	3.90 ± 0.76	9.40 ± 0.22	7.00 ± 0.87	3.43 ± 0.14	1.08	1.10	5.83
Total root number	57.00 ± 1.25	56.00 ± 2.64	57.00 ± 1.66	73.00 ± 2.04	47.00 ± 2.00	17.90	11.00	98.00
Root–shoot ratio	0.49 ± 0.21	0.59 ± 0.11	0.51 ± 0.05	0.49 ± 0.11	0.55 ± 0.02	0.18	0.15	1.05
85 DAS, well-watered								
Plant height (cm)	39.47 ± 5.20	66.37 ± 6.40	31.67 ± 1.45	77.40 ± 1.63	45.94 ± 1.17	8.73	31.20	67.60
Number of tillers	14.00 ± 1.00	5.00 ± 0.33	16.00 ± 1.20	4.00 ± 0.33	7.00 ± 0.32	2.36	3.00	14.00
Root length (cm)	17.93 ± 2.00	40.67 ± 1.33	18.60 ± 0.81	22.60 ± 2.10	24.22 ± 1.01	7.59	12.17	54.80
Root volume (mL)	17.00 ± 4.50	20.00 ± 2.31	21.00 ± 1.33	8.00 ± 1.16	13.60 ± 0.69	5.16	4.67	26.00
Root thickness (mm)	0.82 ± 0.10	1.25 ± 0.03	0.66 ± 0.02	1.31 ± 0.02	0.98 ± 0.01	0.11	0.72	1.26
Root dry weight (g)	3.70 ± 0.86	3.40 ± 0.25	5.00 ± 0.12	1.70 ± 1.06	2.00 ± 0.13	0.96	0.70	6.13
Shoot dry weight (g)	4.13 ± 0.17	7.03 ± 0.45	7.23 ± 0.15	4.87 ± 0.27	4.80 ± 0.25	1.90	1.63	10.40
Total dry weight (g)	7.83 ± 1.02	10.43 ± 0.54	12.23 ± 0.15	6.57 ± 1.32	6.80 ± 0.30	2.25	2.67	12.63
Total root number	103.00 ± 1.73	73.00 ± 3.47	68.00 ± 1.20	78.00 ± 2.03	76.00 ± 3.00	23.39	16.00	128.00
Root–shoot ratio	0.88 ± 0.18	0.49 ± 0.07	0.69 ± 0.03	0.33 ± 0.19	0.57 ± 0.10	0.71	0.15	5.43
85 DAS. low-moisture stress								
Plant height (cm)	35.90 ± 2.10	65.43 ± 5.66	32.95 ± 1.55	73.80 ± 5.87	47.36 ± 1.28	9.60	33.03	66.40
Number of tillers	12.00 ± 0.58	5.00 ± 0.88	12.00 ± 1.29	5.00 ± 0.88	6.00 ± 0.26	1.98	3.00	12.00
Root length (cm)	27.00 ± 1.85	45.07 ± 2.49	20.63 ± 0.61	45.33 ± 4.92	25.47 ± 1.11	8.33	17.20	52.53
Root volume (mL)	14.00 ± 2.31	25.33 ± 1.20	8.17 ± 1.41	13.67 ± 0.33	8.41 ± 0.56	4.19	1.50	24.00
Root thickness (mm)	0.80 ± 0.10	1.08 ± 0.04	0.76 ± 0.04	1.06 ± 0.1	0.89 ± 0.01	0.08	0.70	1.12
Root dry weight (g)	2.80 ± 1.50	1.87 ± 0.78	1.83 ± 0.17	1.20 ± 0.4	1.73 ± 0.11	0.80	0.23	4.47
Shoot dry weight (g)	5.30 ± 1.29	4.13 ± 0.74	5.23 ± 0.19	7.30 ± 0.13	3.91 ± 0.20	1.49	1.20	9.23
Total dry weight (g)	8.10 ± 2.72	6.00 ± 1.00	7.06 ± 0.19	8.53 ± 0.43	5.62 ± 0.26	1.97	1.53	11.53
Total root number	62.00 ± 2.72	98.00 ± 1.16	40.00 ± 1.33	25.00 ± 3.84	50.00 ± 2.57	19.24	14.00	102.00
Root–shoot ratio	0.45 ± 0.18	0.49 ± 0.26	0.35 ± 0.12	0.16 ± 0.06	0.49 ± 0.03	1.23	0.10	0.22
Leaf rolling (1–5)	4.00 ± 0.10	4.00 ± 0.28	4.00 ± 0.24	2.00 ± 0.15	4.00 ± 0.14	1.03	1.00	5.00
Drought score (1–5)	3.00 ± 0.17	2.00 ± 0.19	3.00 ± 0.16	2.00 ± 0.13	2.69 ± 0.09	0.66	1.00	4.00

Table 2. Mean values of traits under 85 d after sowing (DAS), well-watered condition and 85 DAS, low-moisture stress and t statistic.

Trait	Units	85 DAS WW[a]	85 DAS S[b]	Paired t-test[c]
Shoot characters				
Plant height	cm	46.46	47.67	ns
Number of tillers	#	7.62	6.52	*
Shoot dry weight	g	4.87	4.01	*
Root characters				
Max. root length	cm	24.27	26.13	ns
Root volume	cm^3	13.87	8.87	**
Root dry weight	g	2.10	1.73	ns
Root thickness	cm	0.98	0.89	**
Number of roots	#	76.64	50.30	**
Whole-plant characters				
Total dry weight	g	6.97	5.74	ns
Derived characters				
Number of roots $tiller^{-1}$	#	11.73	9.20	*
Root-shoot ratio	–	0.59	0.48	ns
Root dry weight $tiller^{-1}$	g	0.31	0.30	**

[a]WW = well-watered treatment. [b]S = low-moisture stress. [c]* & ** = significant at 5% and 1% level of significance, ns = not significant.

genetic drag on marker-aided selection for QTLs may vary considerably depending on the nature and magnitude of the genetic drag.

Differences in trait sensitivity to moisture stress were also made evident by the magnitude of the increase or decrease in each trait value compared with (1) the absolute value of the 85 DAS sample time under well-watered condition and the 85 DAS sample time under moisture-stress conditions and (2) the numerical increase above values obtained at the 65 DAS sampling in the well-watered condition. Table 4 presents the percent increase or decrease at 85 DAS in the well-watered condition and moisture treatment compared with the 65 DAS treatment for the genotypes studied. For most traits, across genotypic classes, the absolute values decreased in the moisture-stress treatment. IR20, the susceptible check, showed higher sensitivity than either IR64 or Azucena because most traits showed a decrease at 65 DAS.

There was consistent behavior across genotypes for three traits—number of tillers $plant^{-1}$, total root number, and root length. Number of tillers and total root number decreased in the DH lines, the parents, and all the checks. The two traits are known to be strongly correlated to each other. Here again, IR20 showed greater sensitivity than other genotypes. Azucena showed a low level of sensitivity for these traits. In IR64, the number of roots was more sensitive to stress than the number of tillers. IR64, Azucena, and IR20 showed an increase in root length under moisture stress compared with the well-watered condition. The magnitude of the increase was the highest in IR64, followed by Azucena, IR20, and the DH line mean (data not shown). For root-to-shoot ratio, all genotypes and DH lines exhibited a significant reduction as indicated by the negative values (change in %) under moisture stress. Interestingly, Azucena

Table 3. Phenotypic (P) and genotypic (G) correlation coefficients among root and shoot characters of IR64/Azucena population under moisture stress. Above the diagonal are moisture-stress conditions, below the diagonal are well-watered conditions.

		PHT	NOT	RTL	RTV	TRN	SDW	RDW	R/S	TDW	RTT	LFR	DRS
PHT	P		–0.08	0.19	0.34**	0.25*	0.34**	0.25*	–0.04	0.38**	0.40**	0.20	0.11
	G		–0.47**	0.24	0.42**	0.25*	0.32**	0.31*	0.14	0.40**	0.61**	0.35**	0.18
NOT	P	–0.31*		0.19	0.20	0.24	0.20	0.26*	0.05	0.27*	4.01	0.27*	0.31**
	G	–0.55**		0.24	0.19	0.29*	0.10	0.33**	0.21	0.19	–0.44**	0.47*	0.20
RTL	P	0.16	0.04		0.44**	0.27*	0.16	0.16	0.10	0.19	0.17	0.15	–0.04
	G	0.14	0.05		0.53**	0.33**	0.14	0.35**	0.33**	0.18	0.22	0.20	–0.05
RTV	P	–0.06	0.40**	0.37**		0.56**	0.38**	0.54**	0.21	0.52**	0.33**	0.16	0.03
	G	–0.19	0.52**	0.43**		0.64**	0.44**	0.76**	0.27*	0.58**	0.73**	0.23	0.05
TRN	P	–0.04	0.20	0.03	0.18		0.34**	0.40**	0.11	0.41**	0.23	0.26*	0.17
	G	–0.07	0.21	0.04	0.14		0.41**	0.81**	0.25*	0.53**	0.46**	0.44**	0.31*
SDW	P	0.18	0.25*	0.31*	0.28*	–0.11		0.40**	–0.33**	0.91**	0.31*	0.13	–0.03
	G	0.22	0.26*	0.38**	0.31*	–0.17		0.37**	–0.46**	0.98**	0.51**	0.25*	–0.01
RDW	P	0.08	0.33**	0.09	0.29*	0.12	0.19		0.55**	0.68**	0.21	0.09	0.14
	G	0.03	0.47**	0.09	0.28*	0.10	0.21		0.58**	0.58**	0.48**	0.39**	0.39**
R/S	P	–0.06	0.05	–0.16	–0.02	0.06	4.43**	0.65**		–0.02	4.01	–0.02	0.26*
	G	–0.13	0.14	–0.24	–0.12	0.09	–0.49**	0.62**		–0.29*	0.24	0.01	0.56**
TDW	P	0.13	0.10	0.13	0.07	–0.02	0.44**	0.30*	–0.04		0.33**	0.15	0.04
	G	0.28*	0.36**	0.33**	0.26*	–0.04	0.86**	0.69**	0.09		0.63**	0.37**	0.14
RTT	P	0.42**	–0.35**	0.16	–0.08	–0.18	0.01	–0.16	–0.06	–0.16		0.12	0.09
	G	0.61**	–0.72**	0.15	–0.18	–0.21	0.01	–0.29*	–0.15	–0.13		0.22	0.20
LFR	P	–	–	–	–	–	–	–	–	–	–		0.33**
	G	–	–	–	–	–	–	–	–	–	–		0.56**

[a] PHT = plant height, NOT = no. of tillers, RTL = root length, RTV = root volume, TRN =total root no. plant^{-1}, SDW = shoot dry weight, RDW = root dry weight, R/S = root-shoot ratio, TDW = total dry weight, RTT = root thickness, LFR = leaf rolling, DRS = drought-resistance score.

Table 4. Percent increase in root and shoot parameters from 65 days after sowing until 85 DAS in the well-watered (WW) and moisture-stress treatments.

Traits	DH lines		Azucena		IR64		IR20		Moroberekan	
	WW	S	WW	S	WW	S	WW	S	WW	S
Plant height	21.11	25.06	31.50	29.70	43.50	30.50	30.10	35.40	33.10	26.90
Number of tillers plant^{-1}	41.39	20.79	77.80	55.60	68.00	44.00	32.70	22.70	0.00	27.30
Root length	18.05	24.39	37.40	62.40	9.80	65.30	32.90	47.40	–31.70	37.10
Total root number plant^{-1}	62.66	7.30	90.40	76.00	81.80	9.41	18.70	–30.40	7.80	–66.50
Root volume	57.59	–2.55	100.00	111.00	25.90	3.70	48.80	–43.00	–11.00	51.90
Root dry weight	75.44	50.00	137.00	30.20	131.00	75.00	56.30	–42.70	–26.00	–47.80
Shoot dry weight	110.53	71.49	185.00	67.60	27.80	63.90	16.00	–16.00	4.29	57.10
Total dry weight	98.83	64.32	168.00	53.80	62.10	67.60	29.70	–25.10	–5.70	22.50
Root thickness	0.00	–9.18	9.26	–5.26	1.92	–0.41	–2.90	12.80	0.40	–18.50
Root-shoot ratio	165.45	–10.91	–16.00	–16.10	80.40	–7.91	34.60	–30.90	–33.00	–66.80

showed no change in the root-to-shoot ratio. Azucena appeared to be able to maintain a certain ratio even under the moisture stress, although there was a reduction in both shoot and root dry weight.

Under the well-watered treatment, IR64 had greater shoot and total dry matter than Azucena and IR20. In contrast, Azucena had a greater total root volume than IR64 under moisture stress.

QTL mapping of traits under the well-watered condition

For the 65 DAS treatment, 15 QTLs controlling the measured traits were detected (Table 5). Two QTLs for plant height on chromosomes 1 and 3 individually explained 22.1% and 27% of the phenotypic variance, respectively. A total of five genomic segments on chromosomes 1, 6, 7, and 10 were associated with total root number. Azucena alleles at all QTLs but the one on chromosome 6 caused a decreased total root number (Table 5, Fig. 1). Four QTLs affecting root thickness were identified on chromosomes 2, 5, 8, and 9. The QTL flanked by RG157 and RZ318 on chromosome 2 was detected at a LOD of 3.21 and explained 26.7% of total phenotypic variance (Fig. 1).

For the 85 DAS treatment, we also identified 15 QTLs for the traits recorded in this data set (Table 5). Three QTLs for plant height were mapped to three of the 12 chromosomes (Fig. 1). Two QTLs were mapped to chromosomes 1 and 3 (LOD scores of 3.63 and 3.42, respectively) and accounted for 29.2% and 27% of phenotypic variance, respectively. Only one significant QTL, for maximum root length, was mapped to chromosome 4 at a LOD of 2.07. Azucena alleles at this locus decreased maximum root length by 3.04 cm. Five QTLs controlling number of tillers were mapped, one each to chromosomes 1, 3, and 4 and two to chromosome 8. The QTLs on chromosomes 4 and 8 were detected at LOD scores of 2.51 and 2.80, respectively. The IR64 allele at the QTL on chromosome 4 increased tiller number, while the QTL on chromosome 8 decreased tiller number plant^{-1}. Two QTLs affecting root thickness were mapped to chromosome 2 flanked by RG157 and RZ318, and *Pall* and RZ58.

The interval mapping results of "65 DAS, well-watered" and "85 DAS, well-watered" were compared to identify QTLs that were common for the two data sets representing two developmental stages of the vegetative phase. Two QTLs for plant height were common between the two stages, with one QTL mapping to the RZ730-RZ801 region on chromosome 1. This genomic segment is closely linked to the map position of *sd-1,* a major gene controlling semidwarfism (Cho et al 1994). Similarly, the second common QTL controlling plant height was closely mapped to RZ448 on chromosome 3. Further, a locus influencing root thickness marked by RG157 and RZ318 on chromosome 2 was detected in both stages. In total, 15 QTLs were detected in both the "65 DAS, well-watered" and "85 DAS, well-watered" conditions. Of these, only three QTLs were common between the two stages. It appears that different sets of QTLs "show up" under different developmental stages within the vegetative stage itself.

Table 5. Putative QTLs identified under two growth stages and contrasting moisture regimes imposed during the vegetative stage.

Traits	Sampling[a]	Flanking markers	QTL[b]	Chromo-some	Vari-ance[c]	Addi-tivity[d]	LOD
Plant height (cm)	65 DAS	RZ730-RZ801	*sd1*	1	22.1	4.32	2.53
		RZ448-RZ337A	qPHT3-1	3	27.0	4.74	3.03
	85 DAS	RZ730-RZ801	*sd1*	1	29.2	4.96	3.63
		RZ519-RZ448	qPHT3-2	3	27.0	4.66	3.42
		RG958-CD0344	qPHT12-1	12	16.9	3.75	2.04
	85 DAS[e]	RZ730-RZ801	*sd1*	1	24.9	5.19	2.79
		RZ519-RZ448	qPHT3-2	3	16.1	4.04	1.92
No. of tillers (#)	65 DAS	RZ-143-RG20	qNOT8-1	8	15.3	0.62	1.94
	85 DAS	RG810-RG331	qNOT1-1	1	15.8	-0.96	2.10
		RZ448-RZ519	qNOT3-2	3	15.8	–0.97	1.95
		RG449-RG788	qNOT4-2	4	19.8	-1.15	2.51
		AlOK250-AG8Aro	qNOT-8-1	8	21.3	1.16	2.80
		AC5-RG418B	qNOT8-4	8	14.3	-0.99	1.73
	85 DAS[e]	RG171-RG157	qNOT2-1	2	15.5	-0.99	1.63
		RZ329-RG348	qNOT3-1	3	14.6	-0.82	1.81
		RG91-RG449	qNOT4-1	4	12.4	-0.77	1.61
		RZ617-RG978	qNOT8-3	8	25.7	1.07	3.54
Root length (cm)	65 DAS	RG214-RG143	qRTL4-2	4	12.7	-1.74	1.65
	85 DAS	RG163-RZ590	qRTL4-1	4	16.0	-3.04	2.07
	85 DAS[e]	RG381-RZ19	qRTL1-1	1	13.7	3.25	1.64
		RG171-RG157	qRTL2-1	2	15.4	-4.01	1.62
Total root number (#)	65 DAS	RG472-RG246	qTRN1-1	1	14.1	-6.88	1.81
		W1-RG173	qTRN1-2	1	24.5	-10.93	3.00
		RG477-PGMS0.7	qTRN7-1	7	15.7	-7.25	2.06
		RG134-RZ500	qTRN10-1	10	13.4	-6.79	1.75
	85 DAS	PGMS0.7-CD059	qTRN7-1	7	15.3	-9.88	1.58
	85 DAS[e]	RG171-RG157	qTRN2-1	2	17.4	-9.96	1.80
		Pdl-RZ58	qTRN2-2	2	25.1	-11.02	2.69
		RZ123RG520	qTRN2-3	2	17.7	-8.15	2.26
Root volume (cm^3)	65 DAS	RG403-RZ556	qRTV5-1	5	13.5	-1.51	1.60
		RZ67-RZ70	qRN5-3	5	15.1	-1.70	1.82
	85 DAS	RZ337B-CD0497	qRTV7-1	7	14.3	-2.01	1.74
	85 DAS[e]	RG171-RG157	qRTV2-1	2	15.6	-2.07	1.74
		RG104-RG348	qRTV3-1	3	21.4	-1.99	2.89
Root thickness (cm)	65 DAS	RG157-RZ318	qRTT2-1	2	26.7	0.05	3.21
		RG313-RZ556	qRTT5-1	5	19.1	0.04	2.41
		AC5-RG418B	qRTT8-1	8	21.6	0.04	2.94
	85 DAS	RG157-RZ318	qRTT2-2	2	16.7	0.05	1.66
		Pal1-RZ58	qRTT2-3	2	12.4	0.04	1.59
Root dry weight (g)	85 DAS	RG171-RG157	qRTW2-1	2	13.3	-0.42	1.58
	85 DAS[e]	RG348-RG104	qRDW3-1	3	20.8	-0.38	2.80
Root-shoot ratio	85 DAS	RG173-Amyl8	qRS1-1	1	12.7	-0.31	1.63
Drought score (1-5)	85 DAS[e]	RG908-RG91	qDRS4-1	4	14.4	-0.27	1.80
		RG181-RG958	qDRS12-1	12	16.1	0.27	2.03
Leaf rolling (1-5)	85 DAS[e]	PGMS0.7-CD059	qLFR7-1	7	11.9	0.36	1.55

[a]QAS = days after sowing. [b]QTL names as suggested by McCouch et al (1997). [c]Percentage of phenotypic variance explained by the locus. [d]Phenotypic effect because of substitution of the IR64 allele by the Azucena allele. [e]Represents low-moisture-stress treatment.

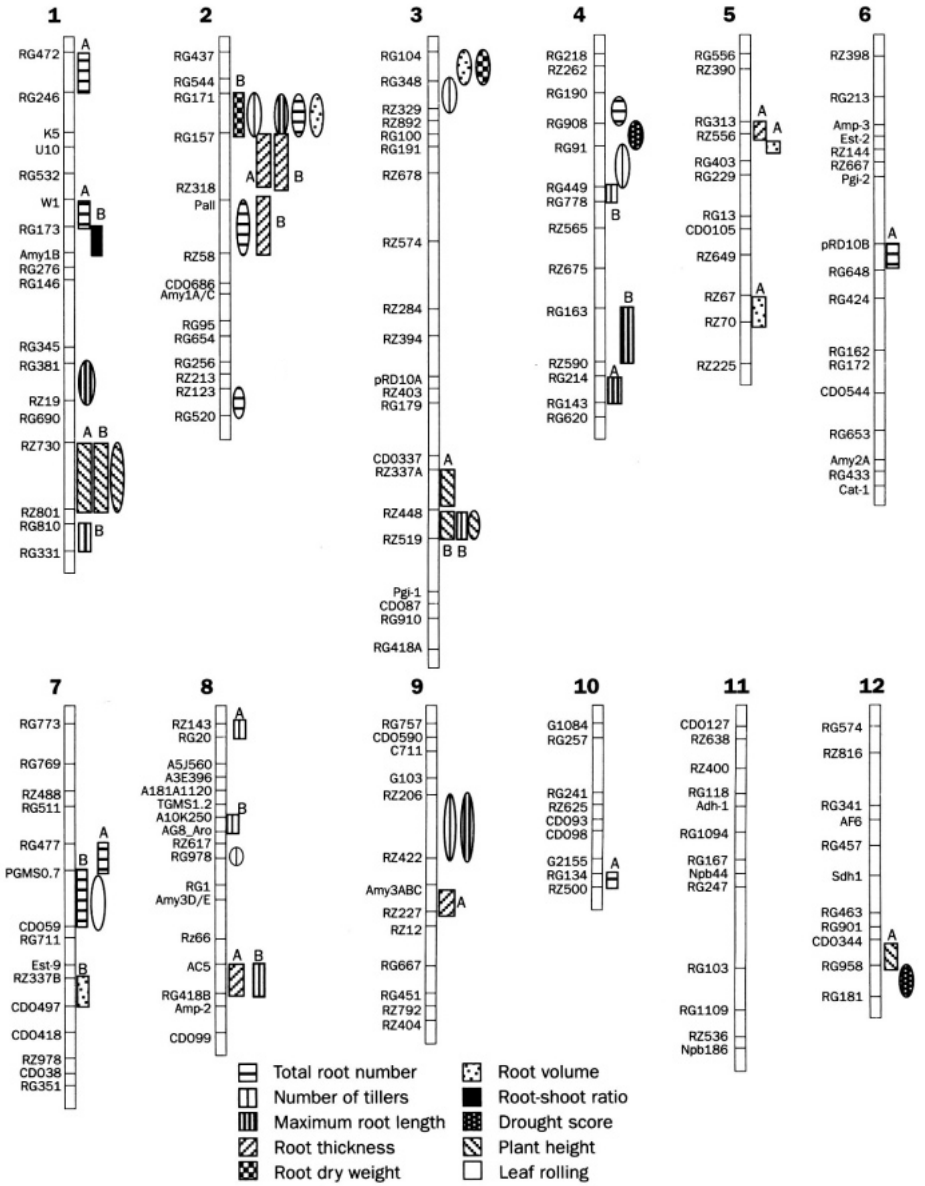


Fig. 1. Putative QTLs associated with root and shoot morphological traits at two different developmental stages and two moisture regimes in doubled-haploid lines of the IR64/Azucena mapping population. DAS = days after sowing.

QTL mapping of traits—85 days after sowing, moisture-stress condition

A total of 20 QTLs controlling different traits were distributed on 11 out of 12 rice chromosomes; however, no QTL was found for root thickness. Two QTLs were associated with plant height on chromosomes 1 and 3 and were within the intervals RZ730-RZ801 and RZ519-RZ448, respectively (Table 5, Fig. 1). Chromosomes 2, 3, 4, 8, and 9 contained a total of five QTLs for number of tillers. The QTL on chromosome 8 flanked by RZ617 and RG978 was detected at a LOD of 3.54 and explained 25.7% of total phenotypic variance. Two QTLs affecting drought score were detected on chromosomes 4 and 12 and explained 14.4% and 16.1% of phenotypic variance, respectively. Three QTLs controlling maximum root length were mapped to chromosomes 1, 2, and 9. The locus on chromosome 9 flanked by RZ206 and RZ422 accounted for 14.7% of phenotypic variance. The allele from the male parent (Azucena) increased the maximum root length by 7.02 cm. Similarly, the allele of Azucena at the locus flanked by RG171 and RG157 (chromosome 2) decreased the maximum root length by 4 cm. Two QTLs affecting root volume were mapped to chromosomes 2 and 3. The locus on chromosome 3 explained 21.4% of total phenotypic variance for root volume and had an additive effect of 1.99 cm^3. Three genomic segments on chromosome 2 and one on chromosome 4 were associated with total root number, all of which had decreasing effects on the traits compared to the phenotypic means of plants carrying the IR64 alleles. Four QTLs of decreasing effect on total root number $plant^{-1}$ were mapped to chromosomes 2 and 4. One of the QTLs on chromosome 2 flanked by *Pal1* and RZ58 was detected at a LOD of 2.69 and explained 25.1% of phenotypic variance. Only one locus had a decreasing effect on root dry weight and it was located on chromosome 3. The genomic segment flanked by PGMS0.7 and CDO59 on chromosome 7 was associated with leaf rolling. It accounted for 11.9% of phenotypic variance for the trait.

QTLs specific to moisture-stress condition

The interval analysis results of "85 DAS, well-watered" and "85 DAS, moisture stress," representing two moisture regimes, were compared to identify QTLs specific to moisture stress. In total, 21 QTLs controlling different traits were detected under the moisture-stress condition, and 15 QTLs were detected under the 85 DAS, well-watered condition at a threshold of 1.5. Of these, the only locus common between the two contrasting moisture regimes was for plant height on chromosomes 1 and 3. The nonoccurrence of common QTLs for root traits between the two moisture regimes may have been due to the existence of a parallel biochemical, physiological, or genetic pathway for plant growth and development. The study consisted of only 56 genotypes from an indica-japonica cross, but it involved two contrasting moisture regimes and sampling at two developmental stages. Ceccarelli and Grando (1993) reported that yield under stress and yield potential (under well-watered growth conditions) are mutually exclusive events by repeated testing of a large number of genotypes in barley. Beavis and Keim (1996), in a study with the F_{2-4} generations of maize, reported that "no QTLs were detected consistently across all environments. QTLs

were identified that were unique to either stress or nonstress environments." Austin and Lee (1998) reported similar results in their study involving yield and yield components under stress and nonstress environments. Yamaguchi-Shinozaki and Shinozaki (1997) observed four parallel signal transduction pathways for the perception of stress stimuli and triggering expression of stress-responsive genes.

QTLs that are expressed specifically under a moisture-stress environment need to be observed for their coincidence with QTLs for yield under drought. The ultimate aim is to design an ideotype that is drought-resistant and at the same time produces an acceptable grain yield under moisture-limiting growth conditions. Therefore, it is important to conduct yield trials to detect QTLs for yield traits and to test the coincidence of map positions of QTLs for yield under stress, root morphological traits, shoot morphological traits, and physiological traits under moisture-stress conditions induced at the appropriate developmental stage of the crop.

Evidence of pleiotropy or linkage

Interval analysis revealed chromosomal regions where QTLs affecting more than one trait were mapped. This congregation may be due to tight linkage, pleiotropy, or a causal relationship between traits. Developmentally correlated traits (such as number of tillers and number of roots in rice) mapping to the same chromosome regions have been reported by Paterson et al (1991) in tomato, Lebreton et al (1995) in maize, and Xiao et al (1996) and Yadav et al (1997) in rice. From the results of interval mapping of "85 DAS, well-watered," we found that the chromosomal segment flanked by RZ519 and RZ448 on chromosome 3 contained QTLs for plant height and number of tillers plant^{-1} (Table 5, Fig. 1). The alleles on this locus had an increasing effect on plant height but a decreasing effect on number of tillers plant^{-1} . It is interesting to note that these two traits show a significant negative correlation with each other in the works of Yoshida and Hasegawa (1982) and Hemamalini (1997). Chromosomal regions with multiple QTLs were also found under moisture-stress conditions. A locus flanked by RG171 and RG157 (chromosome 2) contained QTLs for total root number plant^{-1} , number of tillers plant^{-1} , maximum root length, root dry weight, and root volume. Researchers have found that these traits possess strong correlations among themselves (Ekanayake et al 1985, IRRI 1982, 1984, Salam and Subramanian 1988, Shahid et al 1994). In the current study, a locus on chromosome 9 flanked by RZ206 and RZ422 was associated with number of tillers and maximum root length, two traits known to show a strong positive correlation with each other. Interestingly, the locus contributed positively to both traits. Fine-mapping of such chromosomal regions will help us discern the actual genetic control of these congruent traits. If common primers or other markers can be identified for such traits, combined selection for them is a possibility.

Identifying primers associated with root length

In a related study from this laboratory, we adopted the strategy of bulk segregant analysis using 10 contrasting accessions differing for root length (± 2 standard devia-

tions from the mean) and 500 random primers (10mer), and we were able to identify one primer co-segregating with root length. The 500-kb band was associated with deep rooting habit. QTL mapping indicated that the derived polymorphism maps to RG381 on chromosome 1 and G1084 on chromosome 10. The loci on chromosome 1 flanked by RG381-RZ19 is associated with root length under moisture stress in this population (Hemamalini 1997). Mapmaker/QTL showed that these loci accounted for 24.4% and 21.4% of the phenotypic variance for maximum root length, respectively. The LOD values were 4.2 and 4.6, respectively.

Stable QTLs for root traits over different environments and populations

We compared QTLs detected in our investigation with those identified by Yadav et a1 (1 997) in DH lines of IR64/Azucena to identify stable QTLs over environments. Three QTLs responsible for root length located on chromosomes 1, 2, and 9 were also detected by Yadav et al (1997). These regions were located in the regions flanked by RG381 and RZ19 (chromosome 1) and by RG171 and RG157 (chromosome 2), and in the region flanked by RZ206 and *Amy3ABC* (chromosome 9). Similarly, two QTLs for root thickness were common between the two studies. They were on chromosomes 2 and 8 flanked by *Pal1* and RZ58 and AC5 and RG418B, respectively. One locus flanked by RZ422 and *Amy3ABC* on chromosome 9 influencing root-to-shoot dry weight was also common. We found that common QTLs exhibited allelic effects in the same direction. The consistency of QTLs across different environments and mapping populations is essential for marker-aided selection to be practiced. Our study identified some molecular markers linked to root traits and moisture-stress-induced root responses, which will help in molecular fine-mapping to identify tightly linked markers. This can help us understand the genetic interactions among traits contributing to drought resistance and probably allow us to answer questions on pleiotropic or tight linkage. In our molecular marker-assisted breeding program, where the locally well-adapted, deep-rooted, drought-resistant, poor-yielding genotype is used as a female, we need to select for higher grain yield, which we expect to be contributed by an improved semidwarf male parent.

A marker-assisted selection strategy for drought resistance should therefore also consider the trait-specific loci expressed under moisture stress. Certain chromosomal regions or loci may influence several traits simultaneously, because of either pleiotropy or tight linkage. Specialized plant materials such as chromosomal substitution lines for specific QTLs can be developed to distinguish the underlying genetic causes. We are using flanking markers associated with root length and root thickness on chromosomes 1 and 2 to identify YAC and BAC clones from genomic and cDNA libraries. The DH lines from the mapping population are being tried in farmers' fields and under artificially imposed moisture-stress situations to quantify the association of desirable root morphology with drought tolerance and quantify grain yield loss under drought conditions.

References

Ali L. 1999. Mapping quantitative trait loci for root traits related to drought resistance in rice (*Oryza sativa* L.) using AFLP markers. Ph.D dissertation. Texas Tech University, Lubbock, Texas, USA.

Arraudeau MA. 1989. Breeding strategies for drought resistance. In: Baker FWG, editor. Drought resistance in cereals. Wallingford (UK): CAB International. p 107-116.

Austin DF, Lee M. 1998. Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and nonstress environments. Crop Sci. 38:1296-1308.

Basten, CJ, Zhao BZ, Bruce SW. 1996. QTL Cartographer: a suite of programs for mapping QTL. Abstracts. Plant Genome IV International Conference on Status of Plant Genome Research, San Diego, Calif., USA, 14-18 Jan. 1996.

Beavis WD, Keim P. 1996. Identification of QTL that are affected by the environment. In: Kang MS, Hugh HG, editors. New perspective on genotype-by-environment interactions. Boca Raton, Fla. (USA): CRC Press, Inc.

Bidinger FR, Witcombe JR. 1989. Evaluation of specific drought avoidance as selection criteria for improvement of drought resistance. In: Baker FWG, editor. Drought resistance in cereals. Wallingford (UK): CAB International. p 151 - 164.

Brady NC. 1985. The nature and properties of soils. New York (USA): MacMillan Publishing Company.

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J. Wu K, Xiao JZ, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population genetics. Genome 138:1251-1274.

Ceccarelli S, Grando S. 1993. From conventional plant breeding to molecular biology. International Crop Science 1. Madison, Wis. (USA): Crop Science Society of America. p 533-538.

Champoux MC, Wang G, Sarkarung SS, Mackill DJ, O'Toole JC. Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Chen X, Temykh S, Xu Y, Cho YG, McCouch SR. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet. 95:553-567.

Cho YG, Eun MY, McCouch SR, Chae YA. 1994. The semidwarf gene, *sd-1,* of rice (*Oryza sativa* L.). II. Molecular mapping and marker-assisted selection. Theor. Appl. Genet. 90:54-59.

Chopra RK, Sinha SK. 1998. Prospects of success of biotechnological approaches for improving tolerance to drought stress in crop plants. Curr. Sci. 74(1):25-34.

Doerge RW. 1993. Statistical methods for locating quantitative trait loci using molecular markers. Ph.D dissertation, North Carolina State University, Raleigh, North Carolina, USA.

Ekanayake IJ, O'Toole JC, Garrity DP, Masajo TN. 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Sci. 25:927-933.

Fukai S, Cooper M. 1995. Development of drought resistant cultivars using physiomorphological traits in rice. Field Crops Res. 40:67-86.

Guiderdoni E, Galinato E, Luistro J, Vergara G. 1992, Anther culture of tropical japonica × indica hybrids of rice. Euphytica 62:219-224.

Hanson AD, Peacock WJ, Evans LT , Arntzen CJ, Khush GS. 1990. Drought resistance in rice. Nature 234:2.

Hemamalini GS. 1997. Molecular mapping of low moisture stress induced response in rice (*Oryza sativa* L.) roots at peak vegetative stage. M.Sc. thesis submitted to the University of Agricultural Sciences. GKVK, Bangalore, India.

Huang N, McCouch SR, Mew S, Parco A, Guiderdoni E. 1994. Development of an RFLP map from a double haploid population in rice. Rice Genet. Newsl. 11:134-137.

Huang N, Parco A, Mew T, Magpantay G, McCouch SR, Guiderdoni E, Xu J, Subudhi P, Angeles RE, Khush GS. 1997. RFLP mapping of isozymes, RAPD and QTLs for brown planthopper resistance in a doubled haploid rice population. Mol. Breed. 3:105-113.

Ingram KT, Bueno FD, Namuco OS, Yambao EB, Beyrouty CA. 1994. Rice root traits for drought resistance and their genetic variation. In: Kirk GJD, editor. Rice roots: nutrient and water use. Manila (Philippines): International Rice Research Institute. p 67-70.

IRRI (International Rice Research Institute). 1980. Annual report for 1979. Los Baños (Philippines): IRRI. p 73-99.

IRRI (International Rice Research Institute). 1982. Annual report for 1981. Los Baños (Philippines): IRRI. p 77-99.

IRRI (International Rice Research Institute). 1984. Annual report for 1983. Los Baños (Philippines): IRRI. p 85-100.

IRRI (International Rice Research Institute). 1995. Challenges and opportunities in a less favorable ecosystem: rainfed lowland rice. IRRI Information Series No. 1. Los Baños (Philippines): IRRI. p 8-12.

Kurata N, Nagamura Y, Yamamoto A, Harushima Y, Sue N, Wu J, Antonnio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimona T, Kuboki Y, Momma Y, Umehara Y, Yano M, Sasaki T, Minobe Y. 1994. A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet. 8:365-372.

Lander ES. 1993. MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1. Cambridge, Mass. (USA): Whitehead Institute.

Lebreton C, Lazic-Jancic V, Steed A, Pekic S, Quarrie SA. 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. J. Exp. Bot. 46:853-865.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Ludlow MM, Muchow RC. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.

McCouch, SR, Cho YG, Yano M, Paul E, Blinstrub M, Morishima H, Kinoshita T. 1997. Report on QTL nomenclature. RGN 14:11-13.

McWilliam JR. 1989. The dimensions of drought. In: Baker FWG, editor. Drought resistance in cereals. Wallingford (UK): CAB International. p 1-11.

Nguyen HT, Chandra Babu R, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37: 1426-1434.

O'Toole JC. 1989. Breeding for drought resistance in cereals. In: Baker FWG, editor. Drought resistance in cereals. Wallingford (UK): CAB International. p 107-116.

O'Toole JC, Moya JB. 1978. Genotypic variation in maintenance of leaf water potential in rice. Crop Sci. 18:873-876.

Paterson AD, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Tanksley SD. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127: 181-197.

Price AH, Tomas AD, Virk DS. 1997. Genetic dissection of root growth in rice (*Orysa sativa* L.). I. A hydroponic screen. Theor. Appl. Genet. 95:132-142.

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice. Theor. Appl. Genet. 92:627-633.

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata M, Sugiura M. 1991. Linkage map of restriction fragment polymorphism loci in rice. Jpn. J. Breed. 41:665-670.

Salam MA, Subramanian S. 1988. Relationship between root weight and plant characters in rice in different seasons. Indian J. Agric. Sci. 58(9):710.

Shahid M, Latif T, Iqbal N, Khan MA. 1994. Genetic studies on drought tolerance in rice. Sarhad J. Agric. 10(6):617-674.

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES. 1992. Identification of genetic factors contributing to heterosis in hybrids from two elite maize inbred lines using molecular markers. Genetics 132:823-839.

Xiao J, Li J, Yuan L, Tanksley SD. 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor. Appl. Genet. 92:230-244.

Yadav RS, Courtois B, Huang N. 1997. Mapping genes controlling root morphology in a doubled haploid population of indica × japonica cross of rice. Theor. Appl. Genet. 94:619-632.

Yamaguchi-Shinozaki K, Shinozaki K. 1997. Gene expression and signal transduction in water-stress response. Plant Physiol. 115:327-334.

Yoshida S, Hasegawa S. 1982. The rice root system: its development and function. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 53-68.

Zheng HG, Pathan MS, Chandra Babu R, Ali L, Huang N, Courtois B, Nguyen HT. 1996. Molecular mapping of QTLs associated with root penetration ability in rice. Plant Genome IV, The International Conference on the Status of Plant Genome Research, San Diego, Calif. Abstract no. P125.

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Mapping root and shoot traits in rice: experience in UK, IRRI, and WARDA

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A mapping population of 205 recombinant inbred lines derived from a cross between two drought-tolerant upland rice varieties, Azucena and Bala, has been used to identify genomic regions contributing to drought tolerance. We describe analyses of screens conducted in the dry season at the International Rice Research Institute (IRRI) over two seasons and at the West Africa Rice Development Association (WARDA) over one season. Performance under drought was assessed visually as leaf rolling and leaf drying and by measuring relative water content. Using a combined RFLP and AFLP map with 134 markers, quantitative trait loci (QTLs) with LOD >2.0 detected using composite interval mapping were found on all chromosomes except 4, 6, 8, and 11. The same population has been used to locate QTLs associated with root morphology assessed in two different greenhouse experiments in the UK. Maximum root length and root thickness traits were mapped and showed considerable plasticity. We discuss the results in the context of previous work on drought screening and root-morphology mapping with other populations and root penetration QTLs identified in this population. We also discuss progress toward identifying valuable regions for marker-assisted breeding.

Improving drought tolerance in rice is a goal of breeders targeting rainfed environments. The subject has been reviewed by Fukai and Cooper (1995) and Nguyen et al (1997). Identifying quantitative trait loci (QTLs) that contribute to drought tolerance should speed the achievement of that goal by facilitating a marker-assisted breeding approach (see Price and Courtois 1999). One characteristic considered of particular value in conferring drought avoidance in rice is the possession of a deep and thick root system, which allows access to water at depth. The co-location of QTLs for reaction to drought stress in the field with QTLs for root morphology would firmly establish the value of root traits in drought tolerance in rice. This has been successfully achieved in one rice population (Champoux et al 1995), but it has yet to be shown whether other crosses or the use of diverse screening environments will confirm these encouraging results.

An F_2 population derived from a cross of two drought-tolerant upland rice varieties, Azucena and Bala, has been used to locate QTLs theoretically relevant to drought

avoidance in rice (Price et al 1997a,b, Price and Tomos 1997). QTLs were reported for maximum root length, adventitious root thickness, and the speed of both leaf rolling and stomatal closure in excised leaves. The F_2 population has been advanced to F_6 by single-seed descent and used to conduct replicated field assessments of performance under drought. Screens of root morphology have also been conducted under diverse environmental conditions. These studies primarily aimed to identify regions of the genome that may be of value when breeding more drought-tolerant rice and to evaluate the contribution of root growth to drought tolerance. A linkage map with restriction fragment length polymorphism (REP) and amplified fragment length polymorphism (AFLP) markers has been produced and QTLs for root penetration ability have been identified (Price et al, unpublished results). Here we analyze some of the results (which will be published more fully elsewhere) to give an overview of progress being made in mapping for drought tolerance and root growth-related traits in this cross.

Materials and methods

Plant material

F_6 seeds were collected from a total of 205 F_5 plants produced by single-seed descent from an original set of 310 F_2 plants derived from a cross between varieties Bala and Azucena.

Drought screening at IRRI, 1996

A total of 176 lines plus both parental varieties were sown on 23 January 1996. The layout was a factorial design replicated twice with a fully irrigated and a stressed treatment. Each plot consisted of 3.0 × 3.0-m-long rows of plants spaced 0.1 m apart, with 0.25 m between rows. Every 20 plots, four check-variety plots were sown (check varieties IR20, IR50, Moroberekan, and IRAT 104, only data from Moroberekan are presented here). Sprinkler irrigation was given every other morning for 2 h, enough to wet the soil profile. Watering was withheld from two replicates on 2 March (40 d after sowing). On 19 March (after 18 d of stress), 18 mm of rain fell. After 12, 14, 17, 21, and 24 d of stress, the degree of leaf rolling was assessed visually according to the standard evaluation system of IRRI (International Rice Testing Program 1996). These assessments were averaged to give a mean leaf rolling score over the drought period (I96LRA). Table 1 shows the abbreviations used for all the traits presented here. After 26 and 28 d of stress, the degree of leaf drying was assessed visually according to the standard evaluation system of IRRI, and these two assessments were averaged to give a mean leaf dryness score (I96LDA).

Drought screening at IRRI, 1998

A total of 118 lines plus the two parental lines were sown in 0.75 × 2.0-m plots according to an a-lattice design with two replications on 12 December 1997 and irrigated 3 times weekly by sprinkler until 27 January 1998. Thereafter, water was withheld (starting at 45 d after sowing) and no rain fell during the stress period. Leaf rolling (after 14, 16,

Table 1. Abbreviations for traits presented.

Abbreviation	Description
Drought-reaction traits	
196LRA	IRRI 1996 season, leaf rolling average
196LDA	IRRI 1996 season, leaf drying average
198LRA	IRRI 1998 season, leaf rolling average
198LDA	IRRI 1998 season, leaf drying average
198RWCA	IRRI 1998 season, relative water content average
WLDA	WARDA, leaf drying average
WLR16	WARDA. leaf rolling after 16 d drought
Root morphology traits	
ACRL	Aberdeen control, maximum root length
ACRT	Aberdeen control, adventitious root thickness
ADRL	Aberdeen drought, maximum root length
ADRT	Aberdeen drought, adventitious root thickness
BCRL28	Bangor control, maximum root length after 28 d
BCRL35	Bangor control, maximum root length after 35 d
BCDRW	Bangor control, deeproot dry weight
BCRTB	Bangor control, root thickness at base
BCRTD	Bangor control, root thickness deep (at 75-cm depth)
BDRL28	Bangor drought, maximum root length after 28 d
BDRL35	Bangor drought, maximum root length after 35 d
BDDRW	Bangor drought, deeproot dry weight
BDRTB	Bangor drought, root thickness at base
BDRTD	Bangor drought, root thickness deep (at 75-cm depth)

18, and 22 d of drought), leaf drying (after 20, 22, and 23 d of drought), and relative water content (after 18 and 23 d of drought) were assessed and averaged as above to give I98LRA, I98LDA, and I98RWCA.

Drought screening at WARDA, 1997

A total of 142 lines plus the parental varieties were sown in three replicated plots in a plot size, layout, and randomization similar to the screen at IRRI in 1996. After every eight experimental plots, one plot of a check variety (Moroberekan, OS6, or SP14, only data from Moroberekan are presented here) was included. Seeds were sown on 11 January 1997 and watered by boom irrigation every other day. On 5 February (25 d after sowing), water was withheld from two of the replicates for 30 d. After 14, 16, 18, 20, 22, 24, 26, 28, and 30 d of stress, the degree of leaf drying was assessed visually. As with the IRRI data, an average leaf drying score was calculated (WLDA). In addition, data from a single assessment of leaf rolling score taken on the 16th day of drought stress are presented (WLR16).

Aberdeen soil box screen of root growth, 1997

Plants were grown in soil-filled nylon bags within a box of soil, modified from Townend (1995) and Townend and Dickinson (1995). A box 2 × 1 × 1 m (length × depth ×

width) was constructed from plywood. The box contained 170 plants in a 10 × 17 pattern placed at 0.1 × 0.12-m spacing. The roots of each plant were held within a porous nylon fabric bag (0.9 m deep × 0.08 m diameter). Each bag was filled with soil (sandy loam, pH 6.0) and the spaces between bags were filled with the same soil. The soil within the bags was therefore in hydraulic contact with that outside the bags and hence with all parts of the container. Plants were grown in Aberdeen in a greenhouse with air temperatures between 25 and 37 °C, under daylight plus supplementary lighting (16-h photoperiod at >250 μmol m^{-2} s^{-1} PAR), during the summer of 1997. In the first (control) screen, the box was watered regularly (matric potential −5 to −20kPa); in a subsequent (drought) screen, it was not watered (matric potential -490 kPa, decreasing to -1,480 kPa). After 4 wk, the soil was washed from each plant and maximum root length (ACRL and ADRL) and the thickness of the thickest root taken from the base of each plant (near the stem, ACRT and ADRT) were measured.

Bangor thin chamber screen of root growth

A total of 300 soil-filled chambers were made by taping two sheets of 4-mm-thick glass (1.2 × 0.3 m depth × length) 15 mm apart and filling the space with approximately 5 L of soil (sandy loam, pH 5.5). These chambers were grouped in stacks of six, leaned at an angle of 15° from vertical, and covered to prevent light and radiant heat entry. Before sowing, each chamber was saturated with Yoshida's nutrient solution, pH 5.5 (Yoshida et a1 1976). For 140 F_6 lines and five replicates of both parental varieties, two seedlings were grown in each of two randomly arranged chambers. One chamber did not receive further water or nutrient (drought treatment). The other chamber was given 1 L of nutrient solution three times a week for the first 4 wk. Thereafter, it received nutrient every day, and after 6 wk received 1 L of water in addition to 1 L of nutrient every day. Plants were grown for 8 wk in a greenhouse (minimum temperature 25 °C) in Bangor during the summer of 1997, under daylight supplemented by 150 μmol m^{-2} s^{-1} PAR. Twice a week, the position of the chambers within a stack was rotated to remove position-within-stack effects. Many parameters were measured, but only those relevant to this report are detailed here. After 28 and 35 d, the length of the longest visible root was recorded (BCRL28 and 35, BDRL28 and 35). After 8 wk, the roots were washed and sections of the three thickest roots were removed both from the base of the plant (BCRTB and BDRTB) and at 75-cm depth (BCRTD and BDRTD). These were stored in 50% ethanol prior to measurement of thickness. The entire root system was divided into four sections by cutting at 25-, 50-, and 75-cm depth, and each section was dried and weighed. The dry weight of the bottom section (below 75 cm) was used for analysis here (BCDRW and BDDRW).

DNA extraction and marker analysis

DNA of the F_6 recombinant inbred lines was extracted as described in Price and Tomos (1997). DNA was restricted with *Bam*H1, *Dra*l, *Eco*R1, *Hind*III, and *Xba*l enzymes.

A total of 100 RFLP markers were used to build a linkage map. Those with the prefix RG, RZ, or CDO were obtained from Cornell University, USA (see Causse et al 1995), and those with the prefix C, G, or R were from the Rice Genome Project, Japan (see Kurata et al 1994). Probes were labeled with digoxigenin (Boehringer Mannheim) by polymerase chain reaction using M13 primers and detected following the manufacturer's instructions. AFLP analysis was conducted according to Vos et al (1995) using *Mse*1 and *Eco*R1 restriction enzymes and adapters.

Molecular map construction

The linkage map was constructed using Mapmaker 3.0 (Lander et al 1987, Lincoln et al 1992) with the Haldane algorithm after all heterozygote data had been entered as missing data. Chromosomes were oriented with the short arm at the top following the data reported by Singh et al (1996).

Statistical analysis

Initially, the data contained variation because of position as well as variation caused by genotype and other environmental factors. All data except IRRI 1998 data were corrected for spatial variation by using linear regression to fit equations of the form

$$z = aX + bX^2 + cY + dY^2$$

where z is the measured data value and X and Y are the coordinates of a plant within the experimental area. Regressions were fitted separately for drought and control plants for each measured parameter. In each case, the fitted values were subtracted from the measured values. This removes spatial variation. The overall mean for that parameter in either drought or control conditions was then added to these values to give estimates of the measured values, corrected for positional effects in the plot.

All data were normally distributed except ADRL, ADRT, BDRL28, and BDRL35. None of these traits could be improved by transformation.

QTL analysis

QTL analysis was achieved by composite interval mapping conducted using QTL Cartographer (by C.J. Basten, B.S. Weir, and Z.B. Zeng, Department of Statistics, North Carolina State University) with the default settings for model 6 (five background markers and window size of 10 cM). All skewed markers were removed from the map before QTL analysis, including the bottom of chromosome 7. A result was considered significant using a probability of $P < 0.01$ when the F statistic was greater than 9.2 (equivalent to a LOD score of 2.0, C.J. Basten, personal communication). These relatively low threshold values were used because they facilitate comparisons between screens.

Results and discussion

Drought screening at IRRI and WARDA

Leaf rolling, leaf drying, and relative water content can be used as indicators of plant water status and hence drought-stress severity. Table 2 shows the leaf rolling and drying scores of the parents, F_6 population, and drought-tolerant check (Moroberekan) in three drought screens. The Azucena parent displayed greater leaf rolling than Bala. This is expected because Bala contains a QTL that delays leaf rolling in response to loss of leaf water (Price et al 1997b). Leaf drying was also greater in Azucena than in Bala in all screens, which indicates that Bala was more tolerant of the drought treatments applied here. The relative water content data from IRRI98 also indicated that Azucena was the most severely affected of the two parents. The data suggested that the drought-tolerant check, Moroberekan, was either affected similarly or more severely than Azucena in the IRRI96 screen, but less affected than Azucena in the other two screens. The F_6 population mean was generally between the parental means for the traits.

The drought-reaction traits usually correlated with each other, although sometimes not well. Thus, 196LRA correlated with I96LDA (r = 0.202, $P = 0.002$), I98LRA (r = 0.633, $P < 0.001$), I98RWCA (r = –0.247, $P = 0.010$), and WLR16 (r = 0.441, $P < 0.001$). I96LDA correlated with I98LDA (r = 0.407, $P < 0.001$) and I98RWCA (r = –0.249, $P = 0.010$). I98LRA also correlated with I98LDA (r = 0.321, $P = 0.001$) and I98RWCA (r = –0.530, $P < 0.001$), whereas I98LDA and I98RWCA correlated at r = –0.557 ($P < 0.001$). The only other significant correlation between drought-reaction traits was between WLDA and WLR16 (r = 0.386, $P < 0.001$).

Root growth screens

Table 3 presents results for maximum root length and root thickness in well-watered and drought conditions for seedlings grown in a root box at Aberdeen. Table 4 presents results for maximum root length, root thickness, and deep-root dry weight of seedlings grown under well-watered and drought conditions in thin chambers in Bangor. In neither screen was there good evidence that Azucena and Bala differ significantly in maximum root length, in contrast to hydroponic and soil tube data (Price et a1 1997a) and unpublished data for plants grown in soil tubes at IRRI. Azucena does have significantly ($P < 0.05$) thicker roots and a greater amount of root at depth than Bala in the Bangor thin chamber screen for control (well-watered) conditions and significantly thicker roots at depth under drought conditions.

Some, but not all, root traits correlated with each other. Thus, ADRL and ADRT did not correlate with any other traits measured here. ACRT correlated with BCRTB (r = 0.345, $P < 0.001$) and BCRTD (r = 0.254, $P = 0.007$), while ACRL correlated with BCRL28 (r = 0.253, $P = 0.009$), BCRL35 (r = 0.273, $P = 0.005$), BDRL28 (r = 0.341, $P < 0.001$), and BDRL35 (r = 0.357, $P < 0.001$) as well as BCDRW (r = 0.268, $P = 0.005$). The only correlations between drought-reaction traits and root traits were between I96LRA, I96LDA, and I98LRA and BCDRW (r = 0.225–0.274, $P = 0.009$–0.006) and between I98LRA and BCRL35 (r = 0.319, $P = 0.002$), but in each case

Table 2. Parental and population mean and standard deviations for IRRI and WARDA drought screens.

Generation	I96LRA (1–5)	I96LDA (1–5)	I98LRA (1–5)	I98LDA (1–5)	I98RWCA (%)	WLDA (1–5)	WLR16 (1-5)
Azucena (n=2)	3.69 ± 0.05	1.33 ± 0.17	3.49 ± 0.09	2.56 ± 0.07	78.3 ± 3.0	2.73 ± 0.85	2.81 ± 0.72
Bala (n=2)	2.52 ± 0.03	0.75 ± 0.50	2.28 ± 0.32	1.63 ± 0.17	88.1 ± 3.9	2.09 ± 0.36	1.32 ± 0.01
F_6 (n=176 IRRI, 142 WARDA)	3.23 ± 0.42	1.36 ± 0.36	2.97 ± 10.58	1.92 ± 0.57	84.4 ± 5.6	2.01 ± 0.80	2.30 ± 0.85
Moroberekan (n=16 IRRI96, 2 IRR198, and 10 WARDA)	3.33 ± 0.14	1.48 ± 0.22	2.86 ± 0.69	1.97 ± 1.23	86.8 ± 8.4	1.57 ± 0.64	2.39 ± 0.69

Table 3. Parental and population mean and standard deviations for Aberdeen root growth experiment.

Generation	ACRL[a] (cm)	ACRT (mm)	ADRL (cm)	ADRT (mm)
Azucena (n=6 AC, n=1 AD)	52.7 ± 16.7	0.70 ± 0.13	39.0	0.40
Bala (n=6 AC, n=2 AD)	46.6 ± 7.2	0.60 ± 0.18	56.5 ± 10.6 (n=2)	0.30 ± 0.00 (n=2)
F_6 (n=142 AC, n=134 AD)	55.0 ± 9.7	0.70 ± 0.13	58.7 ± 9.8	0.34 ± 0.11

[a]See explanations in Table 1.

Table 4. Parental and population mean and standard deviations for Bangor root growth screen.

Generation	BCRL28[a] (cm)	BCRL35 (cm)	BCDRW (g)	BCRTB (mm)	BCRTD (mm)	BDRL28 (cm)	BDRL35 (cm)	BDDRW (g)	BDRTB (mm)	BDRTD (mm)
Azucena (n=5)	86.3 ± 11.0	101.1 ± 6.4	0.60 ± 0.12	1.164 ± 0.039	0.954 ± 0.082	98.4 ± 6.4	108.4 ± 6.4	0.603 ± 0.211	0.639 ± 0.057	0.771 ± 0.121
Bala (n=5)	84.9 ± 10.7	98.3 ± 9.0	0.20 ± 0.09	0.962 ± 0.078	0.531 ± 0.062	97.7 ± 7.9	110.8 ± 5.4	0.389 ± 0.152	0.552 ± 0.068	0.322 ± 0.120
F_6 (n=140)	85.1 ± 12.2	100.6 ± 9.5	0.37 ± 0.22	1.088 ± 0.140	0.749 ± 0.174	95.8 ± 8.0	108.4 ± 7.2	0.549 ± 0.200	0.650 ± 0.113	0.553 ± 0.182

[a] See explanations in Table 1.

greater root weight at depth was associated with greater drought score. Most of the root growth traits presented do not appear to be highly heritable because the standard deviation of the F_6 population is not greater than that of the parents, except for the root thickness and deep-root weight data from the Bangor thin chamber screens.

QTL mapping

Figure 1 and Table 5 present results of mapping QTLs for reaction to drought and Figure 2 and Table 6 present QTLs for root morphology. A total of 24 QTLs for reaction to drought were detected. Bala was the donor of the positive alleles (those that indicate the least reaction to drought) in 15 cases. There were 28 QTLs detected for root morphology, in 17 of which Azucena was the donor of positive alleles.

Of all QTLs for drought reaction, 17 were concentrated on six genomic regions. We will discuss these now in more detail and make comparisons with drought-reaction QTLs reported in the IR64 × Azucena population (Courtois et al 1996, Courtois et al, unpublished results) and the C039 × Moroberekan population (Champoux et al 1995), facilitated by using the map of Causse et al (1995) as a "bridge" to align markers. The positions of Rice Genome Project RFLP probes are estimated approximately using comparative mapping data presented at Rice Genome III, Manila, 1995 (McCouch, personal communication). The recombinant inbred lines of the C039 × Moroberekan cross were screened at IRRI in one dry season (in the same region of IRRI's upland farm, but a different field from the one used for the screens described here) at three growth stages. The doubled-haploid population of IR64 × Azucena was screened at IRRI in two seasons (the second season was side by side with the IRRI 1996 screen reported here) and in India (Courtois et al, unpublished results). To establish whether drought-reaction QTLs can be related causally to root behavior, we also refer to reports of QTLs for root morphology and root penetration ability in these populations. Both the IR64 × Azucena and C039 × Moroberekan populations have been grown in tubes containing soil (Yadav et a1 1997, Champoux et al 1995). The original F_2 of the Bala × Azucena population has been screened in hydroponics (Price and Tomos 1997). In addition, QTLs for root penetration ability have been evaluated using a petroleum wax layer method for both the C039 × Moroberekan population (Ray et al 1996) and the Bala × Azucena population (Price et al, unpublished results).

Chromosome 1. A region of chromosome 1 at marker e18m43.5 contained weak QTLs for leaf drying at both IRRI in 1998 (I98LDA) and at WARDA (WLDA), in which Bala alleles reduced drying. Data presented here show that Azucena alleles at this locus promoted greater rooting at depth under drought conditions. These contradictory observations are not readily explicable with current data. It is interesting to note that in the C039 × Moroberekan population, there was a QTL for root to shoot ratio in this area (at marker RZ276) (Champoux et al 1995).

The region of chromosome 1 associated with markers RZ14 and C949 contained QTLs of positive effect from Bala that reduced leaf rolling in all screens (I96LRA, I98LRA, and WLR16), reduced leaf drying at IRRI in 1996 (I96LDA), and increased relative water content (RWC) at IRRI in 1998 (I98RWCA). Associated with marker

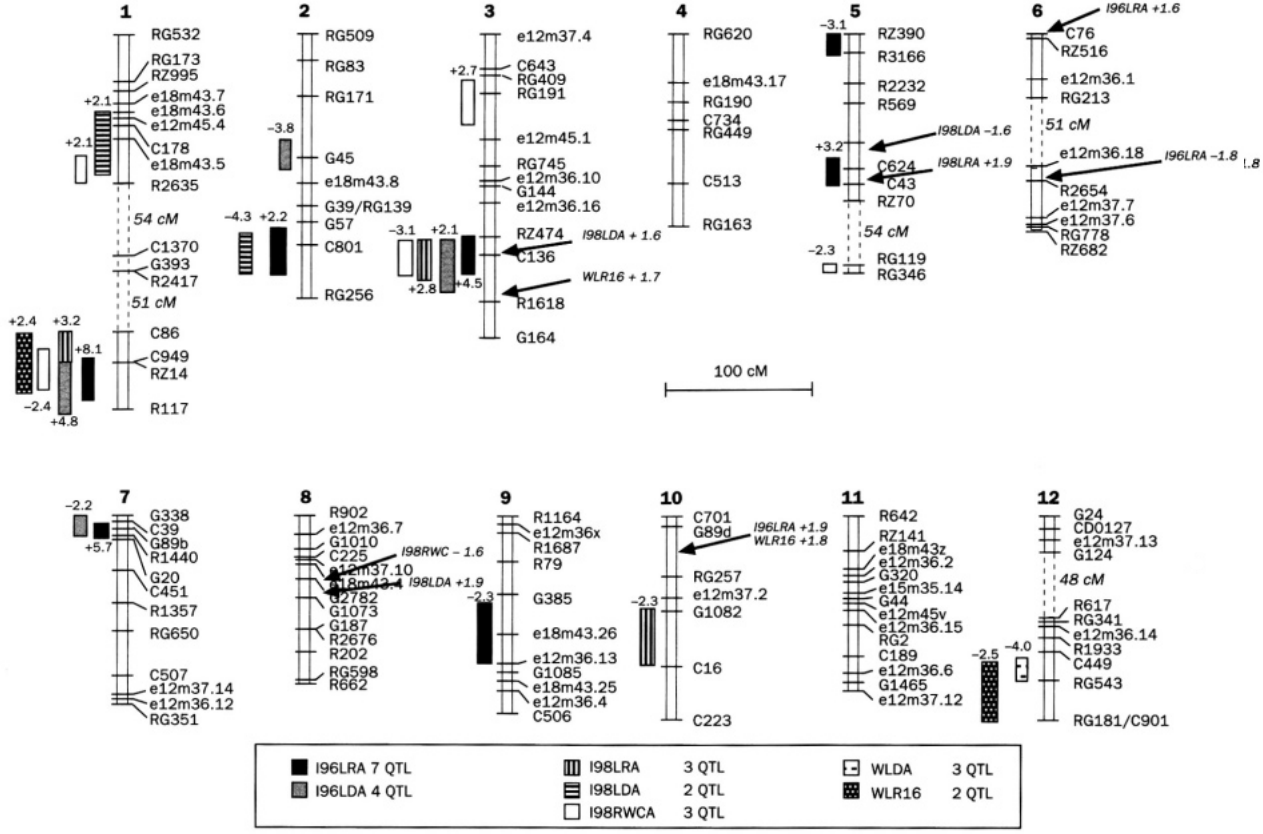

Fig 1. QTL map for reaction to drought treatment in three screens detected by composite interval mapping. Bars represent the 1 LOD confidence interval. Numbers above or below bars indicate the LOD score supporting the QTL; the sign indicates the direction of the effect of Azucena alleles on the trait. Also presented as arrow are the position og high LOD scores below the threshold (LOD = 1.5-1.9

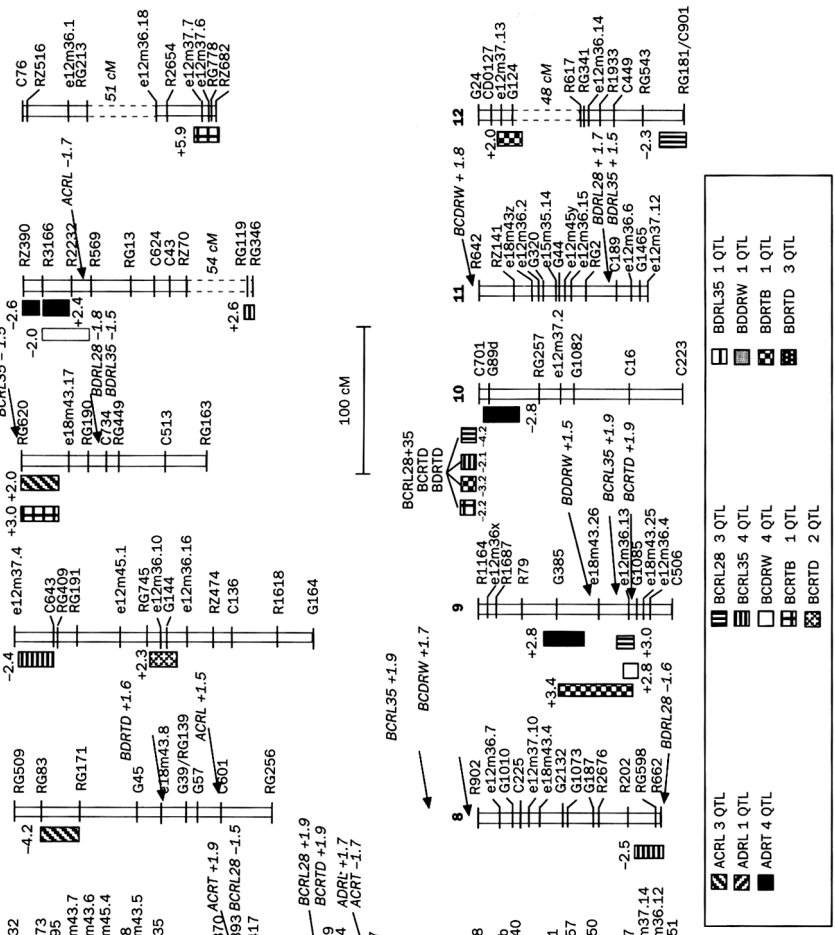

Fig. 2. QTL map for root morphological traits in two screens detected by composite interval mapping. Bars represent the 1 LOD confidence interval. Numbers above or below bars indicate the LOD score supporting the QTL; the sign indicates the direction of the effect of Azucena alleles on the trait. Also presented as arrows are the positions of high LOD scores below the threshold (LOD = 1.5-1.9).

Table 5. Summary QTLs associated with reaction to drought treatment.

Trait	Chromosome	Position[a]	LOD[b]	A[c]	Donor of positive alleles
196LRA	1	RZ14 + 8	8.1	0.18	Bala
	2	C601+ 4	2.2	0.08	Bala
	3	RZ474 + 10	4.5	0.11	Bala
	5	RZ390	3.1	–0.10	Azucena
	5	C624	3.2	0.08	Bala
	7	G89[b]	5.7	0.11	Bala
	9	e18m43.26 + 10	2.3	–0.08	Azucena
196LDA	1	RZ14 + 20	4.8	0.14	Bala
	2	RG171 + 13	3.8	–0.12	Azucena
	3	C136	2.1	0.09	Bala
	7	C39 + 4	2.2	–0.07	Azucena
198LRA	1	C86 + 12	3.2	0.15	Bala
	3	C136	2.8	0.13	Bala
	10	G1082 + 10	2.3	0.13	Bala
198LDA	1	e12m45.4	2.1	0.12	Bala
	2	C601 + 10	4.3	–0.26	Azucena
198RWCA	1	RZ14	2.4	–1.10	Bala
	3	RG191	2.7	1.10	Azucena
	3	C136	3.1	–1.20	Bala
WLDA	1	e18m43.5	2.1	0.16	Bala
	5	RG119	2.3	–0.14	Azucena
	12	C449 + 8	4.0	–0.22	Azucena
WLR16	1	C86 + 12	2.4	0.21	Bala
	12	RG543 + 7	2.5	–0.22	Azucena

[a]Position of QTL relative to the nearest marker to the left (in cM). [b]F statistic from QTL Cartographer divided by 4.6. [c]A = additive effect of Azucena allele.

C86 on chromosome 1 is a QTL at which Azucena alleles promoted longer roots and greater rooting at depth in the control Bangor screen. A QTL in the IR64 × Azucena population for leaf drying and relative water content associated with marker RZ730 (Courtois et al 1996) is likely to be the same region because RZ14 and RZ730 are only 6 cM apart according to Causse et al (1995). As in Azucena × Bala, Azucena alleles increased leaf drying and decreased RWC in the IR64 × Azucena population. Positive root morphology QTLs from Azucena are also present in this region in the IR64 × Azucena population (Yadav et al 1997). This region, however, also contained QTLs for plant size and height associated with the *sd-1* semidwarfing locus (the Bala or IR64 alleles reduce plant height and biomass). In the Bala × Azucena population, there was a QTL for the speed of leaf rolling upon leaf excision (Bala alleles reduce the rate of leaf rolling). Both the *sd-1* locus and the leaf rolling QTLs potentially have a profound effect on drought reaction. The larger the plant, the more water it will use; therefore, Azucena alleles should promote greater water use. The presence of a QTL for the rate of leaf rolling in the Bala × Azucena population introduces potentially confusing concepts. Bala leaves have a reduced tendency to roll as they lose water (relative to Azucena). Thus, reduced leaf rolling *rate* is an indication of the presence of a specific mechanism of drought tolerance. But leaf rolling in the field is used as an

Table 6. Summary of QTLs associated with root morphology.

Trait[a]	Chromosome	Position[b]	LOD[c]	A[d]	Donor of positive alleles
ACRL	2	RG83 + 14	4.2	-5.0	Bala
	4	RG620	2.0	2.2	Azucena
	7	G20 + 20	2.4	2.5	Azucena
ACRT	No QTLs				
ADRL	1	C1370 + 4	2.2	2.9	Azucena
ADRT	5	RZ390	2.6	-0.033	Bala
	5	R3166	2.4	0.056	Azucena
	9	G385 + 6	2.8	0.036	Azucena
	10	G89d + 2	2.8	-0.036	Bala
BCRL28	9	e12m36.13	3.0	3.6	Azucena
	10	C701	4.2	-4.5	Bala
	12	RG181	2.3	-3.0	Bala
BCRL35	1	C86 + 4	2.3	3.1	Azucena
	3	e12m37.4 + 4	2.4	-2.8	Bala
	5	RG119	2.6	2.6	Azucena
	10	C701	2.1	-2.4	Bala
BCDRW	1	RZ14	2.6	0.074	Azucena
	5	R2232	2.0	-0.053	Bala
	7	G20 + 16	2.4	0.068	Azucena
	9	G1085	2.8	0.063	Azucena
BCRTB	6	e12m37.7	5.9	0.061	Azucena
BCRTD	3	G144 + 4	2.3	0.049	Azucena
	10	C701	3.2	-0.056	Bala
BDRL28	No QTLs				
BDRL35	8	R202 + 16	2.5	-2.3	Bala
BDDRW	1	e18m43.6	2.9	0.062	Azucena
BDRTB	4	RG620 + 13	3.0	0.047	Azucena
BDRTD	9	G385 + 16	3.4	0.085	Azucena
	10	C701	2.2	-0.045	Bala
	12	G124	2.0	-0.043	Bala

[a]See explanations in Table 1. [b]Position of QTL relative to the nearest marker to the left (in cM).[c]F statistic from QTL Cartographer divided by 4.6. [d]A = additive effect of Azucena allele.

indication of reduced drought seventy or overall drought tolerance and assumes no inherent difference in leaf rolling rate. When this assumption is not valid, interpreting leaf rolling score can become difficult. The reduced leaf rolling rate from Bala should increase water loss and therefore increase leaf drying. Apparently, delayed leaf rolling caused by the Bala allele is not associated with increased drought score but with reduced drought score, presumably because of linkage to the *sd-1* locus. Without breaking that linkage, the value of the leaf rolling rate QTL will not be assessable. It is worth noting, in addition, that the large effect of this region (associated with plant size and leaf rolling but probably not root growth) on drought reaction explains to some extent the lack of correlation found between root traits and drought reaction scores.

Chromosome 2. Associated with marker C601 on chromosome 2 is a region in which Azucena alleles increased leaf rolling (I96LRA) but reduced leaf drying

(I98LDA). This is probably congruent with a QTL for leaf rolling in IR64 × Azucena reported near marker RG95 (Courtois et al 1996). Although two QTLs on chromosome 2 are reported in the C039 × Moroberekan cross, they are not very close to C601 or RG95. The QTL detected in this region in both populations sharing Azucena as a parent is where a QTL for root penetration ability has been revealed in the Bala × Azucena population (explaining 18% of the variation, Price et al, unpublished results) and in C039 × Moroberekan (Ray et al 1996). It is also a region with weak QTLs for maximum root length in hydroponics in the F_2 (Price and Tomos 1997) and for rooting depth in C039 × Moroberekan. This QTL is possibly related to root growth, with Azucena promoting deeper and more penetrating roots. Because this region promoted leaf rolling in the IRRI 1996 screen, however, this QTL may have both beneficial and detrimental effects in different environments or be due to the presence of two opposing QTLs.

Chromosome 3. There were several QTLs for drought reaction near marker C136 on chromosome 3, where Bala alleles reduced leaf rolling (I96LRA, I98LRA) and drying (I96LDA and a putative QTL for I98LDA, with LOD = 1.6) at IRRI. This region was also associated with higher relative water content in the IRRI 1998 trial (I98RWCA) and may have reduced leaf rolling at WARDA (WLR16, with LOD = 1.7). This QTL may be close to, and therefore related to, a QTL between RG482 and RG910 in the C039 × Moroberekan population. There is no evidence from the Bala × Azucena cross that root growth QTLs exist in this region although a QTL for root penetration was detected only 10 cM above RZ474, in which Bala alleles promoted root penetration (Price et al, unpublished results). A striking observation is that this region (indeed the same marker, C136) has been identified as a QTL for leaf abscisic acid content in a detached leaf test in rice (Quarrie et al 1997). It is also possible that the region is homologous to that of C039 × Moroberekan, in which a lethal osmotic potential QTL was detected by Lilley et al (1996). It must be noted, however, that the C039 × Moroberekan map appears to be condensed in this region compared with other maps, making confident comparisons difficult.

Chromosome 7. In the region near markers G338, C39, and G89b, Azucena alleles promoted leaf rolling (I96LRA) while reducing leaf drying (I96LDA). In both the IR64 × Azucena and C039 × Moroberekan populations, QTLs for drought reaction near the bottom of chromosome 7 were reported. Because of the high skewing toward Azucena alleles in that region in Bala × Azucena, that region was not used for composite mapping. But single-marker regression with skewed markers did not reveal significant association with drought-reaction traits. The QTLs detected here near the top of chromosome 7 were not found in the other crosses. No root growth QTLs have been reported in this region of chromosome 7 in any cross. It is difficult as yet to explain the cause of the drought-reaction QTL, but it is unlikely to be related to root growth.

Chromosome 12. A QTL was detected at the bottom of chromosome 12 only at WARDA, in which Azucena alleles reduced leaf rolling (WLR16) and drying (WLDA). This QTL is in the same place as QTLs for leaf rolling and relative water content

reported in IR64 × Azucena. In both cases, Azucena alleles improved drought performance. No QTLs were detected on chromosome 12 in C039 × Moroberekan. Data shown here revealed a QTL for BCRL28 in this region. In the hydroponic screen of the Bala × Azucena F_2 population, RG181 was associated with a QTL for root mass (Price and Tomos 1997), a result which agreed with observations in C039 × Moroberekan (Champoux et al 1995). It seems quite plausible that this drought-reaction QTL is related to root growth.

Other noteworthy regions. In the region of chromosome 5 near markers C624 and C43, Azucena alleles appeared to both promote leaf rolling (I96LRA and possibly I98LDA, LOD = 1.9) and reduce leaf drying (I98LD, LOD = 1.6). This QTL is in the same place as QTLs in IR64 × Azucena for leaf rolling, drying, and relative water content, which can be concluded by reference to the marker RZ70, which is common to both maps. Because Azucena alleles promoted leaf rolling but increased relative water content and reduced leaf drying in IR64 × Azucena, the two reports are in good agreement (i.e., they both report contradictions in the direction of leaf rolling and other responses). This is a region in which root thickness and root length QTLs of negative effect from Azucena were detected in the F_2 hydroponic screen of the Bala × Azucena population (Price and Tomos 1997). There is also a QTL for root thickness in IR64 × Azucena in this region, in which Azucena alleles reduced root thickness (Yadav et a1 1997). There is a weakly detected root penetration QTL in Bala × Azucena (Price et al, unpublished results), also detected at C624. Together, these data suggest that Azucena alleles at this locus reduce root length, thickness, and penetration ability, but still influence tolerance for drought in a predominantly positive way.

Only one trait was detected as a QTL on chromosome 9 here (I96LRA), but that is likely to be the same as the one detected for leaf rolling in C039 × Moroberekan at RG570 and for leaf rolling, leaf drying, and relative water content in IR64 × Azucena at markers RG667 and RG451. In both populations with Azucena as a parent, Azucena alleles reduced drought reaction. This region contains a cluster of QTLs for root morphology in both Bala × Azucena presented here and in IR64 × Azucena (Yadav et al 1997), indicating that the drought-score QTL may be related to root growth.

Our results clearly indicate a considerable amount (although as yet unquantified) of QTL by environment interaction for both root growth and drought-reaction QTLs. We should expect this because of the complex nature of the drought phenomenon and the known reaction of root growth to environmental conditions. But a primary aim of this research is to identify regions of the rice genome of value in the breeding of varieties with improved drought tolerance. By conducting field screens on one population at several sites and in several seasons and comparing the results with other reports of field evaluation of reaction to drought in other mapping populations, we hope that some regions will be consistently identified as valuable. We originally expected that many of these would be related to root morphology. Our results do identify some regions in which QTLs for drought reaction are identified in similar locations in more than one screen or in other crosses. The magnitude of the effect of these QTLs varies between screens and between populations, as might be expected for an

environmentally and genetically complex trait such as drought tolerance. Moreover, the techniques used for QTL analysis were not identical in all studies and this might have resulted in additional discrepancies.

Champoux et al (1995) found that most drought-reaction QTLs were at root morphology QTLs, suggesting a causal relationship between the two. Our data also provide evidence that some root growth QTLs are co-located with drought-reaction QTLs, despite the lack of correlation between drought reaction and the root traits. For the drought-reaction QTLs on chromosomes 2, 9, and 12 (and perhaps 5) reported here, it seems possible that a deeper, thicker, or longer root system contributes to drought tolerance. These genomic regions might be suitable targets for marker-assisted selection. Some QTLs for drought reaction have been found to be co-located with root morphology QTLs in the IR64 × Azucena population (Courtois et al, unpublished results). But neither Courtois et al nor this report find the co-locations of drought-reaction and root growth QTLs to be as prominent as the co-locations reported by Champoux et al (1995).

We have identified three regions that have drought-reaction QTLs that are not associated with root morphology QTLs. In all cases, Bala alleles have a positive effect. The QTL on chromosome 1 associated with RZ14 is unlikely to be a valuable region for breeding purposes because of the linkage or pleiotropic effects of the semidwarfing gene. The QTLs on chromosomes 3 and 7 are potentially valuable, although the underlying physiology is unknown and should be investigated.

Conclusions

Comparisons of multiple-season and multiple-site field drought screens in several populations have enabled us to identify several genomic regions of potential value for the improvement of drought tolerance in rice. The results from analyses of root growth traits compared between populations suggest that some regions influencing field reaction to drought are related to root thickness, rooting depth, or root penetration ability.

References

Causse M, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SB, Second GA, McCouch SR, Tanksley SD. 1995. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251-1274.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Courtois B, Chaitep W, Moolsri S, Sinha PK, Trébuil G, Yadav R. 1996. Drought resistance and germplasm improvement: on-going research in the Upland Rice Consortium. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. Proceedings of the Upland Rice Research Consortium Workshop, 4-13 Jan. 1996, Padang, Indonesia. Manila (Philippines): International Rice Research Institute. p 154-175.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

International Rice Testing Program. 1996. Standard evaluation system for rice. Fourth edition. Manila (Philippines): International Rice Research Institute. 52 p.

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N. Wu J, Antonio BA, Shoura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y. 1994. A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet. 8:365-372.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ Lincoln SE, Newburg L. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Lincoln S, Daly M, Lander E. 1992. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report. 2nd ed.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiological and molecular genetics considerations. Crop Sci. 37:1426-1434.

Price AH, Courtois B. 1999. Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. Plant Growth Reg. (In press.)

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95:143-152.

Price AH, Virk DS, Tomos AD. 1997a. Genetic dissection of root growth in rice (*Oryza sativa* L.). I. A hydroponic screen. Theor. Appl. Genet. 95:132-142.

Price AH, Young EM, Tomos AD. 1997b. Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). New Phytol. 137:83-91.

Quarrie SA, Laurie DA, Zhu J, Lebreton C, Semikhodskii A, Steed A. Witsenboer H, Calestani C. 1997. QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol. Biol. 35: 155-165.

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen H. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor. Appl. Genet. 92:627-636.

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS. 1996. Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa*). Proc. Nat. Acad. Sci. USA 93:6163-6168.

Townend J. 1995. Effects of elevated CO_2, water and nutrients on *Picea sitchensis* (Bong.) Carr. seedlings. New Phytol. 130:193-206.

Townend J, Dickinson AL. 1995. A comparison of rooting environments in containers of different sizes. Plant Soil 175:139-146.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Homes M, Frijters A. Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution on a double-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Yoshida S, Forno DA, Lock JH, Gomez KA. 1976. A laboratory manual for the physiological studies of rice. Manila (Philippines): International Rice Research Institute.

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Developing near-isogenic lines of IR64 introgressed with QTLs for deeper and thicker roots through marker-aided selection

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A well-developed root system is an important trait contributing to drought tolerance in rice. Preliminary work allowed us to map QTLs involved in the control of several root traits in a doubled-haploid population of rice derived from the cross IR64 x Azucena. We are now developing near-isogenic lines of IR64 introgressed with the major QTLs. Four segments on chromosomes 1, 2, 7, and 9 were initially targeted for single QTL selection. Four doubled-haploid lines with the proper allelic combination at the targeted segments and less than 50% Azucena alleles in the rest of the genome were chosen as donors. These lines were backcrossed on IR64 up to the BC_3F_1 generation and then selfed. Marker-aided selection for the target segments was performed from BC_1F_1 up to BC_3F_2. All the selected BC_3F_2 progenies carried the donor alleles for at least one target region. Plants carrying two target segments were also identified. Phenotypic variation for several nontarget traits was observed in the BC_3F_2 plants, suggesting the presence of genetic drag during introgression. A background survey of the rest of the genome indicated that the BC_3F_2 plants had a very small portion of Azucena alleles in the nontarget regions, close to the expected ~3%. Crosses were made between BC_3F_2 plants carrying different target donor segments to remove possible genetic drag and pyramid different target QTLs.

Rice is the most important staple food crop in the world. Drought is a major constraint for rice production, especially for upland rice (Widawsky and O'Toole 1990). The development of drought-tolerant rice cultivars has not yet been very successful despite the efforts made by breeders. The reasons for this lack of progress are the diversity of rice-growing environments, the complex interactions between genotypes and environments, the complexity of the physiological basis of drought tolerance, and the difficulties in determining suitable criteria on which to base breeding work (O'Toole 1982, Lilley and Fukai 1994, Fukai and Cooper 1995). There is an overall consensus, however, on the fact that a deep and thick root system is one of the main drought-avoidance mechanisms (Nguyen et al 1997). A rice variety with such a root system may be able to reach deep soil water and meet its water requirement under water-limited situations. Although the overall influence of a deeper and thicker root

system on final yield has yet to be fully understood, any progress in breeding rice for a better root system will be a benefit to less-favored, drought-prone rice ecosystems.

Root traits are difficult and time-consuming to measure. Because most measurement methods are destructive for plants, they are difficult to incorporate into conventional breeding programs. Molecular marker technology is seen as a way to overcome these barriers. Several groups used molecular markers to map genes involved in the control of rice root morphology (Champoux et al 1995, Ray et al 1996, Price and Tomos 1997, Yadav et al 1997.)

The next step is introgression of the superior alleles into various genetic backgrounds using molecular marker-aided selection (MAS). This has already been achieved for major rice disease resistance genes. In the pathology field, the results of mapping studies were successfully applied to the development of near-isogenic lines (NILs) carrying various combinations of major genes that allowed rice scientists to test their hypotheses on the durability of blast resistance (Nelson et a1 1996, Hittalmani et al 1999). Their work was facilitated because they were dealing with dominant major genes and phenotyping at each generation was possible and simple.

In contrast, few cases of successful introgression of quantitative trait loci (QTLs) have been reported though overall strategies for QTL introgression were proposed (Tanksley and Nelson 1995, Tuinstra et a1 1997). Simulation studies have provided useful guidelines for methodological choices (Visscher et al 1996a, for animal breeding; Hospital and Charcosset 1997, for plant breeding).

In a previous work, we conducted a QTL analysis for root morphology in a doubled-haploid (DH) population of rice derived from a cross between IR64 and Azucena (Yadav et al 1997). We identified QTLs for maximum root length (MRL), root thickness (THK), deep root/shoot ratio (DR/S), total root weight (TRW), deep root weight (DRW), and deep root weight/tiller (DR/T). Based on these mapping results, we began a marker-aided backcross program for root system improvement. Selected DH lines were backcrossed to the recurrent indica parent IR64 to produce NILs introgressed with japonica alleles from Azucena at the location of the most significant QTLs.

This program has three major objectives:

- to develop NILs of IR64 carrying various possible combinations of QTLs,
- to validate the effects of the QTLs for root morphology identified previously before attempting to transfer them into agronomically relevant materials, and
- to test hypotheses on the role of the root system in drought avoidance. A reciprocal program to introgress indica alleles (IR64) into the japonica background (Azucena) has also begun but is less advanced.

This chapter presents the strategy used for developing NILs and the characteristics of the selected BC_3F_2 plants.

Materials and methods

QTL mapping in an IR64 × Azucena DH population: the need for reanalysis

The mapped population consisted of 135 DH lines derived from a cross between IR64, an irrigated indica variety, and Azucena, an upland tropical japonica variety (Guiderdoni et al 1992). Among those lines, 105 were phenotyped for root system (Yadav et al 1997). The six analyzed traits were DRW, DR/S, DR/T, MRL, THK, and TRW. In that initial study, a genetic map of 175 markers with 141 restriction fragment length polymorphism (RFLP) markers, 14 random amplified DNA (RAPD) markers, 8 isozyme markers, and 12 cloned genes (Huang et al 1997) was used for QTL mapping using the regression on flanking marker method. Later, 113 microsatellite markers were added on the same map (McCouch et al, unpublished results). Polymerase chain reaction-based microsatellite markers are a tool of choice for rapid genotyping and we wanted to use them whenever possible. We therefore constructed a new version of the genetic map that incorporated part of the microsatellite markers and part of the RFLP markers, eliminating a few markers with doubtful genotypes and markers that were not co-dominant (a requirement for our backcross program). The final map now contains 192 markers (Fig. 1). The raw phenotypic data of Yadav et al (1997) were re-analyzed using the composite interval mapping approach with QTL Cartographer software (Basten et al 1997).

Choice of target segments and markers analyzed

Based on QTL mapping results, four segments on chromosomes 1, 2, 7, and 9 were chosen as the target genomic regions for introgression. The following markers flanking the targeted regions were used for the genotyping work:

- RZ19, RG690, RZ730, and RZ801 for the target region of chromosome 1
- RM29, RG171, RG157, and RZ318 for the target region of chromosome 2
- RM234, RZ978, CD0418, CD038, and RM248 for the target region of chromosome 7
- RZ228 and RZ12, replaced by RM201 and RM242 after BC_2 generation, for the target region of chromosome 9

For the sake of simplicity, in the rest of the text we will call “target” the target region on chromosome 1, “target 2” the target region on chromosome 2, “target 7” the target region on chromosome 7, and “target 9” the target region on chromosome 9.

Choice of donor DH lines

For each of the four targeted segments, we chose one DH line carrying Azucena alleles in a relatively broad area around the putative QTLs for root morphology and IR64 alleles on the rest of the carrier chromosome, and having more than the average proportion of alleles coming from the IR64 parent (Table 1). Figure 1 presents the graphical genotype of one of them.

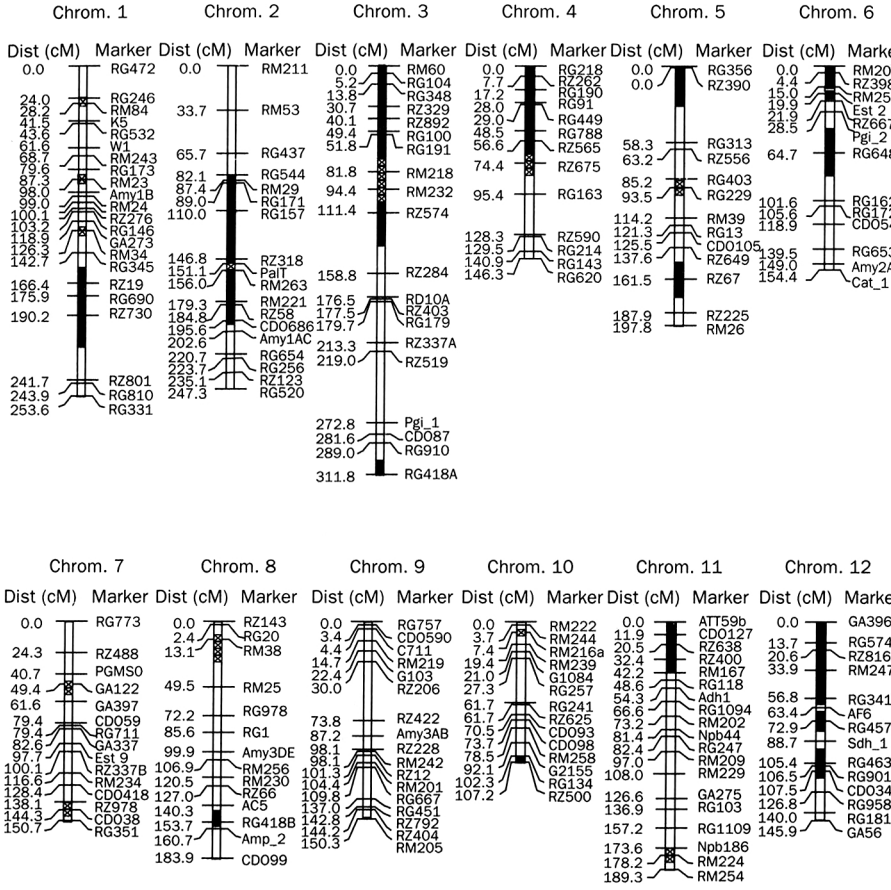

Fig. 1. Genetic map of the DH population with graphical genotype for P0055. The genetic distance unit is centiMorgan; the map function is Haldane's. Regions homozygous for Azucena are shown in black; regions with missing genotype data are shown with a cross.

Three of the four DH lines selected also carried Azucena alleles at one or two other secondary target regions (Table 1). P0055, initially selected for target 1, also carried target 2 (see Fig. 1). P0295, initially selected for target 7, carried targets 1 and 2 as well. P0475, initially selected for target 9, also carried target 7. This fact was used to select backcross progenies that simultaneously retained donor chromosomal segments for several target regions.

Marker-aided selection of backcrossed progenies

Table 2 presents the sequence of operations for the marker-aided backcross scheme. The DH lines were used as donors and IR64 as the recurrent parent. MAS was used to select plants carrying Azucena alleles at markers flanking the target regions in the

Table 1. Doubled-haploid lines selected for backcrossing.

DH line	Primary target			Secondary target		
	Proportion of Azucena alleles in nontarget regions [a] (%)	Chr[b]	Markers	Length of the interval (CM)	Chr[b]	Markers
P0055	33.8	1	RZ19-RG690-RZ730	61.4	2	RM29-CD0686
P0035	38.9	2	RG437-RG171-RG157-RZ318	99.2		None
P0295	44.9	7	RM234-CD0418-RZ978-CD03SRG351-RM248	42.4	1 2	W1-RG331 RM211-RG157
P0475	37.0	9	RZ22SRM242-RZ12-RM201-RG667	30.8	7	Est9-RG351

[a]Nontarget regions means all genome except the primary target region. [b]Chr = chromosome.

BC_1F_1, BC_2F_1, BC_3F_1, and BC_3F_2 generations. In each generation up to BC_3F_1, plants with the desired genotype profile were selected before heading and used as female parents in crosses with IR64. The BC_3F_1 plants were selfed and their BC_3F_2 progenies screened for plants homozygous for the Azucena allele at the target regions. The genotyping was conducted according to published protocols for the RFLP markers (McCouch et al 1988) and microsatellite markers (Chen et al 1997). For microsatellite marker analysis, DNA was isolated by the mini-preparation method of Wang et al (1993).

In the BC_1F_1 and BC_2F_1 generations, no selection for secondary target regions was conducted. In the BC_3F_1 generation, however, plants were genotyped for both primary and secondary target chromosome regions. This was intended to identify plants that may carry several target regions. For target 9, 25 plants were randomly selected for DNA mini-preparation and microsatellite marker analysis. For the other three targets, 45 plants were selected for RFLP analysis.

In the BC_3F_2, the same process was repeated. In this generation, plants were selected if they fit one of three categories:

1. Homozygous for the Azucena allele at markers flanking target 1, 2, 7, or 9.
2. Homozygous for the Azucena allele at markers flanking the QTLs for two or more target regions simultaneously. Possible combinations were targets 1 and 2, targets 1 and 7, and targets 2 and 7.
3. Homozygous for the Azucena allele at different and overlapping chromosome segments around the target segment on one of the chromosomes (1, 2, 7, or 9). These plants are intended for fine mapping of the QTLs.

Seventy-two plants from lines under selection for target 9 were chosen for DNA mini-preparation and microsatellite marker analysis. For the three other target segments, DNA of 12 to 20 plants was isolated for each line and used for RFLP analysis.

Based on the above criteria, 58 plants were selected for whole-genome genotyping.

Table 2. Sequence of operations in the marker-aided selection scheme for introgression of QTL for root traits in rice using four IR64/Azucena doubled-haploid (DH) lines as donors and IR64 as recurrent parent.

Backcross generation	Season[a]	Selection for primary target	Selection for secondary target	Type of plants selected	Product	Theoretical status of the product	Plants genotyped (no.)	Plants with desired genotypes (no.)	Plants selected for further backcrossing or selfing (no.)
Hybridization DH lines/IR64	1996 DS	No	No		F_1 seeds	75.0% IR64 25.0% Azucena			
BC_1	1996 WS	No	No		BC_1F_1 seeds	87.5% IR64 12.5% Azucena			
BC_2	1997 DS	Yes	No	Heterozygous	BC_2F_1 seeds	93.7% IR64 6.3% Azucena	120	33	18
BC_3	1997 WS	Yes	Yes	Heterozygous	BC_3F_1 seeds	96.9% IR64 3.1% Azucena	120	50	15
Selfing	1998 DS	Yes	Yes	Homozygous	BC_3F_2 seeds	96.9% IR64 3.1% Azucena	74	32	32
Whole-genome survey	1998 WS	Yes	Yes		BC_3F_3 seeds		312	66	58

[a]DS = dry season, WS = wet season.

Whole-genome survey of BC_3F_2 progenies with microsatellite markers

To evaluate the proportion of the Azucena genome present in the plants selected from the BC_3F_2 generation, two to five additional markers were analyzed for each chromosome of these plants. Because microsatellite markers are PCR-based and both easy and fast to use, they were preferred for the genome survey of the nontarget regions. First, 35 microsatellite markers giving good genome coverage and evenly distributed throughout the genome were selected among the 113 possible. The information regarding the allelic constitution at these loci in the original DH lines was available (McCouch et al, unpublished results). Only the subset of markers carrying Azucena alleles in the original DH line (respectively, 6, 17, 20, and 10 markers for P0055, P0035, P0295, and P0475) was used to genotype its BC_3F_2 progenies. The number of marker loci possessing the Azucena alleles was determined for each plant. We assumed that the genotyped markers represent the whole-genome profile of the nontarget regions. Therefore, the frequency of Azucena alleles in the nontarget regions was computed as (percentage of markers carrying the Azucena allele in BC_3F_2) × (initial Azucena allele frequency in the nontarget regions of the DH lines) × (probability of the Azucena allele at a given marker locus). Because the original donor DH lines carried less than 50% Azucena alleles across the genome and the proportion of Azucena alleles in the genome was expected to be reduced by 50% in each backcrossing, the expected frequency of the remaining Azucena alleles in BC_3 should be 3.125% or less. Thus, the selected BC_3F_2 plants can be regarded as NILs for the target QTLs.

Preliminary phenotyping of various agronomic traits in BC_3F_2

The plants sampled for DNA extraction were grown in the field. We performed a preliminary assessment of the degree of phenotypic similarity between IR64 and the BC_3F_2 plants. A few characteristics were recorded for all the genotyped plants and for the IR64 recurrent parent. Duration was recorded on a line basis although some segregation for flowering time was observed within lines. Plant height, panicle number, and total grain weight per plant were recorded on an individual plant basis just before harvest. Grain weight was not measured for those plants used for hybridization.

Pyramiding QTLs by crossing BC_3F_2 progenies

To obtain plants carrying Azucena alleles at several target regions, two BC_3F_2 plants carrying Azucena alleles at targets 1 and 7, two plants carrying Azucena alleles at target 2, and one plant carrying the Azucena allele at target 7 were crossed with BC_3F_2 plants homozygous for the Azucena allele at target 9. We expected to recover progenies carrying Azucena alleles at all three target regions.

Results and discussion

Brief summary of QTL mapping results in IR64 × Azucena DH population

A complete description of the QTL mapping results in the DH population was reported previously (Yadav et al 1997). The composite interval mapping method has a

Table 3. Summary of QTLs for root morphology on the target chromosomes.

Trait[a]	Chromosome	Interval	Marker-QTL distance	QTL position	LOD score	R^2	Additive effect
THK	1	RM34-RG345	0.0	126.3	4.7	21.4	–0.042
TRW	1	RG690-RZ730	12.0	187.9	4.6	21.1	–0.125
TRW	1	RZ730-RZ801	12.0	202.2	5.1	35.2	–0.158
DR/S	1	RG690-RZ730	14.0	189.9	3.1	13.3	–0.003
DRW	1	RG690-RZ730	10.0	185.9	4.7	23.0	–0.035
DR/T	1	RZ730-RZ801	0.0	190.2	5.6	22.9	–3.758
MRL	2	RG171-RG157	14.0	103.0	6.3	33.4	–6.160
MRL	2	RZ318-Pal1	0.0	146.8	3.4	14.8	+4.565
MRL	7	CD0418RZ978	0.0	128.4	7.4	28.8	–5.078
DR/S	7	CD0418-RZ978	0.0	128.4	8.1	31.2	–0.005
DR/S	7	RZ978CD038	2.0	140.1	5.5	23.0	–0.004
DRW	7	RG773RZ488	8.0	8.0	3.4	21.5	–0.035
DRW	7	CD0418-RZ978	0.0	128.4	7.0	27.7	–0.039
DRW	7	RZ978-CD038	4.0	142.1	5.3	23.4	–0.036
DR/T	7	CD0418-RZ978	0.0	128.4	5.7	24.4	–3.713
MRL	9	RZ12-RM201	0.0	101.3	4.2	16.8	–3.561
TRW	9	RZ206RZ422	4.0	34.0	3.6	18.4	–0.112

[a]DRW = deep root weight (= root weight below 30 cm); DR/S = deep root weight per shoot; DR/T = deep root weight per tiller; MRL = maximum root length; THK = root thickness; TRW = total root weight; marker-QTL distance = distance in cM from the left marker of the interval: QTL position = distance in cM from the top arm of the chromosomes; R^2 = percentage of phenotypic variability accounted for by the putative QTL. Negative sign of additive effects represents positive phenotypic effects from Azucena alleles.

greater power of QTL detection and a better resolution of linked QTLs and provides a more accurate estimation of QTL effects than conventional interval mapping methods, so we re-analyzed the original phenotypic data using this method. Table 3 presents the QTL mapping results for the four targeted chromosomes. The genome-wide threshold LOD score for a risk of 5% type 1 error is 3.32. QTLs exceeding this value are presented, but putative QTLs with a LOD score of more than 2 were also included if they were located on the target chromosomes. The results from the two analyses in terms of QTL location were not very different and the original choice of target regions was validated. LOD scores and percentage of phenotypic value explained by the QTLs were higher with composite interval mapping, whereas the additive effects were similar with both methods. As in the first study, most of the alleles having a positive effect on the root system were from Azucena. The only exception was the interval RZ318-Pal1 for maximum root length.

Genetic composition of selected BC_3F_2 plants

Table 4 shows the genotypes of the BC_3F_2 plants for all the target regions. Plants homozygous for Azucena alleles in the primary target regions were identified for all four target regions. Plants carrying overlapping segments from Azucena in the target regions were also found. But only a few of the plants with target 7 retained the secondary target 1.

Table 4. Genotype in target regions and preliminary phenotype of selected BC_3F_2 plants.

Plant	C[a]1 RZ 19	C1 RG 690	C1 RZ 730	C1 RZ 801	C2 RM 29	C2 RG 171	C2 RG 157	C2 RZ 318	C7 RM 234	C7 CDO 418	C7 RZ 978	C7 CDO 38	C7 RM 248	C9 RM 201	C9 RM 242	TIL	HGT	DUR	WGT	Prop Az
Target 1																				
IR74392-108-1	3	2	1	1												21	105	129	16.7	0.0
IR74392-108-6	3	2	1	1												13	105	129	8.1	0.0
IR74392-118-4	2	3	3	1												21	148	129	35.1	0.0
IR74392-135-1	3	3	3	1												28	109	125	19.0	5.6
IR74392-135-7	3/2	3/2	3	1												24	111	125	12.6	0.0
IR64	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22	105	124	18.2	0.0
P0055	3	3	3	1	3	3	3	3	1	1	1	1	1	1	1	–	–	–	–	33.8
Target 2																				
IR74392-201-5					nd	3	3	3								26	104	129	hYb	nd
IR74392-201-11					3	3	3	2								26	108	129	10.1	3.4
IR74392-201-12					3	3	3	3								20	99	129	hyb	3.4
IR74392-201-14					3	3	3	2								20	92	129	hyb	4.6
IR74399-204-2					1	2	1	3								23	101	120	25.9	3.4
IR74399-204-3					1	2	3	3								17	106	120	9.3	2.3
IR74399-204-4					nd	2	3	3								20	107	120	22.7	nd
IR74399-204-6					1	2	1	3								27	106	120	21.3	2.3
IR74399-204-7					1	2	2	3								27	110	120	19.7	2.3
IR74399-204-9					1	2	2	3								18	109	120	12.9	nd
IR74399-204-10					1	2	1	3								18	105	120	7.1	0.0
IR74401-215-5					1	1	3	3								27	110	127	17.7	0.0
IR74401-215-13					1	nd	3	nd								26	116	127	18.9	3.7
IR74401-215-17					1	nd	3	nd								21	110	127	10.9	3.7
IR74401-215-18					1	nd	3	nd								50	110	127	24.6	3.7
IR74401-216-3					3	3	3	2								22	102	121	24.8	2.3
IR74401-216-4					3	2	2	2								19	108	121	24.1	4.6
IR74401-216-5					3	3	3	2								18	106	121	13.8	2.3
IR74401-216-7					3	3	3	3								31	110	121	26.7	3.4
IR74401-216-9					3	3	3	3								20	110	121	14.0	2.3
IR64	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	23	110	122	20.7	0.0
P0035	1	1	1	1	3	3	3	3	1	1	1	1	1	1	1	–	–	–	–	38.9

Table continued

Table 4 continued.

Plant	C[a]1 RZ 19	C1 RG 690	C1 RZ 730	C1 RZ 801	C2 RM 29	C2 RG 171	C2 RG 157	C2 RZ 318	C7 RM 234	C7 CDO 418	C7 RZ 978	C7 CDO 38	C7 RM 248	C9 RM 201	C9 RM 242	TIL	HGT	DUR	WGT	Prop Az
Target 7																				
IR74405-711-1	1	1	1	1					1	1	3	1	1			22	105	120	15.3	0.0
IR74405-720-7	1	1	1	1					1	1	3	1	1			21	114	125	22.2	1.2
IR74405-720-12	1	1	1	1					1	1	3	1	nd			14	135	125	8.7	nd
IR74409-730-8	1	1	1	1					1	3	3	1	3			13	92	123	2.7	4.7
IR74409-730-9	1	1	1	1					1	3	3	1	3			26	106	123	5.5	2.4
IR74409-730-10	1	1	1	1					1	3	3	1	3			20	139	123	12.1	3.5
IR74409-734-4	1	1	1	1					1	3	2	1	3			19	111	123	7.2	3.9
IR74409-735-2	3/2	3	3	2					1	3	3	1	3			13	149	118	29.7	5.6
IR74409-735-11	2	2	2	2					1	3	3	1	2			19	131	118	15.7	4.5
IR74409-735-12	2	2	2	2					1	3	3	1	2			17	145	118	29.7	3.4
IR74409-736-5	2	1	1	2					1	1	2	2	1			15	114	123	7.8	0.0
IR74409-736-11	3	3	1	3					1	2	3	0	1			23	135	123	hyb	2.3
IR74409-737-5	2	2	3/2	3					1	2	1	2	2			10	155	127	10.0	5.6
IR74409-737-12	3	1	1	2					1	3	3	3	2			18	141	127	16.2	6.7
IR74409-738-8		?	2	2					1	3	3	2	2			20	150	126	26.0	4.5
IR74409-738-11		2	3/2	2					1	3	3	1	2			24	138	126	hyb	3.4
IR74409-739-4	1	0	3	3					1	1	1	2	1			27	160	126	19.0	3.4
IR74409-739-7	2	2	3	3					1	1	2	nd	1			19	150	126	11.3	1.2
IR64	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	103	123	16.3	0.0
PO295	3	3	3	3	3	3	3	1	3	3	3	3	3	1	1	–	–	–	–	44.9
Target 9																				
IR74418-910-2														3	3	13	83	125	hyb	3.7
IR74418-910-3														3	3	29	98	125	hyb	3.7
IR74418-910-9														3	3	23	113	125	23.1	3.1
IR74418-910-12														3	2	21	111	125	17.9	1.9
IR74418-913-1														3	3	14	110	122	10.9	3.7
IR74418-913-7														3	3	18	102	122	hyb	1.9
IR74418-913-9														2	3	25	112	122	22.4	0.0
IR74418-913-11														3	2	11	115	122	16.0	4.1
IR74418-913-12														1	3	12	120	122	19.6	2.0

Table continued

Table 4 continued.

Plant	C[a] 1 RZ 19	C1 RG 690	C1 RZ 730	C1 RZ 801	C2 RM 29	C2 RG 171	C2 RG 157	C2 RZ 318	C7 RM 234	C7 CDO 418	C7 RZ 978	C7 CDO 38	C7 RM 248	C9 RM 201	C9 RM 242	TIL	HGT	DUR	WGT	Prop Az
IR74419-921-1														3	3	21	82	122	hyb	0.0
IR74419-921-3														3	3	20	112	122	9.6	0.0
IR74419-921-8														3	3	12	96	122	hyb	0.0
IR74419-933-12														3	3	17	108	122	19.0	1.9
IR74419-934-2														3	3	27	100	120	hyb	1.9
IR74419-934-7														3	3	23	113	120	18.4	1.9
IR64	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	113	123	28.0	0.0
P0475	1	1	1	1	1	1	1	1	3	3	3	3	3	3	3	–	–	–	–	37.0

[a]C = chromosome. Allele code: 1 = homozygous for IR64, 3 = homozygous for Azucena, 2 = heterozygous, 3/2 = unclear signal, either homozygous for Azucena or heterozygous, nd = not determined. TIL = tillering, HGT = plant height (cm), DUR = duration of sowing to maturity (in days), WGT = weight per plant (in g), hyb = plant used for further hybridization work, Prop Az = proportion of Azucena alleles in the nontarget regions.

The whole-genome survey with microsatellite markers showed that the frequency of the Azucena alleles in the nontarget regions ranged from 0.0% to 6.7%, with an average of 2.4% (Table 4). This minor deviation from the theoretical expectations may well be explained by chance because, in each generation, only 24 plants were selected for backcrossing. Because the microsatellite marker data only became available in the middle of the MAS process, selection for IR64 alleles in the nontarget regions using microsatellite markers did not start until the BC_3F_2 generation. Such selection would have been more effective if it had started in earlier generations.

Preliminary phenotyping of BC_3F_2 plants

Because of the limited amount of seeds available per plant, it was not possible to conduct replicated experiments in this generation. To get a rough idea of the phenotypes of the selected BC_3F_2 plants, however, plant height, duration, tiller number, and yield per plant were recorded. All traits showed segregation (Table 4). Segregation existed both between different lines and between different plants from the same line. Deviations were observed in both directions, implying the segregation of underlying QTLs for these traits in the BC_3F_2 progenies.

For plant height, segregation occurred only in lines derived from a DH line carrying the Azucena allele at target 1, whereas all plants derived from a DH line carrying the IR64 allele in this region had a plant height similar to that of IR64. We know that *sd-1,* a recessive gene carried by IR64 and causing semidwarfism, is located in the RZ730-RZ801 interval (Huang et al 1996), which corresponds to target 1. For the segregating sets, the plants with Azucena alleles at both locus RZ730 and locus RZ810 were all tall, whereas the plants carrying the IR64 alleles at both loci were small, with two exceptions (IR74405-720-12 and IR74409-730-10). This interval is quite broad (51.5 cM) and double crossovers cannot be excluded.

For duration, differences between lines of up to 1 wk were observed. Variations between plants within lines were also noticed. Some BC_3F_2 plants flowered earlier than IR64. Differences in duration were less marked in the BC_2F_1 and BC_3F_1 generation, indicating that some variations might result from the expression of recessive genes.

Tillering and grain weight per plant also showed a range of variation. These traits, however, generally have a lower broad-sense heritability than plant height and duration. It was not possible with the present experimental design to determine whether the observed variations were genetically controlled or not.

Regardless of the basis of the observed phenotypic variation, these BC_3F_2 plants were largely returned to the IR64 background. Thus, they can easily be converted to NILs for characterization of individual QTLs for the targeted root traits and for other traits of interest. For example, *sd-1* on chromosome 1 is known to be located in the RZ730-RZ801 interval (Huang et al 1996). If we compare the genotypes and height profiles of plant IR74392-118-4 and plant IR74392-135-1 or IR74392-135-7 (Table 4, target 1), we can infer that *sd-1* is probably located in the small subregion of the RZ730-RZ801 interval, which is different between these plants.

Fine mapping of QTLs on different chromosomes

Each of the introgressed regions spanned a large genomic segment to ensure a high probability of inclusion of the QTL. Thus, simultaneous transfer of undesirable donor alleles due to genetic drag was expected to occur. To minimize the impact of genetic drag, fine mapping of the target region is required. This will be achieved more efficiently by phenotyping progenies of the BC_3F_2 plants that have overlapping genomic segments in the target areas. We have identified several useful recombinants for this purpose among the BC_3F_2 plants. For instance, recombinants carrying the Azucena allele at different and overlapping regions in the region RZ19-RG690-RZ730-RZ801 can be used to accurately locate the target QTL to one of these intervals with or without mapping additional markers in the target region.

Pyramiding QTLs by crossing selected BC_3F_2 plants

According to the original study, the effect of each individual QTL was small. To obtain differences for the root system in the progenies, which translate into significant differences for drought tolerance in the field, the association of several QTLs might be necessary. Pyramiding of QTLs can be obtained by crossing plants carrying Azucena alleles at different target regions. For example, BC_3F_2 plants carrying Azucena alleles at both targets 1 and 7 were identified. Two of them, IR74409-736-11 and IR74409-738-11, were crossed to plants homozygous for Azucena at target 9. The cross is expected to yield plants carrying Azucena alleles at all three of these target regions (targets 1, 7, and 9). By crossing the resulting plants carrying targets 1, 7, and 9 to plants carrying target 2, we hope to recover plants combining all major QTLs for maximum root length while minimizing genetic drag.

Yet, the pyramiding approach has practical problems. This type of cross involves two individual plants and not, as in the rest of the program, one individual plant and a recurrent parent for which staggered sowings are easy to realize. Therefore, it is more difficult to synchronize the flowering dates and the number of hybrid seeds obtained is usually low.

Future phenotyping work

Working with the root system, we are in the worst-case scenario for marker-aided selection. The confidence interval for QTL location for traits with low heritability is generally very broad (Hyne et al 1995, Visscher et al 1996b), and QTLs risk being assigned to the wrong intervals. This was the primary reason we selected a large region for introgression for each of the target QTLs. Moreover, phenotypic evaluation during the selection process is impossible because the evaluation is destructive and inaccurate without replication. We have therefore delayed it until the end of the process. We obtained BC_3F_2 plants with the proper genotypes at the target regions, but there is always a risk that individuals displaying the desired genotype may not carry the QTL because the linkage between the markers and the target QTL could have been broken during backcross work.

The final phenotyping work using the BC_3F_3 seeds coming from the selected BC_3F_2 plants will be carried out in three steps. The first step will be to evaluate their root system under greenhouse conditions in comparison with IR64 and see whether they express the expected differences. A second experiment would allow better characterization of the NILs for duration, height, and yield components under field conditions. The third step will be to compare the performance of the best lines with that of IR64 under various water-stress conditions.

References

Basten CJ, Weir BS, Zheng ZB. 1997. QTL Cartographer version 1.12c. Raleigh, N. Car. (USA): North Carolina State University.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet. 95:553-567.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

Guiderdoni E, Gallinato E, Luistro J, Vergara G. 1992. Anther culture of tropical japonica/indica hybrids of rice. Euphytica 62:219-224.

Hittalmani S, Parco A, Mew T, Zeigler RS, Huang N. 1999. Fine mapping and marker-assisted pyramiding of major genes for blast resistance in rice. Theor. Appl. Genet. (In press.)

Hospital F, Charcosset A. 1997. Marker-assisted introgression of quantitative trait loci. Genetics 147:1469-1485.

Huang N, Courtois B, Khush GS, Lin HX, Wang GL, Wu P, Zheng KL. 1996. Association of quantitative trait loci for plant height with major dwarfing genes in rice. Heredity 77:130-137.

Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu J, Subudhi P, Angeles ER, Khush GS. 1997. RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population. Mol. Breed. 3:105-113.

Hyne V, Kearsey MJ, Pike DJ, Snape JW. 1995. QTL analysis: unreliability and bias in estimation procedures. Mol. Breed. 1:273-282.

Lilley JM, Fukai S. 1994. Effect of timing and severity of water deficit on four diverse rice cultivars. II. Physiological responses to soil water deficit. Field Crops Res. 37:215-223.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.

Nelson RJ, Chen D, Mauleon RP, Inukai T, Clavero AC, Zeigler RS. 1996. Recent progress in rice blast research for the uplands. In: Piggin C, Courtois B, Schmit V, editors. Upland rice research in partnership. IRRI Discussion Paper Series 16. Manila (Philippines): International Rice Research Institute. p 125-140.

Nguyen HT, Chandra Babu R, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37:1426-1434.

O' Toole JC. 1982. Adaptation of rice to drought-prone environments. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 195-216.

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95:143-152.

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration. Theor. Appl. Genet. 92:627-636.

Tanksley SD, Nelson JC. 1995. Advanced back-cross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Tuinstra MR, Ejeta G, Goldsbrough PB. 1997. Heterogeneous inbred family analysis: an approach for developing near-isogenic lines that differ at quantitative trait loci. Theor. Appl. Genet. 95:1005-1011.

Visscher PM, Haley CS, Thompson R. 1996a. Marker-aided introgression in backcross breeding programs. Genetics 144:1923-1932.

Visscher PM, Thompson R, Haley CS. 1996b. Confidence interval in QTL mapping by bootstrapping. Genetics 143:1013-1020.

Wang H, Qi MQ, Cutler AJ. 1993. A simple method of preparing plant sample DNA. Nucleic Acid Res. 21:4153-4154.

Widawsky DA, O'Toole JC. 1990. Prioritizing rice biotechnology research agenda for Eastern India. New York (USA): The Rockefeller Foundation.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor. Appl. Genet. 94:619-632.

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Perspectives on molecular marker research: (2) shoot traits and integration

Screening for osmotic adjustment in rice

R. Chandra Babu, A. Blum, Jingxian Zhang, S. Sarkarung, and H.T. Nguyen

Osmotic adjustment (OA) is a major component of drought tolerance. Incorporation of OA in crop improvement programs is limited by its slow and complex measurement procedures. Broadly, three different methods for determining OA in plants are in use. In a recent study, the comparative performance of these methods was tested using diverse rice lines. Significant variation in OA among cultivars was observed in all three methods. More than a fourfold difference in OA among cultivars (0.35 to 1.51 MPa) was observed using the standard regression method. The mean OA over the cultivars was 0.89, 0.51, and 0.72 MPa in the "regression," "full turgor adjusting," and "rehydration" methods, respectively. The rehydration method had a significantly higher correlation with the standard regression method and had an advantage over the full turgor adjusting method in terms of quick measurements of osmotic potential. The rehydration method served as a rapid procedure to screen large amounts of germplasm with some improvements suggested here. OA was not fully expressed under conditions of high temperature and evaporative demand because of the fast development of stress. Because of the sensitivity of the measurement systems and experimental methods employed in this study, rice required about 3 wk of stress duration for solute(s) to accumulate and for cultivar differences to develop in OA. The complexity of measuring OA in selection work tempts us to seek alternate indices for OA that are simple and rapid for measurement. Constitutive capacity for OA is one such example. The initial turgid osmotic potential in rice plants not previously exposed to drought, however, is not associated with OA after the stress treatment. On the other hand, the osmotic potential of plants rehydrated after stress showed a significant positive relation with OA. The need for a proper stress protocol, measurement procedures for plant water relations, and the different methods of OA determination are discussed, with emphasis on rice.

Osmotic adjustment (OA) in higher plants has been defined (Blum 1988) as follows: "As water is being removed from the plant cell, its osmotic potential (OP) is reduced due to the simple effect of solute concentration. However, if during the course of cellular water loss solutes are actively accumulated, OP would be reduced beyond the rate dictated by the mere effect of concentration. Such an active accumulation of solutes during the development of water deficit is termed OA."

OA can allow higher turgor potential (TP) at a given leaf water potential (LWP). Thus, zero turgor will occur at a relatively lower LWP in an osmotically adjusting leaf:

$$LWP = TP + OP$$

To maintain a positive turgor (TP = more than zero), OP must be less than LWP. Solute accumulation (generating a more negative OP) clearly represents a mechanism for maintaining a positive turgor at low LWP. Ideally, the OP is inversely proportional to the cell volume (V) or volume of the solution, and directly proportional to the number of solute molecules (n):

$$OP = -nRT/V$$

where R = gas constant and T = absolute temperature. When water stress occurs, the water potential in the cell walls and conducting vessels falls, creating a potential gradient for the protoplast. The response to re-establish equilibrium may involve either a change in V or n or both. If n does not change, water is lost, that is, the tissue dehydrates. When OA occurs, n increases, either to reduce or prevent a change in V, and turgor is maintained. Thus, analysis of the relationship between OP and V provides a basis for comparing degrees of OA. Because it is difficult to measure V directly, relative water content (RWC) is used as an alternate (Morgan 1983).

The objective of this chapter is to explain to researchers the importance of OA in drought tolerance, the need for a proper water stress protocol for expression of OA, and the various methods for determining OA. These points are discussed, with emphasis on screening rice germplasm for OA.

Osmotic adjustment and drought tolerance

Osmotic adjustment is an effective component of drought tolerance in several crop plants (Morgan 1984, Blum 1988, Ludlow and Muchow 1990, Kramer and Boyer 1995). Generally, OA contributes to turgor maintenance of both shoots and roots as plants experience water deficit. This allows turgor-dependent processes such as growth and stomatal activity to continue to progressively lower LWP. The accumulated compatible solutes may also protect specific cellular functions, irrespective of turgor (Paleg et al 1985, Shen et al 1997). Substantial genotypic variation for OA was observed in wheat (Morgan 1977, Morgan et al 1986), sorghum (Blum and Sullivan 1986, Santamaria et al1 1990, Basnayake et al 1993, Tangpremsri et al 1995), chickpea (Morgan et al 1991), field pea (Rodriguez-Maribona et al 1992), and sunflower (Jamaux et al 1997). OA is known to occur in rice under water deficit to an extent of about 0.5–0.6 MPa in tests using a limited number of cultivars (Cutler et al 1980, Steponkus et al 1982, Hsiao et al 1984, Turner et al 1986). Higher levels of OA were reported under a prolonged stress among diverse rice lines (Lilley and Ludlow 1996).

Unlike most other putative traits, the association of OA with components of yield and determinants of plant survival has been demonstrated (Ludlow and Muchow 1990). OA as an important drought-tolerance component and its positive influence on leaf rolling, tissue death, and retention of green leaf area in rice have been reviewed (Nguyen et al 1997). OA has been associated with yield under drought stress in several crops (Kumar et al 1984, Ludlow et al 1990, Santamaria et al 1990, Morgan et al 1991, Rodriguez-Maribona et al 1992, Morgan 1995). Although a yield benefit of OA has yet to be clearly demonstrated in rice (Lilley and Ludlow 1996), a possible indication to this effect is seen in the literature.

In a pot experiment using 12 rice cultivars (Ramakrishnaiya and Murthy 1993), the soil moisture was allowed to drop to 25% of the field capacity during the vegetative stage (21–49 dafter sowing). In cultivar CR143-2-2, the decline in RWC was relatively slow, but the fall in LWP was more apparent and leaf diffusive resistance was the lowest. The maintenance of a high RWC in spite of the low LWP in CR143-2-2 might have been due to OA because its OP dropped below that of LWP as the stress advanced. The drought index was relatively higher in CR143-2-2 (90.3%) compared to other cultivars assessed in a separate field experiment with soil moisture depletion down to 1/3 of field capacity (8% soil moisture). This cultivar consistently recorded stable yield under periodical moisture stress.

The OA value as a drought-tolerance mechanism is high in heavier soils (Morgan 1984) but is limited under rapid desiccation typical in crops growing on shallow sandy soils (Blum 1982). Except for the need to develop a rapid screening procedure, there is no problem with this trait being used in breeding programs with good prospects for improving yield under stress (Ludlow and Muchow 1990).

Screening for OA

Once OA has been shown to influence yield and crop adaptation, selection should be conducted in the environment where the most effective discrimination is possible. Little research has been conducted on the stress magnitude and conditions required to maximize OA (Begg and Turner 1976). Difficulties with natural agricultural environments include the probability that the relevant environmental challenge(s) will not occur and the possibility that confounding challenges may also occur. Hence, in many situations it may be more appropriate to use contrived environments where some control is exercised over features such as water management, nutrition, photoperiod, or temperature. Glasshouse measurements of OA may thus be an appropriate selection criterion, as in wheat breeding (Morgan et al 1986). Further measurements of OA made on glasshouse-grown plants may be used to select lines that yield higher under conditions of water deficit in the field (Morgan 1983).

Stress protocol and measurement of plant water relations

The methodology for measuring OA in plants under drought stress is strengthened by the fact that OA is strongly affected by the extent and duration of drought stress. OA

is time-dependent (Blum 1982) and the progression of water stress has to be slow enough to allow solutes to accumulate. The slow development of water stress is critical to allowing the genetic potential for OA to be fully expressed (Jones and Rawson 1979). Thus, a protocol for measuring OA in rice under the effect of drought stress must standardize the conditions for development of a slow and prolonged water stress cycle. Growing plants in large containers (e.g., 18-L capacity with a 35 × 30 × 25-cm dimension), filled with 6 kg of potting soil consisting of peat (5–15%), perlite bark (40–50%), Styrofoam (20–30%), and vermiculite (10–20%) (e.g., Ball Growing Mix3, Ball Horticultural Company, USA), and maintaining 2 plants pot^{-1} will permit a prolonged drought stress cycle under relatively mild atmospheric conditions. Because OA is also a function of the extent of plant water deficit, the evaluation of genotypic differences in OA must be performed by subjecting all the genotypes to an adequate and same level of plant water stress (Nguyen et al 1997). Later, OA measurements can be normalized to a standard leaf RWC. Otherwise, comparing genotypes using OA values obtained from plants subjected to different levels of plant water deficit may be misleading, as in the case of barley (Teulat et al 1998).

Determining RWC, OP, and LWP at midday in the midsection of the same second-youngest fully expanded leaf blade will minimize errors arising from comparing one variable on one plant or leaf and another on a second that may have a different water status. RWC is normally determined after 4 h rehydration under light compensation following the standard formula of Barrs and Weatherley (1962) and OP and LWP are measured using thermocouple psychrometry following the procedures outlined by Brown and Collins (1980), as these methods are consistent and standard. Thermocouple psychrometers are generally considered to be reliable and accurate instruments for measuring plant and soil water potentials (Brown and Oosterhuis 1992).

Methods for determining OA

The comparison of OA data from different studies is hampered by differences in methodology and protocol. Different methods for determining OA in plants have been proposed and used (Jones and Turner 1978, Wilson et al 1979, Ludlow et al 1983, Blum 1989, Morgan 1992), and have been reviewed recently (Zhang et al 1999). The various published methods for measuring OA can be classified broadly into three procedures: the "regression" method, the "full turgor adjusting" method, and the "rehydration" method.

The regression method

The regression method (Morgan 1992) is the most comprehensive and is considered the standard because of its underlying theory and the extensive data acquisition it is based on. This method estimates OA from the linear regressions of RWC on OP as derived from consecutive measurements during a drought stress cycle. It partitions between active solute accumulation (i.e., OA) and the "concentration effect" on OP (OP_0) of water loss from tissues. OP_0 was calculated for each RWC data point according to Morgan (1992) and Wright et al (1983):

$$OP_0 = OPi\ ((RWCi/l00)/RWC/l00))$$

where OPi = initial OP at full turgor and RWCi = initial RWC at full turgor.

Osmotic adjustment was calculated from the two regressions as the difference between OP and OP_0 at an RWC of 70%. The regression of RWC on OP could be considered as a single-phase response as was done in rice (Babu et al 1999), wheat (Morgan 1992), and sunflower (Chimenti and Hall 1993), or as a bi-phase response as was done in rice (Lilley and Ludlow 1996, Lilley et al 1996) depending on the trend in the data set. OA at 70% RWC indicates the amount of solutes accumulated at an equivalent level of cell volume for all the genotypes. Leaves die when they reach a critical RWC rather than when they reach a critical LWP (Flower and Ludlow 1986) and photosynthesis, protein synthesis, nitrate reduction, and leaf senescence are often better correlated with changes in cell volume and RWC than with LWP. But the regression method is demanding on time, labor, and plant materials.

The full turgor adjusting method

The full turgor adjusting method (Wilson et al 1979, Ludlow et al 1983) is relatively simpler. It estimates OA from the difference in OP at full turgor (RWC_{100}) between nonstressed and stressed plants. With this method, OP at RWC_{100} is calculated from point measurements of OP and RWC at a given level of plant water deficit stressed (e.g., at about 60–65% RWC for rice), with a correction for apoplastic water content (B). OP at RWC_{100} (OP_{100}) is calculated as follows:

$$OP_{100} = OP((RWC - B)/(100 - B))$$

A B value of 18% has been reported for rice (Turner et al 1986). A constant value of B was used for both stressed and nonstressed leaves of all cultivars because B did not change with cultivars (Turner et al 1986) or dehydration (Wilson et al 1980). A constant value of B was also used by others (Ludlow et al 1983, 1990). OP and RWC measurements are made in nonstressed and stressed plants.

The rehydration method

The rehydration method (Jones and Turner 1978, Blum 1989) also estimates OA by a point measurement of the difference in OP at RWC_{100} between nonstressed and stressed plants. Here, however, OP at RWC_{100} in stressed plants is actually measured after plants or parts thereof are rehydrated to RWC_{100}.

The standard regression method can also be used alternatively as done by Morgan (1992, 1995), where the relative capacity for OA is derived from the regression of RWC on OP and estimated as the RWC at OP of –3.5 MPa, which is at about wilting for rice (Lilley and Ludlow 1996). This is the breakpoint OP near or below the phase of maintenance of RWC in high OA lines in rice. This OP varies with crop; it is –2.5 MPa in wheat (Morgan 1991) and –1.7 MPa in sunflower (Chimenti and Hall 1993). The RWC at an OP of –3.5 MPa gives an estimate of maintenance of cell volume at a

given OP for all the genotypes. This alternate procedure and the standard regression method previously discussed use the same data set. The difference between the two is that the relative capacity for OA as expressed in terms of RWC at a given OP does not account for possible variations among cultivars in the concentration effect on OP during a drought event.

Comparing screening methods

Recently, the advantages and disadvantages of these methods for measuring OA variation were compared in 12 rice genotypes (Babu et al 1999). Very high levels of OA variation among lines were established by the regression method at 70% RWC, ranging from 0.35 MPa in IR52561-UBN-1-1 -2 to 1.51 MPa in IR62266-42-6-2, with a mean of 0.89 MPa across cultivars. Such high values are comparable with the highest values observed in wheat (Morgan 1992), barley (Blum 1989), and sorghum (Blum and Sullivan 1986, Santamaria et al 1990, Tangpremsri et al 1995) and they are higher than those observed earlier in rice (Cutler et al 1980, Hsiao et al 1984, Turner et al 1986). Similar high levels of OA have been recorded in diverse rice lines subjected to prolonged stress (Lilley and Ludlow 1996).

Morgan's (1992) regression method is the most comprehensive, but it is time- and labor-consuming. It is not suitable for screening a large number of genotypes as in molecular mapping work. The high correlation across rice cultivars between OA at RWC of 70% and RWC at –3.5 MPa OP (Babu et al 1999) indicates that the variation among cultivars in concentration effect was not very important for estimating cultivar differences in OA. This seems to be the case in wheat also, where estimating relative capacity for OA based on RWC at a given level of OP is used extensively by Morgan (Morgan 1992, 1995, and personal communication). This reduces only the number of calculations but not labor, time, and plant material; hence, it is not suitable for screening work.

Because the full turgor adjusting and rehydration methods provide a point measurement of the difference in OP at RWC_{100} between well-watered and drought-stressed plants, they are less tedious. But the mean OA value (0.51 MPa) by the full turgor adjusting method is low (Babu et al 1999). This was not due to an insufficient level of drought stress because the plants were stressed to about the same or lower RWC. The reason could be perhaps because of the assumptions on which the calculation to full turgor is based. First, the correction for B may be unnecessary, despite its estimate (18%) for rice (Turner et al 1986). The value and constancy of bound water have only a small effect on OP_{100} and they are sometimes omitted from the equation (Jones and Turner 1978). Second, the correction to an RWC of 100% may be unrealistic because normally grown turgid plants rarely reach an RWC of l00%, as pointed out by Morgan (1995). RWC at full turgor under normal growing conditions was often below 100% in rice (Babu et al 1999). Therefore, these two corrections are not sufficient to explain the relatively low mean OA value by this method.

In comparison to the full turgor adjusting method, the mean OA (0.72 MPa) across cultivars by the rehydration method was relatively higher (Babu et al 1999),

although, compared with the mean OA by the standard regression method, this is lower, perhaps because solutes were diluted during rehydration. Further, respiration during the rehydration period (overnight) may have reduced osmolality. But the ranking of the rice cultivars for OA by the regression method agreed well with ranking by the other methods. Based on the correlation analysis with the regression method, Babu et al (1999) suggested that the rehydration method is more suitable for extensive measurement of OA, as in selection work. Further, the rehydration method may be more useful in extraction of sap for studies involving quantification of osmolytes.

Several improvements can be considered when the rehydration method is used. First, measuring OP using thermocouple psychrometers is too slow and elaborate. A technically faster method is to measure OP of expressed sap of freeze-thawed leaf in a vapor pressure osmometer. Although this method has been debated (Kikuta and Richter 1992, Morgan 1992), the use of sap from uniformly rehydrated tissues can improve the consistency of results compared with the psychrometric measurement of freeze-thawed tissues from stressed plants. Second, results in favor of a shorter rehydration period can be considered. A large increase occurred in the first 24 h in OP of rewatered rice plants (Steponkus et al 1982). Watering intact rice plants in the dark and allowing 2 h for equilibration resulted in full hydration as judged by water potentials near zero and the presence of guttation fluid (Cutler et al 1979). Obviously, the rehydration time must not be too long, especially in plants adapted to water deficits, because the plants may lose or gain solutes during rehydration (Turner and Jones 1980).

Studies with most crop species suggest that rehydration for 3–4 h at light compensation may be enough to regain full turgor without any marked loss of solutes. But OA is known to persist up to 14 d after rewatering in rice (Steponkus et al 1982). We tested the change in OP of stressed plants at different intervals after rehydration and preliminary results indicated that the OP remained fairly unchanged at least during the first 10 h after rewatering (Tripathy, personal communication). Based on this, we suggest rewatering the stressed plants late in the evening and sampling for OP early the next morning.

Time requirement for OA

Besides tissue water deficit, time is a critical variable for the development of OA. This is deduced from the poor development of OA in most cultivars in an experiment where stress duration was shorter (Babu et al 1999). Though plants were grown in an adequately large soil volume, higher ambient temperature and transpirational demand led to faster development of stress and plants wilted earlier in this experiment, which did not allow enough time for solute accumulation. Plants wilted at an LWP of around –2.5 MPa in the second experiment because of a lack of sufficient time for OA, whereas they wilted at an LWP of about –3.5 MPa under prolonged stress in the first experiment. Therefore, it is not the rate of stress development that is important for OA but the time available for solute accumulation before the plants wilt as a function of tissue water loss on the one hand and the rate of solute accumulation on the other. The maximum

extent of OA in rice is independent of the rate of stress development over a range of LWP of 0.05 to 0.6 MPa d^{-1} (Steponkus et a1 1982). The degree of OA was correlated with cumulative stress days above a threshold cumulative LWP of –16 to –17 MPa days (Turner et al 1986). Rice evidently requires about 3 wk of drought stress (from initiation of stress to wilting) to express its full capacity for OA, which seems to be longer than what is required by wheat (Morgan 1992) or sorghum (Basnayake et al 1993).

The time required for OA may depend on the nature of the osmoticum and the rate of its accumulation, which could differ among plant species. The compatible solutes—various sugars, organic acids, amino acids, sugar alcohols, or ions (most commonly K^+)—differ with plant species and genera (Morgan 1984). The main solutes responsible for OA in rice under water-deficit conditions have not yet been elucidated. Rice does not accumulate glycine betaine as do barley and other plants (Ishitani et al 1993) because of a deficiency in choline monooxygenase and betaine aldehyde dehydrogenase (Rathinasabapathi et al 1993). Rice does accumulate proline (Dingkuhn et al 1991). The extent of proline accumulation and its contribution to OA, however, have not been evaluated. Because proline accumulation is proportional to the rate of plant water deficit, genotypic differences in proline accumulation may simply reflect respective differences in LWP (Dingkuhn et al 1991).

Alternative indices for OA

The complexity of measuring OA in selection work makes it tempting to take shortcuts. The constitutive accumulation of solutes can also be an effective component of turgor maintenance under drought stress, as seen in natural vegetation (Walter 1965), where OPi can range from –0.5 to –2.2 MPa (Larcher 1980). Constitutive accumulation (by overexpression of the responsible gene) of a cellular osmoticum is regarded as an effective approach to increasing crop drought resistance by genetic engineering (Bohnert et al 1995). The OPi values in rice cultivars differed significantly from –1.5 to –2.0 MPa (Babu et a1 1999). The OPi was independent of RWCi and it may therefore represent a long-term constitutive adaptive component, as argued by O'Toole (1982). But the constitutive accumulation of solutes may be totally unrelated to the capacity for OA in response to drought stress, as seen by the lack of any significant correlation across cultivars between OPi and OA (Babu et al 1999). A nonsignificant range in OPi (–1.29 to –1.39 MPa) was also reported from diverse and recombinant inbred lines (RILs) in rice (Lilley and Ludlow 1996, Lilley et al 1996).

Desiccation tolerance as measured by lethal OP and/or lethal RWC is suggested to be related to OA. A significant but low correlation coefficient, however, indicates that factors other than OA are involved in dehydration tolerance in rice (Lilley et al 1996). Selection for increased desiccation tolerance has a penalty for OA (Basnayake et al 1993). The OP of rehydrated leaves had a significant positive correlation with OA in rice (Zhang et al 1998). Relative water loss (water loss of excised leaves) is suggested as a physiological marker for OA in sunflower (Jamaux et al 1997).

Molecular approaches

Selection based on molecular markers, known as marker-assisted selection, alleviates difficulties associated with low heritability, recessiveness, and screening procedures (e.g., OA). Selection of suitable breeding populations and a stress protocol and reliable phenotypic evaluation requiring a multidisciplinary team effort will be crucial for the success of this approach. A quantitative trait locus (QTL) linked to OA has been mapped in rice using the regression method with 52 RILs (Lilley et al 1996). Population size plays an important role in identifying the number of QTLs (Tanksley 1993). Thus, by employing the rehydration method, QTLs linked to OA in rice have been mapped recently using 154 doubled-haploid lines (Zhang et al 1998).

Genetic engineering has the potential to improve plant tolerance rapidly (Bartels and Nelson 1994). Transgenic rice plants showed better water-stress tolerance and recovery than nontransformed ones (Xu et al 1996). Transgenic rice was found to accumulate glycine betaine and acquired salt and cold resistance (Sakamoto et al 1998). The mechanism whereby solute accumulation provided stress tolerance has not been examined comprehensively (Chopra and Sinha 1998), especially with current knowledge on plant water relations (Blum et al 1996). Most studies have used readily available promoters for constitutive expression, whereas the introduction of a pathway to enable stress-responsive control of transgenes would be preferable, because constitutive expression of a stress protection would probably compromise productivity (Bohnert and Jensen 1996). Further experiments are needed, however, to determine whether the improved stress tolerance would be great enough to be applicable to agriculture.

References

Babu RC, Pathan MS. Blum A, Nguyen HT. 1999. Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Sci. 39:150-158.

Barrs H.D, Weatherley PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15:413-428.

Bartels D, Nelson D. 1994. Approaches to improve stress tolerance using molecular genetics. Plant Cell Environ. 17:659-667.

Basnayake J, Ludlow MM, Cooper M, Henzell RG. 1993. Genotypic variation of osmotic adjustment and desiccation tolerance in contrasting sorghum inbred lines. Field Crops Res. 35:51-62.

Begg JE, Turner NC. 1976. Crop water deficits. Adv. Agron. 28:161-217.

Blum A. 1982. Evidence for genetic variability in drought resistance and its implications in plant breeding. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 53-68.

Blum A. 1988. Plant breeding for stress environments. Boca Raton, Fla. (USA): CRC Press.

Blum A. 1989. Osmotic adjustment and growth of barley cultivars under drought stress. Crop Sci. 29:230-233.

Blum A, Sullivan CY. 1986. The comparative drought resistance of landraces of sorghum and millet from dry and humid regions. Ann. Bot. 57:835-846.

Blum A, Munns R, Passioura JB, Turner NC, Sharp RE, Boyer JS, Nguyen HT, Hsiao TC. 1996. Letters to the editors: genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations. Plant Physiol. 110:1051-1053.

Bohnert HJ, Nelson DE, Jensen RG. 1995. Adaptations to environmental stresses. Plant Cell 7:1099-1111.

Bohnert HJ, Jensen RG. 1996. Metabolic engineering for increased salt tolerance: the next step. Aust. J. Plant Physiol. 23:661-667.

Brown RW, Oosterhuis DM. 1992. Measuring plant and soil water potentials with thermocouple psychrometers: some concerns. Agron. J. 84:78-86.

Brown RW, Collins JM. 1980. A screen-caged thermo-couple psychrometer and calibration chamber for measurement of plant and soil water potentials. Agron. J. 72:851-854.

Chimenti CA, Hall AJ. 1993. Genetic variation and changes with ontogeny of osmotic adjustment in sunflower (*Helianthus annuus* L.). Euphytica 71:201-210.

Chopra RK, Sinha SK. 1998. Prospects of success of biotechnological approaches for improving tolerance to drought in crop plants. Curr. Sci. 74:25-34.

Cutler JM, Shahan KW, Steponkus PL. 1979. Characterization of internal water relations of rice by a pressure-volume method. Crop Sci. 19:681-685.

Cutler JM, Shahan KW, Steponkus PL. 1980. Dynamics of osmotic adjustment in rice. Crop Sci. 20:310-314.

Dingkuhn M, Cruz RT, O'Toole JC, Turner NC, Doerffling K. 1991. Response of seven diverse rice cultivars to water deficits. III. Accumulation of abscisic acid and proline in relation to leaf-water potential and osmotic adjustment. Field Crops Res. 27:103-117.

Flower DJ, Ludlow MM. 1986. Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeonpea (*Cajanus cajan* (L.) *millsp.*) leaves. Plant Cell Environ. 9:33-40.

Hsiao TC, O'Toole JC, Yambao EB, Turner NC. 1984. Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). Plant Physiol. 75:338-341.

Ishitani M, Arakawa K, Mizuno K, Kishitani S, Takabe L. 1993. Betaine aldehyde dehydrogenase in Graminae: levels in leaves of both betaine-accumulating and nonaccumulating cereal plants. Plant Cell Physiol. 34:493-495.

Jamaux I, Steinmetz A, Belhassen E. 1997. Looking for molecular and physiological markers of osmotic adjustment in sunflower. New Phytol. 137:117-127.

Jones MM, Rawson HM. 1979. Influence of rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency and osmotic potential in sorghum. Physiol. Plant. 45:103-111.

Jones MM, Turner NC. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol. 61:122-126.

Kikuta SB, Richter H. 1992. Leaf discs or press saps: a comparison of techniques for the determination of osmotic potentials in freeze-thawed leaf material. J. Exp. Bot. 43:1039-1044.

Kramer PJ, Boyer JS. 1995. Water relations of plants and soils. San Diego, Calif. (USA): Academic Press.

Kumar A, Singh P, Singh DP, Singh H, Sharma HC. 1984. Differences in osmoregulation in *Brassica* species. Ann. Bot. 54:537-541.

Larcher W. 1980. Physiological plant ecology. Berlin (Germany): Springer-Verlag.

Lilley JM, Ludlow MM. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. Field Crops Res. 48:185-197.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Ludlow MM, Muchow RC. 1990. A critical evaluation of the traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Ludlow MM, Chu ACP, Clements RJ, Kerslake RG. 1983. Adaptation of species of *Centrosema* to water stress. Aust. J. Plant Physiol. 10:119-130.

Ludlow MM, Santamaria FJ, Fukai S. 1990. Contribution of osmotic adjustment to grain yield of *Sorghum bicolor* (L.) Moench under water-limited conditions. II. Post-anthesis water stress. Aust. J. Agric. Res. 41:67-68.

Morgan JM. 1977. Differences in osmoregulation between wheat cultivars. Nature 270:234-235.

Morgan JM. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Aust. J. Agric. Res. 34:607-614.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35:299-319.

Morgan JM. 1991. A gene controlling differences in osmoregulation in wheat. Aust. J. Plant Physiol. 18:249-257.

Morgan JM. 1992. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol. 19:67-76.

Morgan JM. 1995. Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. Field Crops Res. 40:143-152.

Morgan JM, Hare RA, Fletcher RA. 1986. Genetic variation in osmoregulation in bread and durum wheats and its relationship to grain yield in a range of field environments. Aust. J. Agric. Res. 37:449-457.

Morgan JM, Rodriguez-Maribona B, Knights EJ. 1991. Adaptation to water deficit in chickpea breeding lines by osmoregulation: relationship to grain yields in the field. Field Crops Res. 27:61-70.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37:1426-1434.

O'Toole JC. 1982. Adaptation of rice to drought prone environments. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 195-213.

Paleg LG, Stewart GR, Starr R. 1985. The effect of compatible solutes on proteins. Plant Soil 89:83-94.

Ramakrishnaiya G, Murthy KS. 1993. Influence of different levels of soil moisture stress on physiological parameters of upland rice cultivars. Indian J. Plant Physiol. 34:387-391.

Rathinasabapathi B, Gage DA, Mackill DJ, Hanson AD. 1993. Cultivated and wild rice do not accumulate glycine betaine due to deficiencies in two biosynthetic steps. Crop Sci. 33:534-538.

Rodriguez-Maribona B, Tenorio JL, Conde JR, Ayerbe L. 1992. Correlation between yield and osmotic adjustment of peas (*Pisum sativum* L.) under drought stress. Field Crops Res. 29:15-22.

Sakamoto A, Alia, Murata N. 1998. Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. Plant Mol. Biol. 38:1011-1019.

Santamaria JM, Ludlow MM, Fukai S. 1990. Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) under water-limited conditions. I. Water stress before anthesis. Aust. J. Agric. Res. 41:51-65.

Shen B, Jensen RG, Bohnert HJ. 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol. 113:1177-1183.

Steponkus PL, Shahan KW, Cutler JM. 1982. Osmotic adjustment in rice. In: Drought resistance in crops with emphasis on rice. Los Baños (Philippines): International Rice Research Institute. p 181-194.

Tangpremsri T, Fukai S, Fischer KS. 1995. Growth and yield of sorghum lines extracted from a population for differences in osmotic adjustment. Aust. J. Agric. Res. 46:61-74.

Tanksley SD. 1993. Mapping polygenes. Ann. Rev. Genet. 27:205-233.

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A. 1998. Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 96:688-698.

Turner NC, Jones MM. 1980. Turgor maintenance by osmotic adjustment: a review and evaluation. In: Turner NC, Kramer PJ, editors. Adaptation of plants to water and high temperature stress. New York (USA): John Wiley Inc. p 87-103.

Turner NC, O'Toole JC, Cruz RT, Yambao EB, Ahmad S, Namuco OS, Dingkuhn M. 1986. Responses of seven diverse rice cultivars to water deficit. II. Osmotic adjustment, leaf elasticity, leaf extension, leaf death, stomatal conductance and photosynthesis. Field Crops Res. 13:273-286.

Walter H. 1965. The water economy and hydrature of plants. Annu. Rev. Plant Physiol. 6:239-252.

Wilson JR, Fisher MJ, Schulze ED, Dolby GR, Ludlow MM. 1979. Comparison between pressure-volume and dew point-hygrometry techniques for determining the water relations characteristics of grass and legume leaves. Oecologia 41:77-88.

Wilson JR, Ludlow MM, Fisher MJ, Schulze ED. 1980. Adaptation to water stress of the leaf water relations of four tropical forage species. Aust. J. Plant Physiol. 7:207-220.

Wright GC, Smith RCG, Morgan JM. 1983. Differences between two grain sorghum cultivars in adaptation to drought stress. III. Physiological responses. Aust. J. Agric. Res. 345:637-665.

Xu D, Duan X, Wang B, Hong B, Hua T, Ho D, Ray Wu. 1996. Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol. 110:249-257.

Zhang J, Zheng HG, Tripathy JN, Aarti A, Sarial AK, Sarkarung S, Blum A, Nguyen HT. 1998. Mapping QTLs for osmotic adjustment in rice (*Oryza sativa* L.). Abstracts of Plant & Animal Genome VI, 18-22 Jan. 1998, California. p 117.

Zhang J, Nguyen HT, Blum A. 1999. Genetic analysis of osmotic adjustment in crop plants. J. Exp. Bot. 50:291-302.

Notes

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Progress on the molecular mapping of osmotic adjustment and root traits in rice

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The advent of molecular mapping technology makes it possible to dissect complex traits such as drought tolerance and yield. Because drought is a major limiting factor for grain production in rainfed rice ecosystems, this chapter reviews the literature on molecular mapping of drought tolerance in rice. Research progress has been made at Texas Tech University on tagging quantitative trait loci (QTLs) for two important drought-tolerance components in rice, osmotic adjustment and root traits. When QTLs for drought-tolerance traits were compared across populations, we found seven genomic regions where QTLs for various traits were clustered and shared by two or more populations. The practical applications of identified QTLs and the candidate gene ap proach are discussed.

As the most important food crop in the world, rice (*Oryza sativa* L., 2n=24) is receiving more attention than ever before. It is considered a model monocot plant for molecular genetics research because of its small genome size (430 Mb), well-developed genetic maps (Causse et al 1994, Harushima et al 1998), and high level of synteny and colinearity with other cereal genomes (Devos and Gale 1997).

Rice is grown under diverse environmental conditions in terms of topography, soil type, water regime, and climatic factors. About half of the world's rice area is under rainfed culture where drought is the most important abiotic stress limiting grain yields. Rainfed rice area includes 41 million ha of lowland and 17 million ha of upland ecosystems (Khush 1997). Drought, which often occurs because of unpredictable, insufficient, and uneven distribution of rainfall in these areas during the rice-growing seasons, drastically reduces rice production. Incorporating higher levels of drought tolerance into this major cereal is essential.

Significant advances have been made in understanding the physiology of drought tolerance in rice. Current knowledge on physiology suggests that drought tolerance in rice depends on one or more of the following components: (1) the ability of the roots to exploit deep soil water to provide for evapotranspirational demand, (2) the capacity for osmotic adjustment (OA) that allows plants to retain turgor and protect meristems from extreme desiccation, and (3) control over nonstomatal water loss from leaves (Nguyen et al 1997). Our research focuses on two of these drought-tolerance components: osmotic adjustment capacity and root-related traits.

Role of QTL mapping approach in drought-tolerance research in rice

Genetic modification of plants to grow and yield under unfavorable conditions solves problems of environmental stress. Figure 1 illustrates current strategies used to create more drought-tolerant plants, such as a genetic engineering approach, a quantitative trait loci (QTL) mapping approach, and a conventional breeding approach. Although the strategies focus on OA as an example, the concepts are applicable to other traits related to drought tolerance.

Genetic improvement of adaptation to drought is usually addressed through the conventional breeding approach by selecting for yield and its stability over locations and years. Although this approach has been successful to some degree in developing drought-tolerant varieties, such selection programs are expensive and slow in making progress. It is almost impossible to incorporate components such as OA and root-related traits into a plant breeding program via the conventional approach, mainly because of serious methodological constraints (Zhang et al 1999).

New emerging gene transfer technologies have enormous potential for plant improvement through the introduction of foreign genes into plant cells or tissue. A gene from any source can be transferred to plants if it can be cloned, transformed, and expressed, and if the transformed tissue can be regenerated into plants. Genes encoding enzymes involved in metabolic pathways for biosynthesis of osmolytes, for example, have been successfully introduced into several crops (for details, see the review by Zhang et al 1999) and some of these transformed plants showed increased

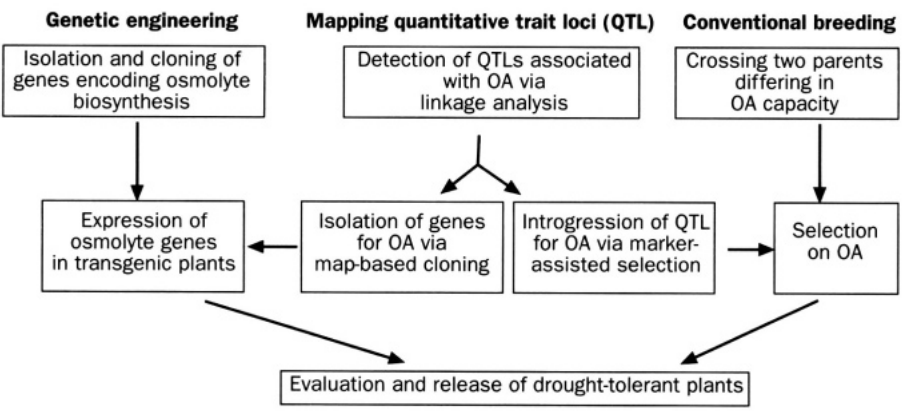

Fig. 1. Genetic avenues for developing drought-tolerant plants by manipulating osmotic adjustment (OA) and its components.

tolerance for osmotic stress. Although this approach holds great promise in the long run for improving tolerance for abiotic stresses, we believe that the successful development and release of drought-tolerant cultivars requires multiple-gene metabolic engineering or expression of a transcription factor that regulates the expression of several stress-related genes (Kasuga et al 1999).

The development of molecular marker technologies during the past ten years has revolutionized the genetic analysis of crops and made it possible to dissect complex quantitative traits. Genetic linkage maps composed of various DNA markers for rice have been constructed and widely used for tagging QTLs for diverse traits. In principle, QTL mapping is used to identify associations between a quantitative trait of interest and marker alleles segregating in a mapping population. The prerequisites for QTL detection are (1) to have a mapping population segregating for a trait of interest, (2) to genotype each individual of the population with molecular markers, and (3) to phenotype each individual of the population for the trait.

When QTL analysis is conducted, it has two essential stages: mapping DNA markers and associating the trait with the markers. Mapping markers is to put markers in linear order, indicate the relative distances between them, and assign them to their linkage groups on the basis of the recombination frequency between markers. Estimates of recombinant frequency and construction of the genetic map can be easily carried out by statistical software such as Mapmaker.

Several approaches have been proposed to detect associations between markers and traits. The most widely used approach is interval mapping using Mapmaker-QTL (Lincoln et al 1993). Intervals between adjacent pairs of markers along a chromosome are scanned. The LOD (Likelihood ODds ratio) score profile (of there being one QTL versus no QTL at a particular point) will be determined in each interval. Maximum LOD scores that exceed a specified threshold indicate the most likely sites of the QTLs.

It is not surprising that molecular mapping of genes that control plant tolerance for drought stress lags behind other work (e.g., disease resistance, morphological characters). Drought tolerance is complex and is associated with thickness of cuticles, stomatal opening and closing, root depth and extent, hormonal composition, OA, and antioxidant capacity. For example, OA, as an important component of drought tolerance, was just recently mapped in rice (Lilley et al 1996, Zhang et al 1998 and in preparation) and wheat (Morgan and Tan 1996).

QTLs for drought tolerance in rice

Because of the complex nature of drought tolerance, information on QTL mapping for this trait in rice is limited. To our best knowledge, Table 1 summarizes all the reported QTLs in the literature for drought-related traits in rice, along with the population used and its size, phenotypic variation explained by an individual QTL, and references. Because precision of QTL detection depends largely on accurate evaluation of phenotypes, caution should be taken in selecting mapping populations. For

Table 1. Summary of identified QTLs for drought-tolerance components in rice.

Traits	Populations (size).	No. of QTLs	R^2(%)[b]	References
Osmotic adjustment	C039/Moroberekan RILs (52)	1	32–34	Lilley et al (1996)
	CT9993/IR62266 DHLs (154)	4	7–17	Zhang et al (in preparation)
Dehydration tolerance	C039/Moroberekan RILs (52)	5	27–36	Lilley et al (1996)
Abscisic acid accumulation	IR20/63-83 F_2 (79)	10	–	Quarrie et al (1997)
Root penetration index	CO39/Moroberekan RILs (202)	6	7–13	Ray et al (1996)
	IR64/Azucena DHLs (109)	4	9–14	Zheng et al (1999)
	CT9993/IR62266 DHLs (154)	6	7–11	Zhang et al (in preparation)
	IR58821/1R52561 RILs (166)	3	13–26	Ali (1999)
Root thickness	C039/Moroberekan RILs (203)	18	15–33	Champoux et al (1995)
	Azucena/Bala F_2 (178)	3	7–21	Price & Tomos (1997)
	IR64/Azucena DHLs (135)	5	5–10	Yadav et al (1997)
	IR64/Azucena DHLs (109)	4	10–16	Zheng et al (1999)
	CT9993/IR62266 DHLs (154)	10	6–37	Zhang et al (in preparation)
	IR58821/1R52561 RILs (166)	5	6–14	Ali (1999)
Total root number	C039/Moroberekan RILs (202)	19	8–19	Ray et al (1996)
	IR64/Azucena DHLs (109)	2	8–9	Zheng et al (l999)
	IR58821/1R52561 RILs (166)	2	9–12	Ali (1999)
Root length	Azucena/Bala F_2 (178)	5	5-30	Price & Tomos (1997)
	IR64/Azucena DHLs (135)	8	4–21	Yadav et al (1997)
	C039/Moroberekan RILs (203)	0	0	Champoux et al (1995)
	IR64/Azucena DHLs (109)	0	0	Zheng et al (1999)
	IR58821/1R52561 RILs (166)	5	6–13	Ali (1999)
Total root dry weight	IR64/Azucena DHLs (135)	6	5–11	Yadav et al (1997)
Deep root dry weight	IR64/Azucena DHLs (135)	5	4–15	Yadav et al (1997)
	C039/Moroberekan RILs (203)	8	6–17	Champoux et al (1995)
Root pulling force	CT9993/IR62266 DHLs (154)	8	7–24	Zhang et al (in preparation)
Stomatal behavior	Azucena/Bala F_2 (178)	4	6-18	Price et al (1997)

[a] RILs = recombinant inbred lines, DHLs = doubled-haploid lines. [b] Phenotyplc variation explained by an individual QTL.

quantitative traits, doubled-haploid line (DHL) or recombinant inbred line (RIL) populations are preferred over F_2 and BC_1 populations because replicated measurements are usually required. Table 1 shows that most studies chose DHLs or RILs as mapping populations. Exceptions were the studies by Quarrie et al (1997), Price et al (1997), and Price and Tomos (1997), which used F_2 populations.

Osmotic adjustment and dehydration tolerance

Osmotic adjustment is the plant's capacity to actively accumulate solutes in the cells under drought. Of all putative drought-tolerance traits, OA has been associated with sustained yield under drought stress in crops such as wheat and sorghum, although information on rice is lacking (Zhang et al 1999). Other than the studies by Lilley et al (1996) and Zhang et al (1998 and in preparation), there appears to be no work that maps QTLs for OA in rice. Lilley et al (1996) evaluated OA on 52 RILs. A single QTL explaining one-third of the phenotypic variation for OA was identified (Table 1) and mapped at the RG1 region on chromosome 8 in rice. Comparative mapping indicated that this region was homologous with a segment of wheat chromosome 7, where a single locus putatively associated with OA was identified (see Fig. 1 in Zhang et al 1999). Using the same population, Lilley et al (1996) located five QTLs for dehydration tolerance (lethal osmotic potential), with phenotypic variation ranging from 27% to 36% (Table 1). But none of these QTLs overlapped with the QTL for OA. This suggests that OA and desiccation tolerance are two independent mechanisms of drought tolerance.

One factor limiting mapping of QTLs for OA is the lack of rapid procedures to phenotype large numbers of experimental materials. Recently, three commonly used protocols for OA determination have been compared and the rehydration method has been suggested for rice (Babu et al 1999). For detailed theories and equations related to the protocols, readers are referred to Zhang et al (1999) and Babu et al (1999, and this volume). Using a large DHL population derived from a cross between CT9993-5-10- 1-M and IR62266-42-6-2 (abbreviated as CT9993 and IR62266 in later text and table), we phenotyped OA based on the rehydration method in a greenhouse (Box 1). Preliminary analysis with the linkage map constructed for this population (Box 2) identified four QTLs for OA (Table l), one of which was located in a genomic region similar to that of the QTL detected by Lilley et al (1996).

Abscisic acid accumulation

Accumulation of abscisic acid (ABA) is a common response of plants to drought and this generally helps plants to survive stress better. Only one report in the literature deals with mapping of QTLs for ABA accumulation in rice. Quarrie et al (1997) conducted a detailed genetic analysis of ABA accumulation in droughted rice leaves using a population of 79 F_2 plants generated from the lowland × upland cross of IR20 (high-ABA) and 63-83 (low-ABA). Ten QTLs for ABA accumulation were identified (Table l), located on chromosomes 2, 3, 4, 6, 7, and 9, respectively. These QTLs accounted for 55% of the total phenotypic variation.

Box 1: Phenotyping CT9993/IR62266 DHL population for osmotic adjustment

Osmotic adjustment capacity was evaluated with the rehydration method (Babu et al 1999). It was calculated as the difference in osmotic potential at full turgor between well-watered and drought-stressed plants. Osmotic potential at full turgor in stressed plants was measured after the plants were rehydrated. The choice of the value of the water status at which plants were rehydrated was important because the magnitude of OA depended on the degree of water stress. In practice, leaf relative water content (RWC) was monitored during the stress period. When RWC reached a value where wilting occurred in most crop plants, rice plants were rehydrated and samples were collected after the plants regained full turgor.

Box 2: Genotyping CT9993/IR62266 DHL and IR58821/IR52561 RIL populations

Genotyping a DHL population (154 lines) derived from a cross between CT9993 and R62266 was done with restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and microsatellites (Zhang et al, in preparation). A genetic linkage map was then constructed, consisting of 315 molecular markers (145 RFLPs, 153 AFLPs, and 17 microsatellites). The map information was then used for QTL analysis on the phenotypic data collected at Texas Tech University (USA), IRRI (Philippines), the Ubon Rice Research Center (Thailand), and the Volcani Center (Israel) (Zhang et al, Kamoshita et al, Blum et al, this volume).

Genotyping a RIL population (166 lines) derived from a cross between two indica types — IR58821-23-B-2-1 and IR52561-UBN-1-12 (abbreviated as IR58821 and IR52561 in later text and table) — was done with RFLPs and AFLPs (Ali 1999). A genetic linkage map was then constructed, consisting of 383 AFLP and 106 RFLP markers. The map information was then used for QTL analysis on the phenotypic data collected at Texas Tech University and IRRI (Philippines) (Ali 1999, Kamoshita et al, this volume).

Root penetration ability and other root traits

Root penetration ability is an important trait for rice growing under rainfed lowland conditions. This is because compacted soil layers or hardpans are often found in lowland rice fields and genotypes with strong root penetration ability through the compacted layer can facilitate water uptake. Screening rice genotypes for root penetration ability using wax-petrolatum layers was developed at Texas Tech University (Box 3) and genetic variation in root penetration among rice cultivars was found (Yu et al

1995). Using the wax-petrolatum layer system, we phenotyped four mapping populations (CT9993/IR62266 DHLs, IR58821/IR52561 RILs, IR64/Azucena DHLs, CO39/Moroberekan RILs) (Table 1). Based on statistical analysis, we identified three to six QTLs for root penetration ability, which accounted for phenotypic variation ranging from 7% to 26% (Table 1). It is worthwhile to point out that the QTL on chromosome 2 was located in a similar genomic region across three of the four populations and might be more useful for marker-assisted selection (MAS) across different genetic backgrounds.

Box 3: Phenotyping root penetration index (ability)
Root penetration index was calculated as the ratio of penetrated root number through a hardpan to total root number. The wax-petrolatum layers (diameter 13.5 cm, thickness 0.5 cm) used to simulate the hardpan were made of 66.7% wax and 33.3% petrolatum white (Yu et a1 1995). They had a strength of 1.7 MPa at 27 °C. Plants were grown in pots having wax-petrolatum layers inside. To prevent penetrated roots from drying, these pots were suspended in wooden tanks with water filled to half of the pot height.

QTLs associated with other root morphological characters were also mapped (Table 1). Champoux et a1 (1995) located QTLs associated with five parameters of rice root morphology (root thickness, ratio of root to shoot, root dry weight per tiller, deep root dry weight per tiller, and maximum root length). More than 50% of the putative QTLs associated with root characters in the greenhouse study mapped to the same chromosomal locations as the QTLs associated with drought-avoidance scores in field experiments. Similarly, Ali (1999), Ray et a1 (1996), Price and Tomos (1997), Yadav et a1 (1997), Zhang et a1 (in preparation), and Zheng et a1 (1 999) also identified QTLs for various root characters (Table 1).

Genomic regions with QTLs clustering for drought-related traits

When we compared the QTLs detected from the above studies (Table 1), we found seven genomic regions where QTLs for various traits were clustered and shared by two or more populations (Fig. 2). Of the seven genomic regions, six were associated with drought-avoidance scores evaluated in the field. The RG437-RG157 and RG662-RZ12 regions on chromosomes 2 and 9 had QTLs for root thickness, root weight, and maximum root length. The genomic region RG104A-RG450 on chromosome 3 is associated with root penetration ability, root thickness, root weight per tiller, deep root weight per tiller, and deep root to shoot ratio, but not drought-avoidance score in the field. The RG214-RG476c region on chromosome 4 had QTLs for root penetration ability, root thickness, root pulling force, and ratio of root to shoot. The RG351 - RG146 region on chromosome 7 had QTLs for root thickness, root weight, root penetration ability, and maximum root length. The RG1-RZ649 region on chromosome 8

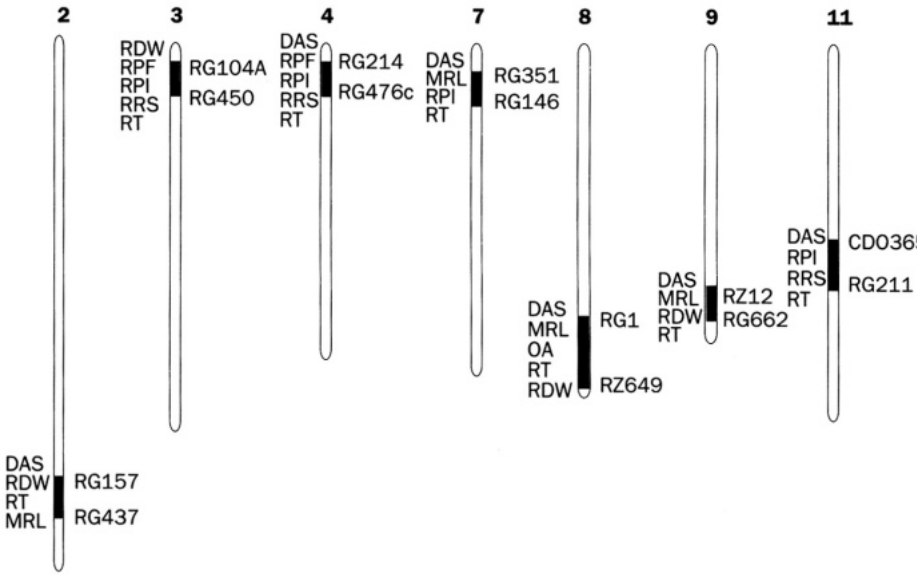

Fig. 2. A schematic chromosome map showing genomic regions where QTLs for drought-related traits are clustered and shared by two or more mapping populations in rice. The numbers above each chromosome indicate chromosome numbers. The map corresponds to that of Champoux et al (1995). DAS = drought-avoidance score, RDW = root dry weight, RPI = root penetration index, RT = root thickness, MRL = maximum root length, RPF = root pulling force, RRS = ratio of root to shoot, OA = osmotic adjustment.

controlled osmotic adjustment, root thickness, root weight, and maximum root length. The CDO365-RG211 region on chromosome 11 controlled QTLs for root penetration index, ratio of root to shoot, and root thickness. QTLs for ABA could not be placed on Figure 2 because no anchor markers matched between the population used by Quarrie et al (1997) and the other populations used in drought-tolerance research (Table 1).

Applications of mapped QTLs and future perspectives

Because of the complicated nature of quantitatively inherited traits, much more attention should be paid to the quality and quantity of trait data. The power to resolve locations of QTLs is limited first by mapping population size and then by genetic marker coverage of the genome and accuracy of phenotypic values of the mapping progenies (McCouch and Doerge 1995). Because current molecular marker techniques are quite standardized, it is not difficult to generate marker data and construct linkage maps. Emphasis should be placed on phenotyping, however, if the trait of interest is complex. For example, compared with morphological and phenological traits, drought-

tolerance traits are more difficult to evaluate. To guarantee that quantitative traits can be measured as precisely as possible, a medium-size mapping population with more than 100 individuals is acceptable for QTL detection (Tanksley 1993) and also manageable when three or four replications are needed for phenotyping.

Mapped QTLs have wide applications in genetic and physiological studies. Perhaps the most practical application of the identified QTLs is to perform MAS aiming at the efficient pyramiding of favorable QTL alleles to improve drought tolerance in rice. Applying MAS to major QTLs for drought tolerance will make it easier for drought tolerance to be incorporated into a breeding program. For MAS to be successful, the following practical points need to be considered. First, the markers linked to the QTLs for a trait of interest must be close enough (<1 cM). Otherwise, the efficiency of MAS may not be guaranteed because of possible crossovers. Greater genetic gain for a trait can be made when flanking markers are used instead of single markers. Fine-mapping of specific QTL regions can be achieved via various strategies, such as adding more markers in the regions, DNA-pooling coupled with polymerase chain reaction (PCR)-based markers, substitution mapping, and developing near-isogenic lines. Second, the markers used for MAS must be converted to easily handled PCR-based markers (such as STS, sequence-tagged sites, and CAPS, cleaved amplified polymorphic sequences) because all markers linked to QTLs for OA, ABA accumulation, and root traits are either RFLPs or AFLPs. Although AFLPs fall within the PCR-based family, their use for MAS is still limited because the techniques involved are complicated and time-consuming.

During the past decade, QTL mapping has improved our knowledge of the genetic architecture of quantitative traits in crops. But the exact genetic nature of the QTLs involved in drought tolerance is still poorly understood. Recently, a candidate gene approach (Rothschild and Soller 1997) was proposed to link the genes in relevant metabolic pathways to QTLs. Because many genes or cDNAs related to osmolyte biosynthesis have been isolated and cloned, emphasis should be placed on mapping them to the current populations used for drought-tolerance research in rice and determining whether there is any correlation between these genes and the identified QTLs for drought tolerance. For drought-tolerance traits whose metabolic pathways are not clear, state-of-the-art high-throughput microarray technology can be employed to detect genes related to drought responses (Schena et al 1995). Once these genes are available, they can then be used for mapping. With the routine integration of newly characterized genes obtained via expressed sequence tag (EST) databases or conventional molecular cloning, the candidate gene approach will become an important and powerful tool to uncover the nature of QTLs underlying drought tolerance in rice.

References

Ali ML. 1999. Mapping quantitative trait loci for root traits related to drought resistance in rice (*Oryza sativa* L.) using AFLP markers. Ph.D. dissertation. Texas Tech University, USA.

Babu RC, Pathan MS, Blum A, Nguyen HT. 1999. Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Sci. 39:150-158.

Causse MA, Fulton TM, Chao YG, Ahn SN, Chunwongse J, We K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251-1274.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969-981.

Devos KM, Gale MD. 1997. Comparative genetics in the grasses. Plant Mol. Biol. 35:3-15.

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata Nori, Khush GS, Sasaki T. 1998. A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479-494.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotech. 17:287-291.

Khush GS. 1997. Origin, dispersal, cultivation and variation in rice. Plant Mol. Biol. 35:25-34.

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Lincoln SE, Daly MJ, Lander ES. 1993. Mapping genes controlling quantitative traits using MAPMAKER/QTL Version 1.1: a tutorial and reference manual. A Whitehead Institute for Biomedical Research Technical Report, second edition. Cambridge, Mass. (USA): Whitehead Institute.

McCouch SR, Doerge RW. 1995. QTL mapping in rice. Trends Genet. 11:482-487.

Morgan JM, Tan MK. 1996. Chromosomal location of a wheat osmoregulation gene using RFLP analysis. Aust. J. Plant Physiol. 23:830-806.

Nguyen HT, Babu RC, Blum A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci. 37:1426-1434.

Price AH, Tomos AD. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95:143-152.

Price AH, Young EM, Tomos AD. 1997. Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). New Phytol. 137:83-91.

Quarrie SA, Laurie DA, Zhu J, Lebreton C, Semikhodskii A, Steed A, Witsenboer H, Calestani C. 1997. QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol. Biol. 35:155-165.

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor. Appl. Genet. 92:627-636.

Rothschild MF, Soller M. 1997. Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe (Newsl. Agric. Genom.) 8:13-20.

Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467-470.

Tanksley SD. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233.

Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor. Appl. Genet. 94:619-632.

Yu L, Ray JD, O'Toole JC, Nguyen HT. 1995. Use of wax-petrolatum layers for screening rice root penetration. Crop Sci. 35:684-687.

Zhang J, Zheng HG, Tripathy JN, Aarti A, Sarial AK, Sarkarung S, Blum A, Nguyen HT. 1998. Mapping QTLs for osmotic adjustment in rice (*Oryza sativa* L.). Abstracts of Plant & Animal Genome VI, 18-22 January 1998, California. p 117.

Zhang J, Nguyen HT, Blum A. 1999. Genetic analysis of osmotic adjustment in crop plants. J. Exp. Bot. 50:291-302.

Zheng HG, Babu RC, Pathan MS, Ali ML, Huang N, Courtois B, Nguyen HT. 1999. Quantitative trait loci for root penetration ability and root thickness in rice: comparison of genetic backgrounds. Genome. (In press.)

Notes

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Drought tolerance of a doubled-haploid line population of rice in the field

A. Blum, J. Mayer, G. Golan, and B. Sinmena

A doubled-haploid (DH) F_2 line population of rice (cross CT9993-5-10-1-M/IR62266-42-6-2, designated IR68586) was developed for the molecular mapping of drought-tolerance traits in rice. This study was performed to evaluate a subset of 100 lines of this population for drought tolerance in terms of plant production in the field. Lines were planted at Bet Dagan, Israel, in 1997 under fully irrigated and drought-stress conditions. Drought-stress conditions were imposed by subjecting plots to two consecutive drying cycles between tillering and heading growth stages, followed by full irrigation until harvest. Data were collected on plant production, phenology, and several indicators of plant water stress.

Lines differed significantly for all measured plant phenology and production traits, under both control and stress conditions. Mean heading date was delayed by 16.7 d under stress compared with the controls. The integrated final effect of the two consecutive drying cycles was outstanding, causing an average reduction of 44% in biomass and 71% in yield. Drought stress as imposed here was therefore severe. There was a significant ($P<0.05$) environment by line interaction for biomass and yield. Yield of lines under stress ranged from nil to 140% of that in the controls, whereas yield drought-tolerance index ranged from 0.04 to 5.06.

Lines differed significantly for all measured indicators of plant water stress—namely, midday canopy temperature, leaf rolling score, leaf death score, and leaf relative water content (RWC, measured only in a subset of 20 lines). For example, RWC at the peak of the first drying cycle ranged from 64.8% to 27.2%. Plant stress indicators were significantly correlated across lines. Significant correlations across lines were found between biomass and yield under stress and canopy temperature, leaf death score, and RWC. These associations support the conclusion that the results of this test indeed reflected real and large genetic variation in drought tolerance within this population.

A doubled-haploid (DH) F_2 line population of rice (cross CT9993-5- 10-1M/IR62266-42-6-2, designated IR68586) was developed at IRRI and adopted for the mapping of quantitative trait loci (QTLs) associated with drought tolerance of rice. This population is being subjected to field and laboratory tests in several collaborating institutions in order to phenotype and tag drought tolerance. Thus far, data have been acquired for osmotic adjustment, root penetration ability, root developmental traits, and various traits evaluated in field tests under relatively nonstress conditions in Thailand.

A critical issue is the level of segregation for drought tolerance in this population in terms of plant production under drought stress, and its physiological and genetic association with osmotic adjustment and root attributes. It was therefore essential to subject this DH line population to a critical test of drought tolerance in terms of plant production in the field.

It is difficult to evaluate drought tolerance for biomass or yield production in the field. This difficulty stems from the inherent spatial variability of soil moisture stress in the field, the "complex" inheritance of biomass and yield, and the large impact of plant phenology and potential productivity on the performance of genotypes under drought stress. The problem can be resolved by carefully selecting a homogeneous testing site, controlling the conditions of drought stress, and using numerical analytical methods to normalize drought-tolerance data for variations in potential productivity and plant phenology (e.g., Blum 1988).

This study was performed under controlled drought-stress conditions in the field at Bet Dagan, Israel. The site is characterized by very homogeneous and deep soil with ideal topography. Rice can be grown at the site during the summer season. Rice has not been grown locally for some 30 years, so the disease and pest status for the crop was expected to be favorable in general. There is no rainfall during the summer and plants depend on stored soil moisture from winter rainfall, or they must be irrigated. The water-holding capacity of the soil is known and root growth into deep soil is potentially unobstructed. Hence, a reasonable level of control over the crop water regime can be achieved in a summer crop such as rice.

Drought tolerance in terms of plant production can be assessed by biomass or yield or by a measure of biomass or yield reduction under stress compared with nonstress conditions. An important index is the "drought-tolerance index," which is the reciprocal of the "susceptibility index" of Fischer and Maurer (1978). Yield under stress is affected by the genotype yield potential (as expressed under nonstress conditions) and by phenology. Yield under stress can therefore be normalized for these effects by calculating the residuals of the multiple regression of yield under stress on yield under nonstress and days to heading under nonstress, as suggested by Bidinger et al (1982).

Materials and methods

Lines tested

The total number of DH lines of this population is 219. A replicated field test cannot accommodate this number. Therefore, a subset of 100 lines was selected. The most variable and representative lines were selected out of a total of 152 lines for which sufficient data were available. A cluster analysis was performed to select the most diverse lines in terms of their measured physiological attributes. The most significant data for this analysis were obtained from previous laboratory tests at Texas Tech University and a field test in Thailand, as follows (values in parentheses represent the range found in the 100-line subset for field testing):

Data from laboratory and greenhouse tests at Texas Tech University
Osmotic adjustment (1996 data by the rehydration method; 1.67 to 11.71 MPa)
Root penetration index (1st data set 1996; 0.02 to 0.54)
Penetrating root thickness (1st data set 1996; 0.57 to 1.20 cm)

Data from afield test in Thailand
Drought score on 21 March 1996 (4.9 to 8.6 on a scale of 1 to 10)
Canopy temperatures on 28 February 1996 (30.1 to 35.7 ºC)
Root-pulling force in 1997 dry season (27.9 to 101.9 kg)

The field experiment

The rainfall of winter 1996-97 terminated before planting date and no rain occurred during the crop cycle. Total available stored soil moisture (to a depth of 1.5 m) at planting was 197 mm, which is roughly 15–25% of the estimated total seasonal potential evapotranspiration of sprinkler-irrigated rice at the site.

Full NPK fertilization was incorporated before planting and additional granular urea fertilizer was applied twice during the growing season. Lines were drill-planted on 19 April 1997 and emergence occurred on 27 April 1997. All lines were tested under two treatments, designated as fully irrigated (nonstress controls) and stress, with two replicates. Each experimental plot was 4 m long and 2.3 m wide, with 15 cm between drilled rows. Weeds were controlled by spray preemergent application of bentazone ("Bazagran") and "Ronstar." No serious disease or pest problem occurred throughout the crop cycle.

All plots were irrigated by sprinklers twice a week according to estimated potential evapotranspiration at the site. At this high frequency, the soil surface barely dried between irrigations. In the stress treatment, two drying cycles were imposed by stopping irrigation. The first cycle was imposed from 25 July to 10 August. The second cycle was imposed from 13 August to 30 August. The decision on stress cycle duration was made by visually inspecting the plots every day, rather than by a predetermined timing. Each stress cycle was terminated when some of the lines appeared to be killed by stress.

Measurements of indicators of plant water stress were taken during these stress cycles, always at midday. These were canopy temperature with the infrared thermometer, leaf rolling score (0 = no rolling, 5 = extreme rolling), leaf desiccation score (0 = no leaf desiccation, 5 = all leaves desiccated), and leaf relative water content (RWC). The latter measurement was performed for 20 random lines only. Heading date was recorded for each plot. Each plot was hand-harvested at its own maturity. Harvest was performed by cutting whole plants at the soil level. The central part of the plot (2.0 × 1.5 m), excluding all four borders, was harvested (the border effect under stress conditions was outstanding). A subsample (1 m of row) of plants was taken for total aboveground dry matter (biomass) determination by oven drying. All panicles were threshed and grain weighed after air drying for more than one week.

Results and discussion

Plant productivity

Table 1 contains plant production data. Plants of one DH line did not emerge well and it was excluded from the trial. Four lines did not head at all. These were not included in the analysis of yield and biomass. Judging by plant type, appearance, foliage color, growth habit, and phenology, this population was extremely variable, appropriate to an F_2 population of a cross between very different parents.

All treatment and genotype effects and their interactions were significant ($P<0.05$) for biomass, yield, and phenology variables (summary in Table 2). Few of the DH lines did not yield at all under stress conditions because of their acute response to water deficit.

The earliest DH line flowered on 25 August, 128 d after planting in the irrigated treatment. The variation in days to heading under irrigated conditions was very high (from 128 to 172 d), representing the high genetic variability in this population. The stress treatment resulted in an average delay in heading of 16.7 d compared with the controls. DH lines varied in this respect by up to 40 d of delay. A delay in heading in response to a transient preflowering stress is to be expected, but the range of variation among DH lines in this respect was beyond expectation. The delay tended to be greater in potentially late-flowering lines, as the correlation between heading delay and days

Table 1. Means, ranges, and standard deviations (SD) for plant production and plant phenology traits in doubled-haploid lines as tested at Bet Dagan, 1997.

Trait	Lines measured in test	Mean	Minimum	Maximum	SD
Plant production					
Control (irrigated) biomass (g m^{-2})	95	1,197	728	2,077	257.78
Stress (stress) biomass (g m^{-2})	95	868	241	1,711	289.42
Stress biomass as % of controls	95	74.8	24.5	157.1	26.98
Biomass resistance index	95	1.02	0.35	2.28	0.38
Residuals for biomass (g m^{-2})[a]	95	0	–231.1	315.6	123.68
Control yield (g m^{-2})	95	164	0	503	91.34
Stress yield (g m^{-2})[b]	94	43	0	199	42.73
Stress yield as % of controls	94	29.2	0	140.5	26.97
Yield resistance index	94	1.18	0.04	5.06	1.02
Residuals for yield (g m^{-2})[a]	94	0	–61.8	158.8	42.27
Percent headed plants in plot (controls)	95	86.1	12.5	100.0	17.88
Percent headed plants in plot (stress)	95	58.4	0	100.0	31.17
Percent headed in stress as % of controls	95	66.8	0	136.9	32.62
Plant phenology					
Control days from planting to heading	99	149	128	172	10.22
Stress days from planting to heading	95	165	139	179	9.13
Heading delay under stress (d)	95	16.7	–1	40	8.49

[a]Residuals of the multiple regression of stress biomass (or yield) on control biomass (or yield) and on days to heading under stress (see text). [b] Lines failed to produce measurable yield under the severe stress condition.

Table 2. Summary of the analysis of variance (F statistic and probability) for selected plant production traits.

Trait	Treatment F *(P)*	DH line F *(P)*
Biomass (g m^{-2})[a]	115.4 (<0.0001)	1.98 (<0.0001)
Yield (g m^{-2})[a]	127.9 (<0.0001)	3.38 (<0.0001)
Days to heading[a]	10.8 (<0.0001)	5.29 (<0.0001)
Biomass in stress as % of controls	—	2.29 (<0.0001)
Yield in stress as % of controls	—	2.96 (<0.0001)
Biomass susceptibility index	—	2.77 (<0.0001)
Yield susceptibility index	—	3.16 (<0.0001)
Delay in days to heading		2.86 (<0.0001)

All treatment by doubled-haploid line interactions for these traits were significant at $P<0.05$.

to heading under irrigated conditions was r = –0.56 ($P<0.000$l). The delay in heading under stress was negatively associated with stress yields across lines (r = –0.31,P = 0.003), which is to be expected. The delay in heading is an expression of growth retardation during the drying cycle and upon recovery. The greatest delay (40 d) was longer than the combined duration of the two drying cycles (33 d). This delay is a strong indication of susceptibility to stress.

The integrated final effect of the two consecutive drying cycles was outstanding, causing an average reduction of 44.0% in biomass and 70.8% in yield. These reductions represent high levels of stress, which brought stress yield to the range where "crossover interactions" are normally observed in cereals and where true differences in genotype adaptation to stress are well expressed (e.g., Blum 1993).

Drought tolerance in terms of plant production of the different DH lines can be assessed by several parameters—namely, yield (or biomass) under drought, yield under drought as a percentage of yield under irrigation, and drought tolerance index (Fischer and Maurer 1978). These terms were evaluated in this trial and the variability among DH lines for each was found to be very high and very significant (Table 1). For some lines, there were no differences in yield between irrigated and stress conditions, whereas in other lines yield failed completely under stress.

Stress yield and control yields were significantly (although not very strongly) correlated across lines (r = 0.26, P = 0.013). Stress yield was negatively correlated with stress days from emergence to heading (r = –0.40,P = 0.0001). Yield tended to increase with longer growth duration under irrigation and with shorter growth duration under drought stress, as expected. Hence, the yield of lines under stress may have been affected by their potential yield and phenology and not only by physiological response to stress. To normalize stress yield for the effect of potential (control) yield and phenology, a multiple regression was performed for stress (stress) yield on irrigated (controls) yield and stress days to heading across lines (Bidinger et al 1982). The model was significant (R^2 = 0.20). The residuals of this regression for each line represent how lines deviated in their actual yield from the expected (Table 1). A positive deviation indicates that the actual stress yield of the given line was higher than

the expected yield according to its potential yield and phenology. Lines differed significantly for these yield deviations, from –62to 159 g m^{-1}. The same calculation was performed for biomass (Table 1).

Yield under stress was very well correlated across lines with the residuals for yield (r = 0.97, P<0.000l). Yield was also well correlated with the tolerance index (r = 0.88, P<0.000l). Therefore, absolute yield of the lines under stress represents quite well their relative drought tolerance in terms of plant production, despite its association with potential yield and phenology. This may have occurred because phenology was changed drastically in response to stress (heading delay) and this in itself represented a physiological response to stress.

It was noticeable that heading was not always complete in all plots, so that a visual estimate of percent heading was recorded for each plot. Mean percent heading was higher under irrigation (86%) than under stress (58%), with a very large range of difference among lines in this respect (Table 1). Percent heading was negatively correlated across lines with days to heading under irrigated (r = –0.41, P<0.000l) and stress (r = –0.54) conditions. Therefore, reduced heading was associated with late flowering. But percent heading was positively correlated with yield across lines only under stress (r = 0.043, P = 0.0001) but not under irrigated conditions (r = 0.18, P = 0.091). The late genotypes with less heading were probably compensating in grain weight per head under irrigation. Under stress conditions, incomplete heading became a stress-responsive factor, which influenced yield. It is interesting, however, that the delay in heading was not correlated across lines with percent heading under stress.

Plant water stress

Very large variation among DH lines in symptoms of plant water stress could be easily discerned in both drying cycles. Figure 1 shows examples of such distinct differences between adjacent plots of different genotypes in the stress treatment. Although plant size and growth habit may have affected the variation among lines in plant water deficit as seen in Figure 1, large differences in visual stress symptoms could also be seen among lines of similar growth habit and plant size. A numerical summary of this variation is represented in Table 3.

Canopy temperatures were used as the main indirect measurement of plant water stress in this study, based on previous favorable results with rice (Ingram et al 1990, Garrity and O'Toole 1995). On the average, canopy temperature under stress conditions increased to 114.5% and 117.0% of that in the controls in the first and second stress cycles, respectively. Variation in this respect among all lines ranged from 102.4% to 148.9% on the last date of measurements (25 August). On this date, canopy temperatures under stress ranged from 29.5 to 38.2 °C among extreme lines. This range was 27.1 to 34.7 °C on 19 August. Analysis of variance indicated very significant differences (P<0.0l) among lines in canopy temperatures under stress conditions on all measurement dates. In contrast, the range of variation among lines in canopy tem-

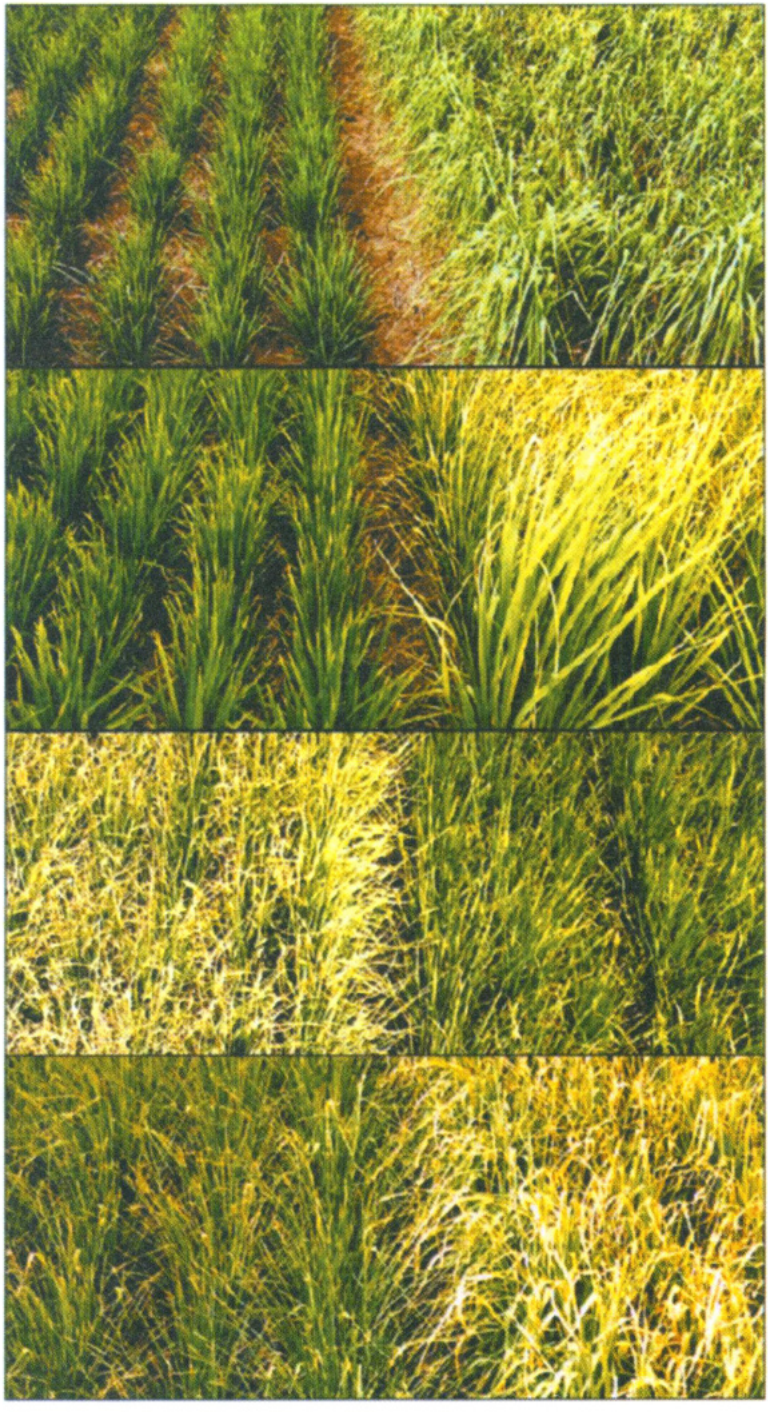

Fig. 1. Apparent differences between adjacent plots of different rice doubled-haploid lines in plant water-stress symptoms during the second drying cycle in the field. All photographs were taken on the same day between 11 a.m. and 12 noon.

Table 3. Means, ranges, and standard deviations (SD) for various indices of plant water stress in doubled-haploid lines as tested at Bet Dagan, 1997.

Trait	Lines in test	Mean	Minimum	Maximum	SD
Plant water stress—cycle I					
Control canopy temperature (°C) on 30 Jul	99	27.0	25.3	29.7	0.87
Stress canopy temperature (°C) on 30 Jul	99	29.4	26.6	32.0	1.27
Canopy temperature in stress as % of control on 30 Jul	99	108.6	98.0	120.1	4.76
Control canopy temperature (°C) on 4 Aug	99	26.2	24.0	28.8	0.88
Stress canopy temperature (°C) on 4 Aug 1998	99	31.5	27.8	35.2	1.46
Canopy temperature in stress as % of control on 4 Aug	99	120.3	108.3	137.3	6.03
Stress canopy temperature (°C) on 7 Aug	99	33.1	28.8	38.0	1.47
Leaf rolling on 7 Aug	99	1.9	0	3.5	0.74
RWC[a] on 10 Aug	20	64.8	27.2	79.7	13.89
Leaf desiccation on 10 Aug	99	1.7	0	4.5	0.93
Plant water stress—cycle II					
Control canopy temperature (°C) on 19 Aug	99	27.1	25.1	29.3	0.82
Stress canopy temperature (°C) on 19 Aug	99	29.8	27.1	34.7	1.47
Canopy temperature in stress as % of control on 19 Aug	99	109.9	97.5	128.9	6.36
Control canopy temperature (°C) on 25 Aug	99	26.8	23.5	29.9	1.32
Stress canopy temperature (°C) on 25 Aug	99	33.1	29.4	38.2	1.77
Canopy temperature in stress as % of control on 25 Aug	99	124.1	102.4	148.9	8.52
RWC on 28 Aug	20	75.6	52.2	96.8	13.25
Leaf desiccation on 28 Aug	99	2.2	0	5.0	1.38

[a]RWC = relative water content.

peratures under irrigated conditions was relatively small (Table 3), around 4 °C between extreme lines.

The variation among DH lines in leaf rolling and leaf desiccation on the different dates (Table 3) was very significant and large and it can be visually appreciated in Figure 1.

Relative water content was determined only for 20 randomly selected DH lines. Mean RWC on 10 August, at peak stress of the first cycle, was 64.8%, whereas lines varied significantly from 27.2% to 79.7%. In the second cycle, 2 d before peak stress, mean RWC was 75.6%, whereas lines varied from 52.2% to 96.8%. The large variation among a limited number of lines (20) in RWC is outstanding. It could arise mainly from the corresponding difference among lines in soil moisture extraction and/or osmotic adjustment.

Canopy temperatures, canopy temperature differentials between controls and stress conditions, and canopy temperatures under stress as a percentage of controls were all well correlated with RWC, leaf rolling, and leaf desiccation as expressions of plant water stress and deficit. Correlation coefficients were generally high and significant. In this respect, there was no advantage to canopy temperature differentials or canopy temperature under stress as a percentage of controls over canopy temperature under stress conditions. The latter was therefore taken to represent the infrared thermal sensing

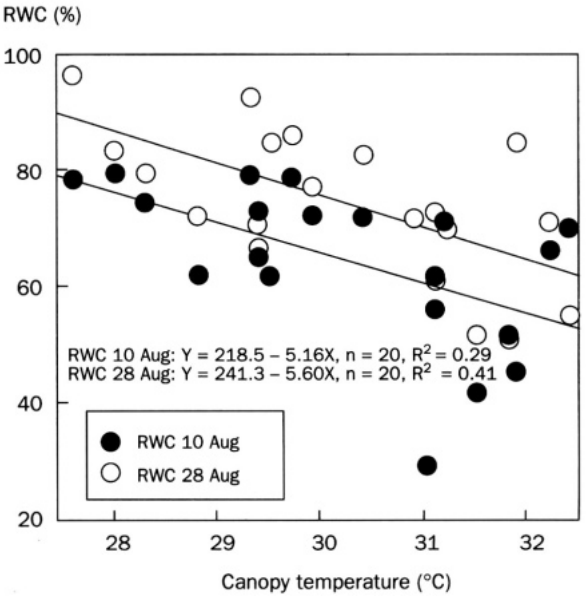

Fig. 2. The linear regression of relative water content (RWC, on two dates of measurement) on canopy temperature under stress treatment on 19 August over 20 doubled-haploid lines.

of canopies, and the measurement taken on 19 August (2nd cycle) was chosen as the most representative.

Canopy temperature under stress on 19 August was well associated with RWC over 20 DH lines (Fig. 2). Temperatures increased with the reduction in RWC, as expected. This correlation indicates that the variation seen in plant water status in terms of RWC over 20 lines may be extended for all lines. Canopy temperature under stress was also well associated with leaf rolling and leaf desiccation scores over all lines (Fig. 3). Canopy temperatures increased as leaf rolling and leaf desiccation scores increased with stress. Therefore, both Figures 2 and 3 uphold the value of canopy temperature measurements as an indirect screen for plant water status in drought-stressed rice.

Finally, canopy temperature under stress conditions was significantly and negatively correlated with stress biomass and stress yield across all DH lines. Biomass and yield tended to be lower in lines with high canopy temperature (Fig. 4). As can be expected, this association was stronger for biomass than for yield, because yield is strongly affected by the partitioning of total biomass production. Correspondingly, stress biomass and yield were negatively correlated with leaf desiccation scores (Fig. 5) and leaf rolling score (not shown) across all DH lines.

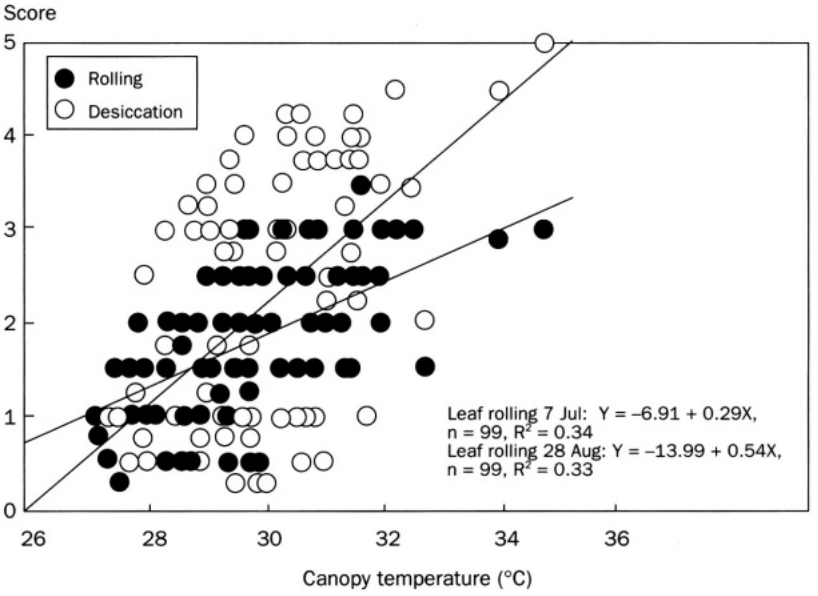

Fig. 3. The linear regression of leaf rolling (1st stress cycle on 7 July) and leaf death scores (2nd stress cycle on 28 August) on canopy temperature under stress treatment on 19 August over all doubled-haploid lines.

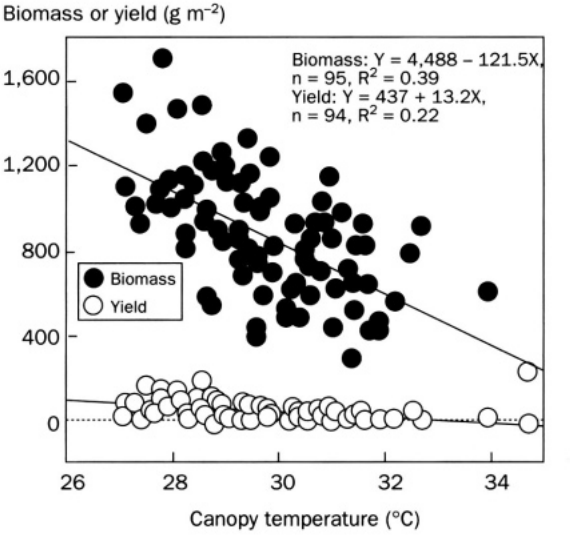

Fig. 4. The linear regression of stress biomass and stress yield on canopy temperature on 19 August during the 2nd drying cycle across all doubled-haploid lines.

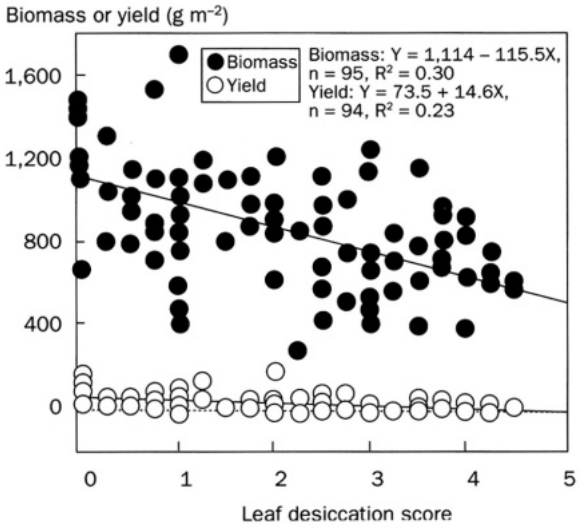

Fig. 5. The linear regression of stress biomass and stress yield on leaf desiccation score during the 2nd cycle across all doubled-haploid lines.

Conclusions

The purpose of this trial was to serve as a critical test of the relative drought tolerance of different DH lines in terms of plant production in the field. This purpose has been achieved. First, stress was severe enough to be expressed clearly both visually and instrumentally. Stress was also severe enough to reduce yield below one-third of the potential, which is the general range where a crossover interaction occurs, such that yield of lines was affected more by their adaptive responses than by their potential yield. The level of stress was appropriate for the excellent separation of lines by indicators of plant water stress and criteria of plant productivity under stress. The statistical associations discussed above demonstrate a consistent difference among lines in their plant water stress and deficit as developed under stress. The consistent association across lines among different stress indicators is a solid proof that these lines are different in their physiological responses to drought stress. The delay in heading under stress was seen as an outstanding expression of rice response to stress, which was negatively associated with plant production under stress. The association among stress indicators such as canopy temperature and yield or biomass under stress supports the conclusion that the results of this test reflect genetic variation in real drought tolerance within this population.

References

Bidinger FR, Mahalakshmi V, Talukdar BS, Alagarswamy G. 1982. Improvement of drought resistance in millet. In: Drought resistance in crop plants with emphasis on rice. Manila (Philippines): International Rice Research Institute. p 357-364.

Blum A. 1988. Plant breeding for stress environments. Boca Raton, Fla. (USA): CRC Press. 208 p.

Blum A. 1993. Selection for sustained production in water-deficit environments. Int. Crop Sci. I. Madison, Wis. (USA): Crop Science Society of America. p 343-347.

Fischer RA, Maurer R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res. 29:897-916.

Garrity DP, O'Toole JC. 1995. Selection for reproductive stage drought avoidance in rice, using infrared thermometry. Agron. J. 87:773-779.

Ingram KT, Real JG, Maguling MA, Obien MA, Loresto GC. 1990. Comparison of selection indices to screen lowland rice for drought resistance. Euphytica 48:253-260.

IRRI (International Rice Research Institute). 1982. Drought resistance in crop plants with emphasis on rice. Manila (Philippines): IRRI.

Notes

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Molecular dissection of drought tolerance in rice: from physio-morphological traits to field performance

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Improving drought tolerance through conventional breeding approaches has been slow because of its complexity. By using current advances in molecular marker technology, we tried to dissect this complex but very important trait. For the first time, we present here a comprehensive report on associations of QTLs for drought tolerance and rice performance under water stress in the field. By using a 154-doubled-haploid line population developed from a cross between CT9993-5-10-1-M and IR62266-42-6-2, we did large-scale phenotyping at Texas Tech University (USA), IRRI (Philippines), the Ubon Rice Research Center (Thailand), and the Volcani Center (Bet Dagan, Israel) under well-watered or drought conditions for traits related to drought tolerance. Yield and biomass under stress were negatively associated with phenotypic expressions of plant water stress in the field. But the locations of QTLs for yield under stress and yield resistance index at Bet Dagan were different from those of QTLs for root penetration ability, osmotic adjustment (OA), and root depth and mass as measured under greenhouse conditions and for canopy temperature and drought scores as measured in the field. These results suggest that drought tolerance in terms of yield and biomass at Bet Dagan was not related to OA or root penetration. Most likely, yield under this field test was related to field rooting depth and the ability to extract deep soil moisture. It is interesting to point out, however, that the genomic region of RZ67-EM15_1 on chromosome 5 harbored QTLs for stress canopy temperature, drought score, and recovery score. Clearly, there is an urgent need for further quality phenotyping of the mapping population in target drought-prone environments in the field where genetic variations in OA and root traits might affect plant productivity. The acquisition of data on maximum root depth in the field and the future development of near-isogenic lines carrying QTLs for different traits might provide a fuller physiological and genetic explanation of drought tolerance in this population.

Drought is a worldwide problem that limits crop growth and productivity. The need for research on drought tolerance was clearly justified (Boyer 1982). Because of the unpredictable occurrence of drought, research on improving drought tolerance in crops is difficult. For example, rice may suffer drought stress any time from early seedling to grain filling (Fukai and Cooper 1995). Research attempts to improve drought tolerance in rice have to develop different strategies for various target environments,

Drought tolerance is a complex trait. Important mechanisms of drought tolerance include at least the following four aspects: (1) drought escape via a short life cycle and developmental plasticity, (2) drought avoidance via enhanced water uptake and reduced water loss, (3) drought tolerance via osmotic adjustment (OA) and antioxidant capacity, and (4) drought recovery via desiccation tolerance (Fukai and Cooper 1995, Zhang et a1 1999a). Various physio-morphological traits have been suggested to be related to drought tolerance and may be used to improve crop performance in the field (Fukai and Cooper 1995, Ludlow and Muchow 1990). The use of such putative traits to improve grain yield under water-limiting conditions has been demonstrated in maize (Fischer et al 1989). Selection based on putative traits such as stay-green leaves has been successfully tested recently in sorghum (Tuinstra et a1 1998).

Several traits related to drought tolerance in rice have been identified (O'Toole 1982, Hanson et al 1990, Nguyen et al 1997). A deep root system may allow the rice crop to extract deep soil moisture during drought. Increased soil strength under reduced soil moisture and the presence of hardpans in the subsoil of rainfed lowlands, however, make it difficult for roots to gain access to the deep soil moisture. Under such conditions, roots with a higher penetration ability may be advantageous for absorbing water from deeper soil layers (O'Toole 1982). Genotypic variation in root penetration and other root traits has been reported in rice (Ingram et al 1994, Yu et al 1995). Increased rooting depth, root density, root to shoot ratio, root pulling force, and penetration ability through hardpans are reported to be the major drought-tolerance traits associated with the root system in rice (O'Toole 1982, Hanson et al 1990).

Osmotic adjustment capacity is an important shoot-related component of drought tolerance in crop plants. It allows turgor maintenance as leaf water potential falls under drought. It also helps delay leaf rolling, yellowing, and death in rice under water stress (Nguyen et al 1997). Wide genetic variation for OA has been documented in rice and other crops (Lilley and Ludlow 1996, Zhang et al 1999b). Genetic analysis of OA in crops has recently been reviewed by Zhang et al (1999b).

Visual leaf rolling score is an efficient method for detecting drought tolerance at the seedling stage (De Datta et a1 1988) and can be used as an indirect estimate of drought tolerance. Visual drought scoring by an experienced researcher based solely on leaf rolling and leaf desiccation is apparently quite effective in discriminating drought avoidance in rice (O'Toole and Moya 1978). Root pulling force showed a significant negative correlation with drought stress score tested at three progressive soil moisture levels (–0.2, –0.4, and –1.0 MPa soil matric potential at 15-cm depth) (O'Toole and Soemartono 1981).

A cooler canopy temperature under stress as measured by infrared thermometry is also reported to be a measure of drought tolerance (Blum et al 1988, and this volume). Canopy temperature is usually lower in plants having a better canopy water status. Water deficit causes partial stomatal closure in a crop canopy, reducing transpiration and allowing sunlit leaves to become warmer (Jackson et al 1981). The apparent ease and rapidity of measuring canopy temperature has made it a useful screening technique for drought tolerance of crop cultivars. The use of infrared ther-

mometry to screen germplasm for drought tolerance based on canopy temperature under stress conditions has been successfully demonstrated in rice (Ganity and O'Toole 1995). Canopy temperature was negatively associated with biomass and yield under drought (Blum et al, this volume).

Despite our increased understanding of stress physiology, the development of drought-tolerant cultivars has been slow in rice. A major reason for the slow progress is the incidence of large genotype by environment (G × E) interactions, which result from a combination of differences in genotypic adaptation and the target areas (Fukai and Cooper 1995). Phenotypic selection for drought-tolerance traits is difficult, especially in large breeding populations. Molecular marker technology has been identified as a powerful tool for selecting traits that are otherwise difficult to screen phenotypically. Where molecular markers are linked to genes that control the expression of drought tolerance, there is a scope to select for the genes without subjecting breeding lines to a drought screen. Because of the difficulty in identifying and conducting reliable drought-tolerance screens, opportunities exist to improve the efficiency of selection for drought tolerance by using molecular markers.

We therefore need to establish the genetic association between defined physiological components of drought tolerance and a rice mapping population under drought stress in the field. Such associations should allow us to perform effective marker-assisted selection to improve drought tolerance in rice. A doubled-haploid line (DHL) population was developed from a cross between CT9993-5-10-1-M and IR62266-42-6-2 (abbreviated as CT9993 and IR62266 in later text) at IRRI. Large-scale phenotyping was done for this population from greenhouse to field conditions and from physiomorphological traits to field performance. Our objective was to identify QTLs for field performance and components of drought tolerance and further test whether there are any relationships between these QTLs.

Materials and methods

The DHL population (154 lines) of CT9993/IR62266 used for the genetic linkage map construction at Texas Tech University was used for field screening of drought tolerance and yield performance at the Ubon Rice Research Center in Thailand. Field screening of drought tolerance was conducted in the dry season of 1996 (Fig. 1). Seeds were sown on 8 December 1995. Irrigation began at seeding and ceased at 45 d after sowing. A randomized complete block design with three replications was used. Plot size was 4 rows × 9 hills, with one plant per hill. Based on a 1–9 scale (good to bad), visual drought score was recorded on 5 and 28 February and 5 and 21 March 1996. Canopy temperature was measured on 5 and 28 February 1996. Recovery ability was scored based on a 1–9 scale (good to bad). Yield was tested in the wet season of 1996. Seeds were sown on 18 May. A randomized complete block design with four replications was used and the harvested area for grain yield was 4 rows × 11 hills (2.75 m^2).

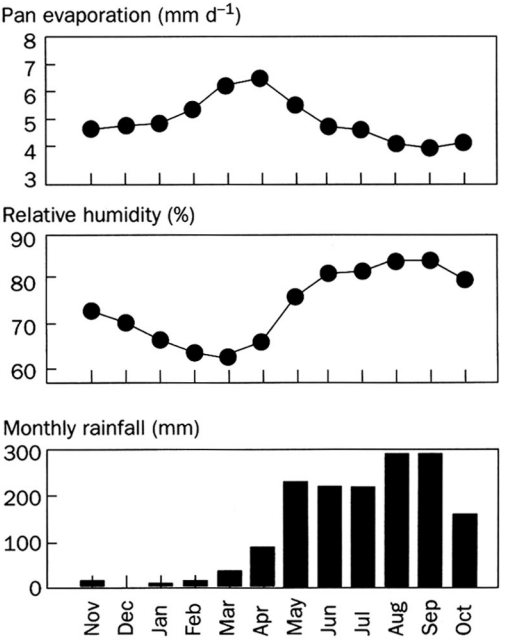

Fig. 1. Long-term (1985-96) monthly pan evaporation, relative humidity, and rainfall at the Ubon Rice Research Center, Thailand. The experimental period for the field screening for drought tolerance was between January and March 1996.

A subset of 100 lines from this population was evaluated for drought tolerance in terms of plant production in the field at Bet Dagan, Israel (Blum et al, this volume). Plants were stressed for two cycles by withholding water before plant heading. Detailed information on this experiment can be found in Blum et al (this volume).

The RFLP/AFLP/SSR genetic map constructed at Texas Tech University was used for QTL analysis. This map consisted of 315 markers: 145 RFLPs, 153 AFLPs, and 17 microsatellites (Zhang et al, in preparation). Using MAPMAKER/QTL software (Lincoln et al 1992), interval mapping was employed to detect QTLs with LOD (Likelihood ODds ratio) score 22. QTL analysis was conducted for OA and root penetration at Texas Tech University (Zhang et al, this volume), root depth and dry weight at IRRI (Kamoshita et al, this volume), and drought tolerance and plant production in Israel (Blum et al, this volume) and Thailand.

Results and discussion

Figure 1 shows long-term monthly (1985-96) pan evaporation, relative humidity, and rainfall at Ubon, Thailand, where the field screening of drought tolerance was conducted. During the screening period of January to March, pan evaporation was the highest, whereas relative humidity and rainfall were the lowest. The maximum and

Table 1. Mean values of CT9993, IR62266, and doubled-haploid lines as tested in Israel and Thailand for plant production, phenology, and plant water stress indices.

Parameters	CT9993 [a]	IR62266	Lines (mean ± SD)
Production, Israel			
Control (irrigated) biomass (g m^{-2})	1,338 a	1,949 a	1,197 ± 257.8
Stress (drought) biomass (g m^{-2})	567 a	1,070 b	868 ± 289.4
Biomass resistance index	0.5 a	0.7 a	1 ± 0.4
Control yield (g m^{-2})	241 a	278 a	164 ± 91.3
Stress yield (g m^{-2})	47 a	173 b	43 ± 42.7
Stress yield as % of control	21 a	62 a	29 ± 27.0
Yield resistance index	0.3 a	0.4 b	1 ± 1.0
Control kernel weight (g 1,000 $kernels^{-1}$)	–	–	21 ± 2.7
Stress kernel weight (g 1,000 $kernels^{-1}$)	–	–	17 ± 5.2
Stress kernel weight as % of control	–	–	80 ± 25.1
Production, Thailand			
Grain yield (t ha^{-1}, 1996)	1.7 a	3.7 b	2.81 ± 0.65
Sterility (%, 1996)	25.0 a	20.0 a	26.56 ± 18.37
Panicles (no. $hill^{-1}$, 1996)	8.9 a	16.5 b	10.86 ± 3.34
Plant water stress indices, Israel			
Canopy temp. under drought (4 Aug)	–	–	31.50 ± 1.45
Canopy temp. under drought (19 Aug)	32.6 a	31.5 b	29.82 ± 1.46
Canopy temp. under drought (25 Aug)	33.9 a	32.1 a	33.15 ± 1.77
Leaf rolling (7 Aug)	3 a	2 a	1.9 ± 0.7
Leaf desiccation (28 Aug)	1 a	2 a	2 ± 1.4
Plant water stress indices, Thailand			
Canopy temp. (5 Feb 1996)	27 a	27.8 a	27.45 ± 0.74
Canopy temp. (28 Feb 1996)	33 a	32.8 a	32.98 ± 1.07
Drought score (21 Feb 1996)	1.7 a	1.6 a	1.25 ± 0.67
Drought score (28 Feb 1996)	4.3 a	3.6 a	3.41 ± 0.68
Drought score (5 Mar 1996)	5.4 a	4.2 a	4.29 ± 1.00
Drought score (21 Mar 1996)	7.6 a	6.5 a	6.85 ± 0.73
Recovery score (1996)	8.7 a	6.0 b	6.83 ± 1.18
Days to heading, Israel			
Control days from planting to heading	139 a	138 a	149 ± 10.2
Stress days from planting to heading	162 a	145 a	165 ± 9.1
Heading delay under stress (d)	23 a	7a	17 ± 8.5
Days to flowering, Thailand			
50% flowering date (d, 1996)	91 a	94 a	87 ± 6.5

[a] Within each row forCT9993 and IR62266, values followed by the same letters are not significantly different (P >0.05).

minimum temperatures for this period were 39 and 11 °C, respectively. These weather conditions made the season dry and suitable for screening drought tolerance.

Traits evaluated in Thailand and Israel fell into three groups: plant production, plant water stress indices, and phenology (Table 1). Table 1 presents mean values for two parents (CT9993 and IR62266) and the DHLs for these traits. Generally, IR62266

had a higher yield and lower canopy temperature and drought score than CT9993 under drought. Among all the physiological and phenological traits, only the canopy temperature recorded on 19 August and recovery score were significantly different between CT9993 and IR62266 (P <0.05) although the variation in the DHLs for these two traits showed a continuous distribution. For biomass and yield-related traits, differences in stress biomass, stress yield, and yield resistance index existed between the two parents (P <0.05).

QTL analyses were conducted for all traits listed in Table 1 although differences in some traits were not significant between the parents. The number of QTLs detected for these traits varied from 0 to 11, with the phenotypic variation (R^2) explained varying from 0 to 32% (Tables 2, 3, and 4).

Table 2 lists QTLs for plant productivity. For grain yield without drought stress, 8 QTLs were identified in Thailand. Each QTL individually accounted for phenotypic variation from 9% to 22%. For panicle number, 8 QTLs were identified, of which 4 were located in genomic regions similar to those for the QTLs for yield. In Israel, 6 and 2 QTLs for grain yield were located for well-watered and drought-stressed plants, respectively. The QTL ME2–6-RM21 on chromosome 11 was common under irrigated and drought-stressed plants as tested in Israel. Similarly, phenotypic variation explained by these QTLs ranged from 9% to 23%. But none of the QTLs for grain yield under irrigation were common between Thailand and Israel, indicating the complex nature of yield and genotype × environment interaction for yield.

For kernel weight, 4 and 2 QTLs were detected for well-watered and drought-stressed plants, respectively, with R^2 from 13% to 32%. No common QTLs for kernel weight were found between the two conditions. The 2 QTLs for kernel weight under drought, however, also controlled stress kernel weight as % of control. But the QTLs for kernel weight and for yield under drought were located on different chromosomes. For control biomass, 2 QTLs were located and explained 11–15% of the variation. For stress yield as % of control and yield resistance index (which is the reciprocal of the susceptibility index of Fischer and Maurer [1978] in terms of grain yield), 3 and 1 QTLs were found, with R^2 of 11-14%. No QTLs were detected for stress biomass and biomass resistance index (which is the reciprocal of the susceptibility index of Fischer and Maurer [1978] in terms of biomass yield).

Table 3 shows QTLs for plant water stress indices. One QTL was detected for leaf rolling under stress. Three QTLs were linked to leaf desiccation. The QTLs for leaf rolling and death were located on different genomic regions. For drought score recorded on different dates, 2 to 5 QTLs were identified. For canopy temperature recorded on different dates, 1 to 3 QTLs were located. Three QTLs were found to be responsible for recovery ability after drought was relieved. Of the three, two overlapped with the QTLs for drought score and one overlapped with a QTL for leaf death. Phenotypic variation explained by these QTLs ranged from 7% to 27%. It is interesting to point out that the genomic region of RZ67-EM15_1 on chromosome 5 harbored QTLs for stress canopy temperature, drought score, and recovery score. Although a negative association existed between canopy temperature and biomass/

Table 2. QTLs for plant production as identified by MapMaker/QTL.

Traits	Markers bordering QTLs	Chromosome #	LOD[a]	R^2 (%)[b]
Israel				
Control biomass[c]	R1843-ME2–7	2	2.2	15
	RM21-CD0365	11	2.4	11
Stress biomass and biomass resistance index[c] (no QTLs have been detected)				
Control yield[c]	RM212-R2417	1	2.6	13
	EM11–9-CD020	3	2.1	10
	G2132-RI394A	8	2.8	13
	ME5–9-ME5–8	9	2.6	12
	ME2–6-RM21	11	2.7	14
	RZ261-ME10–3	12	2.0	9
Stress yield	RG400-RM220	1	2.2	12
	ME2_6-RM21	11	4.9	23
Stress yield as % of control[c]	ME2–8RG333	8	2.4	11
	ME6–7-ME7–2	11	2.3	11
	EM19–5RG901	12	2.3	14
Yield resistance index	EM19–5-RG901	12	2.1	12
Control kernel weight	RG345-WG110	1	2.3	20
	ME9–1-ME2–8	8	3.7	18
	ME2–10-ME9–3	9	2.6	13
	ME4–15-RM17	12	4.7	20
Stress kernel weight	RG158-C1408	2	3.1	32
	RZ602-ME6–9	4	3.4	15
Stress kernel weight as % of control	RG158-C1408	2	2.5	24
	RZ602-ME6–9	4	3.0	14
Thailand				
Grain yield	RG957-RG345	1	2.4	9
	RG157-R26	2	3.2	13
	EM19–11-RZ474	3	2.5	9
	RZ682-EM14–9	6	5.9	22
	RG172-R2549	6	4.9	19
	RG769-EM19–14	7	4.2	14
	EM14–1-ME2–11	8	2.5	12
	RM201-RG667	9	2.4	9
Sterility[c]	EM11_9-CD020	3	5.4	16
	RG358-RZ792	9	2.8	8
Panicle number	CD0345-ME10–14	1	2.3	8
	EMP2–2-ME10–11	4	4.8	17
	RZ565-EMP3–10	4	3.2	11
	RG476-RG214	4	5.4	18
	RZ682-EM14–9	6	5.0	18
	R2549-RG716	6	3.6	14
	EM14–1-ME2–11	8	3.6	17
	RM201-RG667	9	4.4	13

[a] Likelihood of odds. [b] The phenotypic variation explained by an individual QTL. [c] Differences between CT9993 and IR62266 were not significant ($P < 0.05$) (see also Table 1).

Table 3. QTLs for plant water stress indices as identified by MapMaker/QTL.

Traits	Markers bordering QTLs	Chromosome #	LOD[a]	R^2(%)
Israel				
Canopy temp. under drought (4 Aug)	RG345-WG110	1	2.7	17
	RZ67-EM15–1	5	2.2	10
Canopy temp. under drought (19 Aug)[b]	RG345-WG110	1	2.4	14
	EM11_1-BCD855	7	3.5	26
Canopy temp. under drought (25 Aug)	RGlO9-EM11–11	1	3.4	17
	EMP2–2-ME10–11	4	2.2	10
	G1073-G2132	8	2.6	13
Leaf rolling (7 Aug)	RZ401-RG141	9	2.3	10
Leaf desiccation (28 Aug)	RG437-EM10–18	2	2.1	9
	RG190-EM15–3	4	2.5	11
	EM11_1-BCD855	7	3.1	27
Thailand				
Canopy temp. (5 Feb)	ME5–3-C1121	8	2.9	8
	C711-C313	9	2.4	7
	C1176-G103	9	3.2	9
Canopy temp. (28 Feb)	C1419-EM11–10	2	2.8	11
Drought score (21 Feb)	R1843-ME2–7	2	2.5	12
	RG171-EM14–4	2	3.9	11
	RZ67-EM15–1	5	2.5	8
	RZ682-EM14–9	6	2.6	8
	EM17–9-RZ516	6	2.8	8
Drought score (28 Feb)	R1843-ME2–7	2	2.8	13
	RZ67-EM15–1	5	3.1	9
	RM222-EM16–9	10	2.3	7
Drought score (5 Mar)	R1843-ME2–7	2	2.0	12
	RZ67-EM15–1	5	3.8	11
	RZ516-R3139	6	2.0	6
	RG553-EM14–6	9	2.0	6
	RG1109-G1465	11	2.5	11
Drought score (21 Mar)	RG171-EM14–4	2	2.4	7
	RZ67-EM15–1	5	3.6	12
Recovery score (1996)[b]	ME10–18-C106	2	3.6	13
	RZ67-EM15_1	5	2.4	7
	RG553-EM14–6	9	2.7	8

[a]Likelihood of odds. [b]Differences between CT9993 and IR62266 were significant (P <0.05) only for stress temperature under drought (19 August) and recovery score (see Table 1).

Table 4. QTLs for days to heading and flowering as identified by MapMaker/QTL.

Traits	Markers bordering QTLs	Chromosome #	LOD[a]	R^2(%)
Days to heading, Israel				
Days to heading (irrigated)	CD0345-ME10-14	1	3.5	17
	TGMSP2-ME9-7	2	2.2	10
	RG104-EM11-9	3	6.3	30
	RM55-RG1356	3	3.4	17
	RG528-RG769	7	3.7	16
	ME8-4-EM15-10	8	4.9	20
	RM21-CD0365	11	2.8	13
	G1465C950	11	3.3	15
Days to heading (drought)	EM11-11-ME4-18	1	3.1	15
	ME4-3-EM15-11	7	2.7	10
	R1394A-RG978	8	2.6	12
Heading delay under stress (d)	ME5-7-ME10-4	8	2.6	12
Days to flowering, Thailand				
50% flowering date (d, 1996)	CD0345-ME10-14	1	3.5	13
	C1419-EM11-10	2	3.0	14
	TGMSP2-ME9-7	2	3.6	11
	RG104-EM11-9	3	8.4	32
	C235-EM17-9	6	2.5	17
	ME2-15-RG528	7	4.3	14
	ME8-4-EM15-10	8	3.4	11
	ME2-1-ME9-1	8	3.2	12
	RG667-RM215	9	6.1	19
	ME5-16-EMP2-9	10	3.3	10
	EM18-17-G333	10	3.2	11

[a] Differences in days to heading and flowering between CT9993 and IR62266 were not significant (P <0.05) (see Table 1).

yield under drought (Blum et al, this volume), no overlapping QTLs were identified for them (Tables 2, 3; Fig. 2).

Table 4 presents days to heading and flowering. Although the two parents did not differ significantly for phenological traits, QTLs were still identified. Similarly, Li et al (1997) also identified QTLs for grain number per panicle and grain weight per panicle even though the parents were not statistically different. In Thailand, 11 QTLs were located for flowering date. In Israel, 1, 3, and 8 QTLs were associated with heading delay under drought, stress days from planting to heading, and control days from planting to heading, respectively. These QTLs individually accounted for phenotypic variation from 10% to 32%. Under irrigation, when the QTLs for flowering dates in Thailand and heading dates in Israel were compared, 5 common QTLs were located. The QTLs for heading delay under drought and stress days from planting to heading did not overlap with the QTLs for flowering date and heading date under irrigation. Of the QTLs for days to flowering as detected in Thailand, 2 QTLs (C1419-

Table 5. Correlation coefficients across 100 doubled-haploid lines between productivity under stress (Israel) and osmotic adjustment (OA 96 and 98, USA), mean root penetration index (RPI-M, mean of 96, 97, and 98, USA), root pulling force (RPF96 and RPF97, Thailand), and maximum root depth (MRD, Philippines)[a]. The years 1996,1997, and 1998 were abbreviated as 96, 97, and 98, respectively.

Parameters	OA96	OA98	RPI-M	RPF96	RPF97	MRD
Biomass under drought	0.04	0.00	0.00	–0.11	–0.09	0.05
Yield under drought	0.00	–0.01	–0.22	–0.30	–0.15	0.02
Biomass resistance index	0.03	–0.04	–0.06	–0.10	–0.19	–0.02
Yield resistance index	0.06	0.14	–0.27	–0.22	4.07	–0.04

[a]Correlation coefficient that is significant at 5% is 0.29.

EM11_10 on chromosome 2 and C235-EM17_9 on chromosome 6) were also found in other rice genetic backgrounds (Lin et al 1998, Yamamoto et al 1998). The C235-EM17_9 region corresponds to the *Hd-1* QTL, one of the two major QTLs (*Hd-1* and *Hd-2*) for heading date. This QTL region has recently been finely mapped (Fig. 2 in Yamamoto et a1 1998).

A schematic linkage map with QTL positions marked (Fig. 2) was made to compare whether there are any relationships between the QTLs for components of drought tolerance and the QTLs for rice performance in the field. Figure 2 showed that QTLs for drought-related traits were scattered throughout the whole genome. These QTLs were generally not linked to the QTLs for yield and biomass under stress. In terms of location overlapping between QTLs for yield under stress and QTLs for drought-related traits, only QTLs for root pulling force and grain yield on chromosome 11 were located in the same genomic region. Similarly, there was no significant association between yield under stress and drought-tolerance components as measured in the lab/greenhouse except the association between yield under drought and root pulling force in 1996 (Table 5). Based on the author's (Blum) long experience with the research conducted on soil and plant water relations at the site used in Israel, we speculate that the soil at Bet Dagan had very low penetration resistance and that ample deep soil moisture was available. Under these conditions, the capacity of plants to extract deep soil moisture may have dominated genetic variation in stress response at the site, whereas neither OA nor root penetration were effective in this respect.

This study further confirmed the complexity of the associations between yield performance and physio-morphological components of drought tolerance. Clearly, there is an urgent need for further quality phenotyping of the mapping population in target drought-prone environments in the field where genetic variations in OA and root traits might affect plant productivity. Development of near-isogenic lines and acquisition of data on field rooting depth and traits based on physio-morphological mechanisms should provide a fuller physiological and genetic explanation of drought tolerance and QTL relationships.

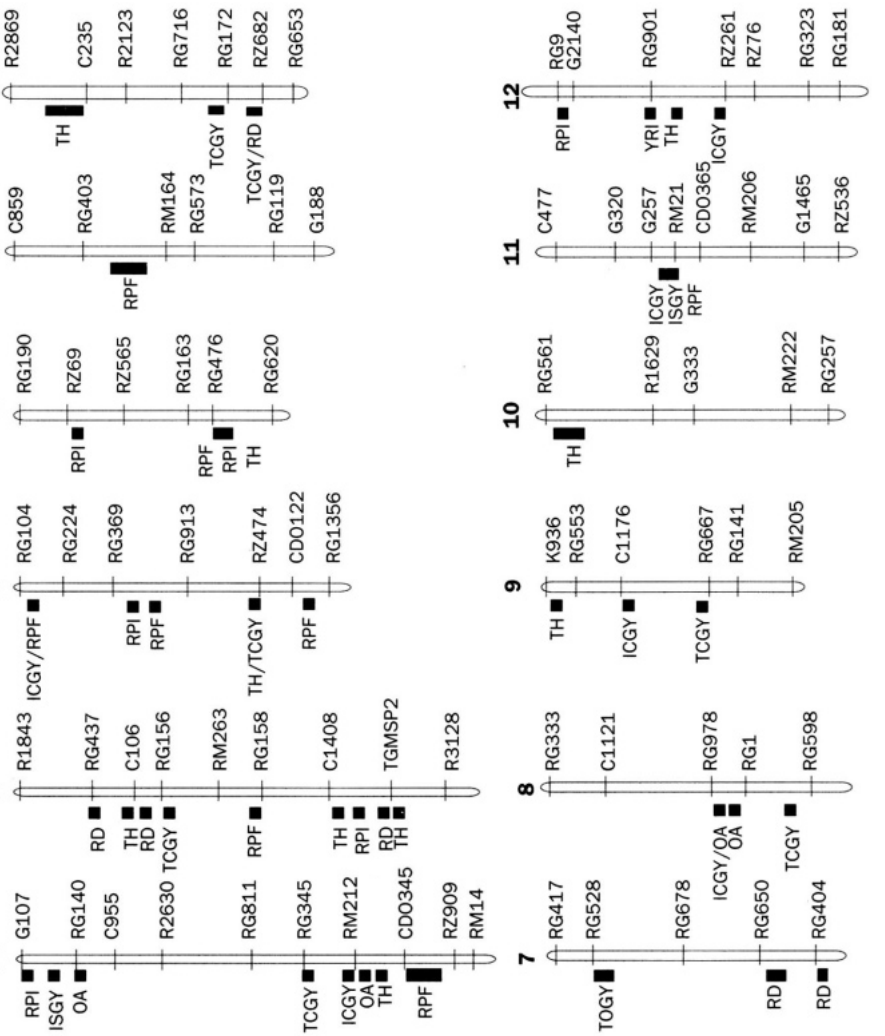

Fig. 2. Locations of QTLs for selected root and shoot traits in a doubled-haploid line population of CT99935-10-1-M and IR62266-42-6-2. For clarity, only a few RFLP markers per chromosome are shown. ICGY = grain yield under control in Israel, ISGY = grain yield under stress in Israel, OA = osmotic adjustment, RD = root depth, RPF = root pulling force, RPI = root penetration index, YRI = yield resistance index, TCGY = grain yield under control in Thailand, TH = root thickness.

References

Blum A. 1988. Plant breeding for stress environments. Boca Raton, Fla. (USA): CRC Press.

Boyer JS. 1982. Plant productivity and environment. Science 218:443-448.

De Datta SK, Malabuyoc JA, Aragon EL. 1988. A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. Field Crops Res. 19:123-134.

Fischer KS, Edmeades GO, Johnson EC. 1989. Selection for the improvement of maize yield under moisture-deficits. Field Crops Res. 22:227-243.

Fischer RA, Maurer R. 1978. Drought tolerance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res. 29:897-912.

Fukai S, Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40:67-86.

Garrity DP, O'Toole JC. 1995. Selection for reproductive stage drought avoidance in rice, using infrared thermometry. Agron. J. 87:773-779.

Hanson AD, Peacock WJ, Evans LT, Arntzen CJ, Khush GS. 1990. Drought tolerance in rice. Nature 345:26-27.

Ingram KT, Bueno FD, Namuco OS, Yambao EB, Beyrouty CA. 1994. Rice root traits for drought tolerance and their genetic variation. In: Kirk GRD, editor. Rice roots: nutrient and water use. Manila (Philippines): International Rice Research Institute. p 67-77.

Jackson RD, Idso SB, Reginato RJ, Pinter Jr. PJ. 1981. Canopy temperature as a crop water stress indicator. Water Resour. Res. 17:1133-1138.

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW. 1997. Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453-465.

Lilley JM, Ludlow MM. 1996. Expression of osmotic adjustment and desiccation tolerance in diverse rice lines. Field Crops Res. 48:185-197.

Lin SY, Sasaki T, Yano M. 1998. Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor. Appl. Genet. 96:997-1003.

Lincoln S, Daly M, Lander E. 1992. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report, 2nd edition. Cambridge, Mass. (USA): Whitehead Institute.

Ludlow MM, Muchow RC. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Nguyen HT, Chandra Babu R, Blum A. 1997. Breeding for drought tolerance in rice: physiology and molecular genetics considerations. Crop Sci. 37:1426-1434.

O'Toole JC. 1982. Adaptation of rice to drought-prone environments. In: Drought tolerance in crops with emphasis on rice. Manila (Philippines): International Rice Research Institute. p 195-213.

O'Toole JC, Moya TB. 1978. Genotypic variation in maintenance of leaf water potential. Crop Sci. 18:873-876.

O'Toole JC, Soemartono. 1981. Evaluation of a simple technique for characterizing rice root systems in relation to drought tolerance. Euphytica 30:283-290.

Tuinstra MR, Ejeta G, Goldsbrough. 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. Crop Sci. 38:835-842.

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M. 1998. Fine mapping of quantitative trait loci *Hd-1, Hd-2* and *Hd-3,* controlling heading date of rice, as single Mendelian factors. Theor. Appl. Genet. 97:37-44.

Yu LX, Ray JD, O'Toole JC, Nguyen HT. 1995. Use of wax-petrolatum layers for screening rice root penetration. Crop Sci. 35:684-687.

Zhang J, Klueva N, Nguyen HT. 1999a. Plant adaptation and crop improvement for arid and semiarid environments. Proceedings of the Fifth International Conference on Desert Development: The Endless Frontier, 12-17 August 1996. Lubbock, Tex. (USA): Texas Tech University, Vol. II. p 604-620.

Zhang J, Nguyen HT, Blum A. 1999b. Genetic analysis of osmotic adjustment in crops. J. Exp. Bot. 50:291-302.

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Future perspectives

Genetic improvement of rice for water-limited environments: constraints and research opportunities

R. Lafitte

The tools available to evaluate genetic variation in tolerance for water stress have expanded tremendously over the past decade. This workshop sought to identify opportunities to apply those tools to reduce the impact of water deficit on rice yields. Many of the previous chapters report results obtained to date. This chapter summarizes the next steps that must be taken to translate these results into improved varieties for rice farmers in regions affected by water stress.

Three working groups identified the major constraints to achieving the core objective of developing and applying molecular strategies and physiological understanding to breed productive, stable rice varieties for water-limited environments in five years. Table 1 summarizes the major constraints, along with the research activities needed to overcome those constraints. The following text summarizes the discussion that led to the development of Table 1.

Understanding drought environments and G x E interactions

Variety characteristics that confer an advantage in some water stress environments may prove to be useless or may even be a liability in other environments. This is reflected in the large G × E interactions in drought trials and in the difficulty of identifying drought-tolerant check cultivars. The most desirable traits are those that are valuable across many stress environments, but even these may incur some loss in yield potential in favorable environments. To exploit specific adaptation, we must link specific desirable traits to their corresponding target environments.

Some simulation models can relate rainfed rice yield to water availability. Although these could benefit from further refinement, they can provide a preliminary estimate of the frequency and severity of stress. Such data will allow breeders to better define their primary target stress environments and thus identify which screening environments and which traits will be most useful.

Field testing in controlled stress environments

We are now able to use molecular techniques to verify whether or not a given line carries a particular allele from a given parent, but we do not yet know which alleles or

Table 1. Constraints to the development of improved rice varieties for water-limited environments and opportunities to overcome each constraint.

Constraint	What is needed to overcome constraint
Understanding of environment	
Poor characterization of target environments for climate and soil.	Apply simulation models and GIS databases.
Inadequate information on G × E (including QTL × E).	Evaluate existing mapping populations in more environments; collect physical data necessary to characterize the site. Apply G × E analytical methods.
Breeding	
Do not know what the gains will be from MAS compared with conventional breeding.	Model predicted gains for traits of different heritability.
Breeding objectives need to include tolerance for water deficit as well as risk, impact, and farmer preference.	Consult end users.
Long time and cumbersome mechanisms to release varieties.	Use farmer participatory breeding networks to allow some rapid distribution and pretesting of varieties.
Poor use of local germplasm.	Identify local varieties and conduct marker-assisted backcrossing to introduce specific traits.
Don't know what is available in the gene bank.	Targeted screening.
Phenotyping	
Inadequate field testing in target population of environments; unrepresentative testing sites.	Identify and develop suitable testing sites. Conduct quality field testing of mapping populations.
Techniques for effective • stress management • experimental design • measurements and data quality • data analysis and interpretation are not widely known or applied	1. Develop key testing sites. 2. Develop standard trial designs. 3. Develop standard evaluation systems for conducting trials and measuring traits. 4. Improve analytical software, and provide documentation/training.
Physiological understanding	
Not sure which physiological/morphological traits are of value in which environment, so do not have prioritized list of secondary traits validated under field conditions or appropriate screening techniques for those traits.	1. Develop appropriate materials for precise testing of individual traits (e.g., NILs), and conduct careful field testing for yield. 2. Identify closely related materials that differ for yield under stress (e.g., mutants, NILs), and evaluate different traits. 3. Evaluate mapping populations in more environments with good precision of phenotyping for target traits and yield. Compare tails of populations for specific traits. Develop homozygous contrasts?

Table continued

Table 1 continued.

Constraint	What is needed to overcome constraint
	4. Apply genome-wide approaches to build on existing biochemical understanding of plant response to stress and identify critical response pathways.
Lack of knowledge on water transport and root function in rice, the basis of reproductive stage sensitivity to stress, and the optimal senescence pattern under stress.	Evaluate component physiological process in carefully selected varieties.
Lack of information on genetic variability for some target traits.	Evaluate traits in varieties selected to span the range of variation present in *Oryza.*
Molecular genetics	
Lack of precision in QTL position.	Use improved analytical software. Increase population size. Use more markers.
Lack of PCR markers (or other cheap and easy markers).	Use Information coming from physical mapping project. Share markers within a consortium of labs working on rice.
Lack of integrated map.	Combine available data using anchor markers such as RFLPs.
Transgenic tools not being used to understand QTL function; promoters needed.	Access databases for structural genes, regulatory genes, and stress-specific transcriptional activators, or use BAC cloning to identify genes detected through fine mapping. Identify promoters operative at relevant stress levels and in specific tissues. Put selected sequences into elite adapted background with appropriate promoters and optimize promoter-gene interaction. Test in target environments (including well-watered conditions). Test To ... Tn for stable inheritance and expression.
Bioinformatics	
Difficult to link genotypic data across populations.	Develop a consensus on data management structure and intellectual property rights issues. Provide software and training for database use.

even which loci confer an adaptive advantage in specific stress environments. Some of the constraints to applying molecular tools are thus qualitatively similar to those that have restricted the application of existing physiological understanding to improving crop performance. This reflects, in part, a limitation to our ability to reliably create and sample a range of stress environments when doing yield testing.

When we want to proceed beyond variety trials and target a specific trait using molecular techniques, multienvironment testing with broad coverage of the target

environments becomes even more difficult. For the evaluation of mapping populations, for example, it is necessary to identify a few well-controlled screening environments and then impose realistic, meaningful levels of stress. The locations available for rainfed rice screening under controlled conditions are few (Courtois and Lafitte, this volume). Even when the physical environment is acceptable, the problem of variable phenology within mapping populations makes it difficult to apply the same level of stress to each line. This is an especially intractable problem for the rainfed lowland environment, where the application of irrigation to individual plots is not yet possible on the scale needed for evaluating mapping populations.

A focused effort will be required to develop adequate field phenotyping facilities and expertise. Three to five locations in Asia must be identified where both a dry period (via a reliable dry season or a large rainout shelter) and supplemental irrigation are available. Soil types should be considered in selecting the locations. Human resources for each site must be trained in details of water management, statistical considerations for large experiments grown with stress, and data collection. There is a need to identify and publish standard operating procedures for water stress screening trials, which will allow greater repeatability across experiments. These procedures must include standard methods to measure plant and soil water status. A series of small workshops was suggested to allow the preparation of a manual of standard evaluation procedures.

Understanding the value of specific traits

Many individual traits have been nominated as routes to improved rice performance under conditions of water deficit. Some traits mentioned at this workshop were leaf responses to stress (rolling and drying; Price et al, Shen and Courtois, this volume), root morphology in aerobic or anaerobic soil (Kamoshita et al, Shashidhar et al, Price et al, this volume), and osmotic adjustment capacity (Babu et al, this volume). Quantitative trait loci (QTLs) have been identified for all of these traits, but their adaptive value in various water stress environments remains unclear. We need quantitative estimates of the value of these and other potentially useful traits. Such estimates can be obtained by associating yield differences with variation in a target trait in the same (or similar) genetic background. This will require wide field testing of rice lines that have been genetically designed for the testing of physiological hypotheses. These rice lines may be near-isogenic lines (NILs) derived from marker-aided backcrossing, or lines that have had specific gene-promoter regions introduced from other varieties.

The number of traits that may confer an advantage under water-limited conditions may be too great to allow the targeted development of NILs for each trait. This problem may be overcome by the use of tolerant donor varieties in the context of a larger breeding project that can generate many sets of NILs (Li, this volume).

Cosegregation analysis of mapping populations for which both yield and potentially valuable traits are measured can be used to link traits to yield. The difficulties in

this approach lie in obtaining realistic yield estimates for populations that are segregating for phenology and plant type, and in working with adequate population sizes to allow precise determination of QTL locations. Increasing the precision of phenotyping in both greenhouse and field experiments is seen as the key to improving the accuracy of QTL placement and QTL stability. At present, few data sets are available that include yield of mapping populations under stress. As an immediate step, a major effort must be made to conduct wider field evaluations in target environments and establish heritabilities and genetic correlations under stress between grain yield and specific traits of interest. This will require both a serious investment in high-quality field screening and sharing of data among groups working on these populations. Working groups need to be truly multidisciplinary, with greater involvement of researchers based in the target environments.

Knowledge gaps in rice stress physiology

Despite the volume of literature available on rice physiology, substantial gaps exist in our understanding of rice response to water deficit. There are indications that rice has a comparatively high internal resistance to water transport and that this may limit water uptake from depth under dry conditions (Kondo et al, this volume), but the basis of this phenomenon remains unclear. No techniques are now available to accomplish high-throughput screening for water uptake characteristics. Membrane stability under stress was also suggested as a possible screening technique for tolerance for water stress at the vegetative stage.

We do not know whether the basis of rice's extreme sensitivity to reproductive stage stress lies in hormonal signals, carbohydrate metabolism (e.g., acid invertase activity, starch reserves in the ovary at pollination), or some other process. Such understanding is greatly needed and might reduce the need for logistically difficult field screens of materials stressed at exactly the same phenological stage.

We have a fairly limited understanding of the optimal stay-green strategy for rice in stress environments and the role of carbohydrate and N remobilization from vegetative parts with terminal stress. We need a clearer understanding of these processes in order to identify additional opportunities for crop improvement.

In identifying additional valuable traits for rice varieties tolerant of water deficit, the needs of breeders should be considered. Screens must allow high throughput and should minimize the use of specialized equipment. This restriction can be overcome to some extent by identifying QTLs for the traits, but localization of the QTLs will still require phenotyping of large populations. Details of the phenotyping strategy should be considered when traits are suggested for mapping. The question whether QTLs identified in one cross will be identified in other crosses subjected to the same environmental conditions also needs to be addressed. Experience with maize suggests that a few QTLs are identified repeatedly in many different crosses, while others are specific to a single cross alone, indicating that QTLs need to be identified in each cross of a (donor × recipient).

Identifying QTLs

The size of most existing mapping populations used in rice water stress studies (100–200 lines) may be too small for precise identification of QTLs. On the other hand, phenotyping larger populations for some of the more difficult traits such as root morphology may be impossible with current resource levels. The cost of genotyping is also high for populations of this size. Statistical models to optimize mapping population size indicate that there is little additional gain in increasing numbers beyond 500 lines, but that populations smaller than 100 lines are of limited value.

Another limitation to rapid progress in QTL identification and use in rice is the lack of inexpensive polymerase chain reaction-based markers. The ideal solution would be to have perhaps 1,000 microsatellite markers well distributed over the genome, and available in the public domain. Appropriate collaborations can also reduce costs. Other co-dominant markers would also be useful. These may emerge as a product of the rice physical mapping project under way in Japan.

Applying QTLs in breeding programs

The wide application of molecular techniques to breeding programs can only begin when breeders have a list of stable and prioritized QTLs across genotypes for specific stress environments. QTLs for yield under stress will show the same large G × E interaction that is observed for yield. It may be possible to reduce the G × E interaction for yield by pyramiding QTLs for several component traits. When a list of stable QTLs 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-aided selection (MAS) and conventional breeding. Simulations have the potential to play an important role in allowing breeders to partition resources between marker-assisted breeding and conventional approaches (Cooper et al, this volume).

In deciding whether or not to use QTLs associated with tolerance for water deficit in a breeding program, breeders must also consult with the end users of the variety. Farmers may perceive the hierarchy of production problems quite differently than researchers do. A promising approach is to introgress QTLs into adapted local varieties, which should be rapidly accepted. This type of rapid varietal improvement recognizes that there may be a need for several cultivars within one region to meet the requirements of different farming systems and consumer preferences, but the approach may well carry linkage drag. When QTLs are transferred to the recipient genome carrying around 5–10% of the genome of the donor (including the QTL of interest), there is ample opportunity for genes that reduce yield in specific environments to be transferred across as well.

One constraint to the rapid release of varieties improved by MAS may be the cumbersome system of varietal testing in many countries. Decentralization of varietal improvement to the village or farm level through farmer participatory breeding programs should speed farmer access to modified local varieties.

Transformation technology could also be applied to improve water stress tolerance. Stress-specific transcriptional activators have been identified in dehydrated *Arabidopsis* plants (Yamaguchi-Shinozaki et al, this volume). Such promoters should be identified in rice plants suffering from milder, agronomically relevant levels of stress. Further investigation will undoubtedly identify promoters that are tissue specific and that function only during certain development stages of the crop.

Managing information

Data management, analysis, and interpretation become increasingly important as the number of selection criteria increases in a breeding program. We need techniques to simplify the extraction of information from databases. The statistical approaches to QTL identification are changing rapidly to include the effect of epistasis, and this aspect needs to be made more user-friendly. We need more accessible software for spatial analyses (e.g., ASREML), and selection indices that allow the incorporation of additional traits. Users need training on how to use some of the rather unfriendly but essential software for managing spatial analyses, identifying QTLs, and gaining access to databases.

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