Abiotic stress tolerance in rice for Asia: progress and the future

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Abstract
Various abiotic stresses limit rice production in rainfed environments, which comprise about 45% of the global rice area. Important stresses include water deficit, submergence, salinity, and deficiencies of P and Zn. In recent years, advances in physiology, molecular biology, and genetics have greatly improved our understanding of how rice responds to these stresses and the basis of varietal differences in tolerance. Progress has relied on the application of rather specific phenotypic screens that allow the effects of stress to be distinguished from general differences in adaptation of diverse parents. QTLs have been identified that explain a considerable portion of observed variation, and in some cases, the genes underlying specific QTLs have been identified. Transformation has been used to assess the effects of altered expression of specific stress-related genes, allowing confirmation of the importance of particular metabolic pathways. Through expression profiling of many genes simultaneously, it has been possible to identify three types of stress-responsive gene networks: early signaling pathways, adaptive responses, and genes that reflect downstream results of damage. For crop improvement, the identification of useful allelic variation for genes in the second group may be the most promising approach. Once such genes or gene combinations are identified, either molecular approaches or trait-specific physiological screens can be used to search for these superior alleles. Marker-assisted backcrossing can then be applied to incorporate these alleles into agronomically superior germplasm.

Media summary
Abiotic stresses such as drought, salinity, submergence, and nutrient deficiencies limit rice production. Recent advances in our understanding of the physiology and molecular biology of stress tolerance in rice are being used to develop improved rice varieties.

Key words
Drought, flooding, salt, *Oryza sativa*, gene expression

Introduction
Over half of the world's population depends on rice as a staple crop; in Asia, rice supplies 30 – 80% of the daily calories consumed (Narciso and Hossain 2002). Rice is an anomaly among the domesticated cereals – a tropical C_3 grass that evolved in a semi-aquatic, low-radiation habitat. As such, rice carries an odd portfolio of tolerances and susceptibilities to abiotic stresses as compared to other crops. Rice thrives in waterlogged soil and can tolerate submergence at levels that would kill other crops, is moderately tolerant of salinity and soil acidity, but is highly sensitive to drought and cold. Even where rice response to stress is superior to other crops, however, many rice-growing environments demand still greater tolerance than is found in most improved germplasm. In tropical regions, rice is grown in monsoon climates that are subject to intermittent submergence (water depths of 0.5 to 1 m that cover the foliage), drought, and, in coastal regions, salinity. Rice is also grown in the tropics during the dry season where adequate irrigation is available, and the crop may suffer from low temperatures at seeding and high temperatures at flowering. In temperate regions, where virtually all rice is fully irrigated, low temperature is also a major abiotic stress affecting rice production. Where rice is grown in unflooded soils in the humid tropics, the crop is affected by water deficit, soil acidity, and deficiency of P and Zn.

Complementing the agronomic need for greater tolerance to abiotic stress in important rice-growing regions is the unique role of rice in the genomic era of plant science. Rice has the smallest genome among the cultivated cereals, and it conserves much of the gene content and, to some extent, gene order present in other species (Gale and Devos 2001). The amplification of the genome in other species appears to have occurred largely through the duplication and rearrangement of an ancestral gene complement, which is most closely preserved in rice. The full rice genome has now been sequenced (Goff et al. 2002), allowing the identification and localization of genes related to stress tolerance. The rice system can be used to

assign function to genes, so that homologues can be identified in other species with more cumbersome genomes, but with possibly greater stress tolerance. The syntenic relationship between genomes has encouraged the application of functional genomic approaches to rice, in order to better understand general plant processes, disease resistance, and tolerance to abiotic stress.

The objectives of this paper are to briefly review recent efforts to better understand rice adaptation to several common abiotic stresses and to highlight efforts to integrate results of advances in physiology and molecular biology into rice breeding programs.

Water deficit
Drought is generally avoided in irrigated rice production systems, but it is a consistent feature across much of the 63.5 million hectares of rainfed rice sown annually, most of which is in tropical Asia, Africa, and Latin America (Narciso and Hossain 2002). Farmers have been selecting those plants that survived drought events for centuries, and there is a wealth of genetic variation for response to water deficit among traditional cultivars (Mackill et al. 1996). There are few examples, however, of improved cultivars that combine acceptable yield potential and drought tolerance. The immediate difficulty lies in reliably measuring drought tolerance. Like other seed-producing crops, rice is more susceptible to damage from water deficit at particular growth stages. A given level of drought at the vegetative stage can cause a moderate reduction in yield, but the same stress can eliminate yield entirely if it coincides with pollen meiosis or fertilization (O'Toole 1982). In some cases, superior response to vegetative stage stress is associated with better performance under reproductive stage stress, but in many cases the strategies that appear to be successful at the reproductive stage may be counterproductive when stress occurs at flowering (Pantuwan et al. 2002). Direct selection for improved yield under drought has been hampered by the unpredictability of drought events, which mean that selection pressure is generally inconsistent, and possibly contradictory, across years. Progress has been made, however, through the inclusion of tolerant parents in crossing (Chang et al. 1982; Pinheiro 2003). More recently, the use of managed environments and targeted multilocation testing has been implemented to facilitate progress in breeding drought tolerant rice (Fischer et al. 2003). The success of these initiatives will be known within the next few years.

Rice varieties differ greatly in their ability to tolerate aerobic soil and moisture deficit. The greatest ability to grow and produce some grain with chronic moderate water deficit is found in japonica varieties from upland ecosystems such as those found in hilly Southeast Asia and Africa (Mackill et al. 1996). Examples include Azucena from the Philippines and Moroberekan from Guinea. Notable levels of drought tolerance are also observed in the early-maturing aus and indica varieties traditionally grown in the plateau region of Eastern India, such as N22 and Dehula (Lafitte and Courtois 2002). While these cultivars usually escape drought through early maturity, they can also produce some grain when rains fail around flowering, indicating that they avoid or tolerate drought. Varieties adapted to anaerobic systems tend to stop growing as soil moisture declines, and leaves roll or senesce, effectively shedding excess radiation. This is associated with limited deep rooting and water extraction in many lowland varieties (Lilley and Fukai 1994). Some lowland cultivars have impressive levels of tolerance of tissue water deficit (Lilley and Ludlow 1996) and perform well in screens for leaf survival with vegetative stage stress (De Datta et al. 1988). Certain cultivars, such as Nam Sagui 19 from Thailand, combine both tissue tolerance and grain yielding ability in an indica genetic background, and these have served as important parental lines in breeding programs.

The physiological basis of genetic variation in drought response is not clear, in part because so many different measures of tolerance have been reported. If tolerance is defined as the ability to maintain leaf area and growth under prolonged vegetative stage stress, the main basis of variation appears to be constitutive root system architecture and its associated tillering habit that allows maintenance of more favorable plant water status (Nguyen et al. 1997), though in the field the impact of root system is easily confounded by plant size effects (Mitchell 1998). Differences have also been observed in the adaptive response of root distribution to soil drying (Azhiri-Sigari et al. 2000; Liu et al. 2004b). The mechanisms underlying genetic variation in both constitutive and adaptive root distribution may be sensitivity to signals, particularly auxin, that influence root elongation and branching (Bao et al. 2004; Ge et al. 2004). When drought tolerance is defined as the ability to flower and produce grain under water deficit, additional mechanisms may become important. Delayed flowering under drought is associated with an

apparent delay in floral development when stress occurs between panicle initiation and pollen meiosis (from 30 to 10 d before heading). With the onset of stress occurring from 10 to 5 d before heading, flowering is slowed mainly due to slower elongation of the panicle and supporting tissues (A. Kathiresan, pers. comm.). Genetic variation in flowering delay under drought has been reported, and only part of this variation depended on measured plant water status (Pantuwan et al. 2002). Drought also affects the process of starch deposition in pollen grains, which normally begins about 3 days before anthesis, contributing to reduced anther dehiscence. Genetic variation for the tolerance of anther dehiscence to low water status has been observed (Liu et al. 2004a). Panicle desiccation can occur when drought coincides with heading; variety-specific mechanisms that can refill cavitated xylem elements in shoots may be important to limit panicle failure (Stiller et al. 2003). Unfortunately, we have little information about genetic variation in the final drought-sensitive processes of fertilization and early embryo establishment, other than the observation that varieties that better maintain shoot water potential have an advantage. As new methods are applied to the study of drought during the reproductive stage, we expect to discover additional mechanisms that allow some varieties to set grain despite unfavorable shoot water potentials.

Molecular approaches to drought tolerance have been widely applied to rice, beginning with QTL analysis. The rice genetic map is well covered by microsatellite markers (McCouch et al. 2003), and rice researchers worldwide have developed diverse mapping populations and related databases (see (Ware et al. 2002)). Mapping studies have been successful in identifying genetic regions associated with highly heritable traits such as plant height and flowering date, and in some cases it has been possible to identify the specific gene underlying a QTL (Ishimaru et al. 2004). QTLs have also been identified for some secondary traits that are expected to be associated with drought response, such as rooting depth, membrane stability, and osmotic adjustment (Table 1). Where tolerance is measured as yield under drought, however, few strong and repeatable QTLs have been identified. As more studies are published using realistic stress levels and adequate documentation of the dynamics of drought development, it should be possible to focus on some key QTLs that appear to be important across environments or populations. To date, however, it has not been possible to identify sufficiently large and discrete QTLs for performance under drought to justify marker-assisted selection. Instead, the results of QTL studies will probably be most usefully applied to the identification of promising genetic regions for the identification of candidate genes. Nonetheless, modifications of QTL mapping strategies still hold promise to deliver a product that will be more directly useful for cultivar improvement – these include linkage disequilibrium studies and the use of the advanced-backcross QTL approach that combines selection and QTL identification in closely related backcross lines.

Many studies report changes in the expression of individual genes when rice is challenged by drought stress, and they frequently respond to other abiotic and biotic stresses as well. These include such diverse genes as MAP kinase (Agrawal et al. 2003), DREB genes (Dubouzet et al. 2003), calcium-dependent protein kinase (Saijo et al. 2001), an endo-1,3-glucanase (Akiyama and Pillai 2001), a translation elongation factor (Li Zi and Chen Shou 1999), and glutathione reductase (Kaminaka et al. 1998). Transformation studies have demonstrated that altering the expression of a number of different genes from different pathways can affect the response of rice to water deficit or dehydration (Table 2). These include genes associated with diverse functions, such as water uptake (aquaporins) (Martre et al. 2002), signaling (kinases) (Saijo et al. 2001) (Liu et al. 2003), membrane integrity (LEA protein) (Xu et al. 1996) (Rohila et al. 2002) (Babu et al. 2004), and carbohydrate metabolism (TPS) (Jang et al. 2003). The effect of transformation on grain production under stress has not been well documented.

Because of the very large number of genes that change expression under drought stress, genomic approaches that can follow transcriptional changes in thousands of genes at a time hold great promise. Few genomic studies on drought have been published for rice, and these are mostly based on seedling responses to desiccation (Rabbani et al. 2003). Other studies have focused on expression profiling after application of other treatments that share some effects with drought, such as exogenous ABA or exposure to salt, usually applied to seedlings or callus (Yazaki et al. 2004). In maize, expression profiling experiments on reproductive tissues has been successful in highlighting important pathways that responded to stress (Yu and Setter 2003; Zinselmeier et al. 2002). For rice, microarray analysis has been used to identify differences in gene regulation in panicles of tolerant and susceptible varieties grown under stress in field conditions (Kathiresan et al. 2004). In addition to direct effects of drought on gene expression, there are changes in post-translational modifications such as protein phosphorylation.

Advances in proteomics and metabolomics provide opportunities to follow these changes as well (Koller et al. 2002).

Excess water
Excess water is a common constraint throughout the rainfed rice production areas as in South and Southeast Asia and tropical Africa. While rice is adapted to waterlogged conditions because of the well-developed aerenchyma that facilitates oxygen diffusion and prevents anoxia in roots, complete submergence can be lethal. Out of 40 million ha in Asia grown under rainfed lowlands, about 15 million ha are frequently damaged by submergence (Huke and Huke 1997). Submergence stress can also damage crops in irrigated areas due to high rainfall and/or impeded drainage, particularly early in the season. The annual average yield loss from submergence is estimated at about 80 kg/ha (Dey and Upadhaya 1996). In general, two types of flooding cause damage to rice: flash flooding that results in complete inundation of short duration, and long-term flooding, where water stagnates for up to few months (deepwater and tidal-flood areas).

The effect of flash-flooding interacts with growth stage. Germination is highly sensitive to flooding. Variability in ability to germinate under water and in coleoptile elongation under anoxia have been observed in rice. This was related to the rate of alcoholic fermentation and enhanced activity of starch degrading enzymes (Setter et al. 1994). During later vegetative development, rice can adapt to complete submergence through processes that provide necessary energy for maintenance metabolism and minimize losses. Under short-term flooding, extension growth is detrimental because it hastens energy depletion and increases mortality. Tall plants also tend to lodge when the water level recedes resulting in additional yield losses and poor grain quality. Mechanisms associated with such tolerance were recently reviewed (Ella et al. 2003; Jackson and Ram 2003; Ram et al. 2002; Setter et al. 1997).

Most existing rice cultivars are seriously damaged if they are completely submerged for more than 3 d; however, a few tolerant cultivars can withstand complete submergence for 10 to 14 d, such as FR13A, FR13B, Goda Heenati, Kurkaruppan, BKNFR76106-16-0-1-0 and Thavalu. FR13A was released in the 1940s in Orissa, India, as a pure line selection from the local variety Dhullaputia (Mackill et al. 1996). This cultivar is probably the most frequently used in genetics and physiological studies and as a standard against which other breeding lines are often compared. Breeding to further improve tolerance to submergence in rice along with yield potential has been on going for over three decades (HilleRisLambers and Vergara 1982; Mackill 1986; Singh and Dwivedi 1996). The initial work focused on transferring tolerance from traditional landraces into semi-dwarf breeding lines. However, the traditional donors were low yielding, tall and had low grain quality. Tolerant breeding lines with improved agronomic characteristics have now been developed (Mackill et al. 1993; Mackill and Xu 1996), and some breeding lines such as IR49830-7 had yield equivalent to the irrigated checks. Other new breeding lines with adequate tolerance to submergence have been recommended for release in India (Singh et al. 1998).

Genetic studies suggested both simple and quantitative inheritance for submergence tolerance (Suprihatno and Coffman 1981). Using a population developed from a cross between an *indica* submergence tolerant line (IR40931-26) and a susceptible japonica line (PI543851) a major QTL was mapped to chromosome 9, designated as *Sub1* (Xu and Mackill 1996). This QTL accounted for about 70% of the phenotypic variation in submergence tolerance in the population studied. The donor line for this QTL was derived from FR13A and had a similar level of tolerance (Mackill et al. 1993). In a subsequent study (Nandi et al. 1997) the importance of *Sub1* in submergence tolerance was confirmed and 4 additional QTLs were identified on 4 different chromosomes. Moreover, the *Sub1* locus has been fine mapped, paving the way for its positional cloning (Xu et al. 2000). Markers linked to this gene are currently being used to incorporate *Sub1* into existing popular varieties. The dramatic effect of *Sub1* on what is essentially a quantitative trait suggests a regulatory locus rather than a specific enzyme. The identity will likely be evident after cloning the putative gene, which is currently underway.

The development of submergence tolerant cultivars has required the use of stress-specific screens, because direct evaluation of tolerance is not as simple as it might seem. Results depend strongly on the depth and duration of submergence, age of seedlings, and water temperature. Because of these complexities, alternative indirect screening approaches have been developed using traits known to be

associated with submergence tolerance such as extent of underwater shoot elongation, shoot carbohydrate storage and extent of chlorophyll retention, all of which are correlated significantly with seedling survival. Our recent studies using cultivars that contrast in initial carbohydrate content as well as in elongation ability showed that initial carbohydrate content is not, on its own, a good indicator for submergence tolerance, though post submergence non-structural carbohydrates, which is the outcome of both elongation ability and the initial carbohydrate contents, is a better indicator of tolerance (Das et al., 2004). Tolerant varieties were also found to have greater ability to retain their chlorophyll content during and after submergence. This is found in studies where chlorophyll degradation was prevented by blocking the action of ethylene that accumulates during submergence (Ella et al. 2003). Monitoring chlorophyll content few days after submergence could constitute an efficient method of screening particularly if it could be done non-destructively. Extent of leaf florescence could also be used indirectly as an indicator of initial chlorophyll degradation. The potential of these traits to provide a reliable screening technique warrant further validation.

In deepwater areas, water depth can exceed 100 cm and stagnate for several months. Elongation ability of leaves and internodes under these conditions are essential to keep pace with the rising water and to escape complete submergence, thus ensuring O_2 supply and access to CO_2 and light (Setter et al. 1997). Flooding increases ethylene concentration in plant tissue both due to enhanced synthesis as well as entrapment. Rice adapted to deepwater conditions appears to differ from lowland rice in its response to interactions between ethylene and GA (Van der Straeten et al. 2001).

Traditional varieties adapted to deepwater environments such as Jalmagna, Baisbish and Rayada 16-3, are low yielding due to their low-tillering ability, long droopy leaves, susceptibility to lodging, and poor grain quality (Mallik et al. 1995). Improved varieties should combine yield and quality attributes with elongation ability, but progress has been rather slow. Artificial screening ponds and rapid generation advancement can accelerate breeding efficiency. Recently, progress has been made in developing lines with facultative elongation ability, broad and thick leaves, heavy panicles and stiff culms for deep-water conditions. Some lines with reasonable yield and grain quality have been released, such as "Prachinburi2" in Thailand. This new class of breeding lines elongate only with rising water and have greater yield.

Using a population developed from a cross between Jalmagna and IR74, three main QTLs for elongation ability were identified. The most important was *QIne1*, which mapped near *sd-1* on chromosome 1. Two other QTLs mapped on chromosomes 4 and 5 (Sripongpangkul et al. 2000). Fine-mapping and tagging of these QTLs should facilitate their efficient incorporation into modern popular varieties using marker-aided selection.

Salt stress
Salt stress is a major constraint to cereal production worldwide. In Asia alone, 21.5 million ha are affected, of which 12 million ha are saline and 9.5 million ha are alkaline/sodic. Rice is a salt-sensitive crop, but it is the only cereal that has been recommended as a desalinization crop because of its ability to grow well under flooded conditions, and because the standing water in rice fields can help leach the salts from the topsoil to a level low enough for subsequent crops (Bhumbla and Abrol 1978). Despite its high sensitivity to salinity, considerable variation in tolerance was observed in rice (Akbar et al. 1972; Flowers and Yeo 1981).

Rice is comparatively tolerant of salt stress during germination, active tillering, and towards maturity and is sensitive during early seedling and reproductive stages. The physiological bases of salt tolerance during early seedling stage are fairly well understood; key traits include high seedling vigor, salt exclusion at the root level, compartmentation of ions in structural and older tissues, high tissue tolerance, responsive stomata that close within minutes after exposure to salt stress but partially reopen after a period of acclimation, and upregulation of antioxidant systems, particularly the ascorbate/glutathione pathway of oxidative stress tolerance. During reproductive development, tolerant genotypes tend to exclude salt from flag leaves and developing panicles (Yeo and Flowers 1986, A. Ismail, unpublished data). Although these traits are essentially independent, none of the known salt-tolerant landraces combine favorably more than few of them and there is considerable variation in the extent of expression of particular traits among cultivars, suggesting the likelihood of identifying even better donors and alleles of useful genes. Salinity tolerance at the seedling and reproductive stages are only weakly associated; hence, pyramiding of

contributing traits at both stages is needed for developing resilient salt-tolerant cultivars (Moradi et al. 2003). Salt tolerance of rice can, therefore, be improved beyond the present phenotypic range by use of physiological criteria to select independently for individual contributing traits or ultimately by tagging genes controlling critical steps in pathways underlying each of these traits to permit their subsequent combination in superior genotypes.

Few attempts have been made to identify QTLs associated with salinity tolerance in rice. For example, seven QTLs for seedling traits associated with salt stress were identified and were mapped to five different chromosomes (Prasad et al. 2000). A major gene for salt tolerance was mapped on chromosome 7, using an F_2 population derived from a salt-tolerant japonica rice mutant, M-20 and the sensitive original variety 77-170 (Zhang et al. 1995). The QTLs associated with different mechanisms of salinity tolerance in rice independently govern the uptake of Na and K and Na:K selectivity and are mapped on different chromosomes (Koyama et al. 2001). A major QTL designated '*Saltol*" was mapped on chromosome 1 using a population generated from a cross between the sensitive variety IR29 and a tolerant landrace, Pokkali. This QTL accounted for more than 70% of the variation in salt uptake in this population (Bonilla et al. 2002) and is now being mapped to within 1 cM using a large set of NILs. Candidate BAC clones from the physical map have also been identified. Marker assisted backcrossing is currently being used to incorporate this QTL into popular varieties sensitive to salt stress.

The genes underlying difference in tolerance are myriad, because of the large number of mechanisms that result in tolerance. One mechanism to avoid toxic concentrations of harmful salts in the cytoplasm is to transport them to the apoplast. This is achieved through active processes involving a gene family of Na^+/H^+ antiporters that transport sodium out of the cell or sequester it in vacuoles (Blumwald et al. 2000). Evidence for the role of these antiporters in tolerance to salt stress has recently accumulated from a number of independent studies. For example, overexpression of the vacuolar Na^+/H^+ antiporters (*AtNHX1*) from *Arapidopsis* in tomato (Zhang and Blumwald 2001) and canola (Zhang et al. 2001) permitted the transgenic plants to grow in up to 200 mM NaCl, which is extremely high for xerophytic plants. A potassium transporter from Arapidopsis (AtHKT1) is involved in Na^+ recirculation from shoots to roots (Berthomieu et al. 2003), probably by mediating Na^+ loading into the phloem sap in shoots and unloading in roots. This mechanism could play a crucial role in plant tolerance to salt stress by removing large amounts of Na^+ from the shoot. The central role of root membrane transporters in determining response to salinity has been demonstrated in large-scale expression studies in roots (Maathuis et al. 2003).

Allelic variation in one copy of a small family of H^+ ATPase genes from 77-170 correlated with a QTL for salt tolerance located on chromosome 12 (Zhang et al. 1999). Transcripts of this gene were found to accumulate in roots of a salt tolerant mutant M-20, suggesting that it may restrict salt uptake into roots. In addition to these examples, there are reports of significant improvements in salinity tolerance associated with over-expression of other genes such as superoxide dismutase in Arabidopsis (Gao et al. 2003) and a calcium-dependent protein kinase in rice (Saijo et al. 2000). The opportunities to improved rice salinity tolerance through the incorporation and pyramiding of superior alleles of these various mechanisms appears very promising.

Phosphorus deficiency
Insufficient plant-available soil phosphorus is a major constraint for rice production. This is particularly apparent under upland conditions, which are commonly characterized by infertile, highly acidic, P-fixing soils, normally in areas where little or no fertilizer is applied. Even under lowland conditions, P deficiency is a main factor limiting performance of modern rice varieties and is likely to become increasingly important as P is removed from soils under intensive rice production (De Datta et al. 1990). The lack of locally available P sources and the high cost of importing and transporting fertilizers prevent many resource-poor rice farmers from applying P. Some rice soils can quickly fix up to 90% of the added P fertilizer into less soluble forms (Dobermann et al. 1998). An attractive, cost-effective and sustainable strategy is to develop rice cultivars capable of extracting higher proportion of fixed P. Genetic variability among lowland (Wissuwa and Ae 2001b) and upland (Fageria et al. 1988) rice cultivars in their ability to exploit soil and fertilizer P were observed. Variation in uptake in the range of 0.6 to 12.9 mg P plant^{-1} was reported, and with the traditional landraces being superior to modern varieties. Hence, genetic variation in tolerance to P deficiency could effectively be exploited for rice improvement.

Two main types of mechanisms confer tolerance to P deficiency; internal mechanisms associated with efficient use of P by plant tissue; and external mechanisms that allow greater P uptake by plant roots. Genetic variation in external efficiency is probably the most important mechanism for P deficiency tolerance in rice (Hedley et al. 1994; Wissuwa and Ae 1999). Morphological characteristics such as root length, surface area, fineness, and density of root hairs are found to influence P uptake in many crop species (Kirk and Du 1997; Otani and Ae 1996). A model was recently developed to critically test the contribution of these traits (Wissuwa 2003). Small changes in root growth-related parameters were found to exert large effects on P uptake. For example, a 22% increase in root fineness or in internal efficiency of root dry matter production could triple P uptake, suggesting that large genotypic differences in P-uptake could be caused by small changes in tolerance mechanisms that are difficult to detect.

Under flooded conditions rice roots can acidify soils in their immediate vicinity through release of H^+ from roots or from oxidation of Fe^{2+} by root-released O_2 (Saleque and Kirk 1995), making soil-bound P more available. Mechanisms of P solubilization in aerobic soils are probably different and mainly involve the secretion of low molecular weight organic acids, such as citrate, that increase P availability through the formation of soluble metal-citrate chelates (Kirk et al. 1999). Chelating agents such as organic acids may help solubilize P in the soil by dissolving Al and Fe solid phases on which P is held. High rates of release of P-solubilizing organic acid anions from roots in response to P-deficiency have been reported (Kirk et al. 1999).

Although genotypic differences in P deficiency tolerance in rice were reported long ago, efforts were limited to screening available varieties rather than developing new genotypes especially adapted to P deficient soils. The fact that traditional varieties were more superior to modern varieties (Wissuwa and Ae 2001b) indicates the need for such breeding programs to incorporate P-deficiency tolerance into modern cultivars. However, tolerance to P-deficiency is quantitatively inherited with both additive and dominant effects (Chaubey et al. 1994) and as with other quantitative traits, progress through conventional approaches will be slow. More rapid progress in breeding may be achieved through the application of modern molecular approaches.

Attempts have been made to detect QTLs controlling P-deficiency tolerance in rice, and four QTLs have been identified for P-uptake. One major QTL (*Pup1*) was mapped on chromosome 12 (Wissuwa and Ae 1999; Wissuwa et al. 1998). *Pup1* was found to triple P uptake under P-deficient soils, with no apparent effect when P was not limiting (Wissuwa and Ae 2001a). At present, *Pup1* is fine-mapped in a 0.4 cM interval, which, on the rice physical map, spans 3 BACs that have been fully sequenced. Physiological studies suggest that the *Pup1* gene is expressed in root tissue, where it either leads to higher root growth per unit P uptake (higher internal efficiency) or improves P uptake per root surface area (external efficiency; Wissuwa and Ismail, unpublished). The availability of rice genome sequence data will facilitate efforts to clone the *Pup1* locus if potential target genes can be identified based on hypothetical gene function or expression. Work is currently ongoing to identify and characterize the putative candidate genes in this region and to develop a MAS strategy for its incorporation into modern varieties.

Understanding the mechanisms by which phosphate is transported across the plasma membrane and into the plant symplast has advanced considerably over the past few years, and genes encoding P transporters were isolated from different plant species (Rausch and Bucher 2002; Smith 2002). Plants have multiple phosphate transporter genes, eight of which have been isolated from barley alone. Two types of phosphate transporters were generally identified; low affinity transporters with constitutive expression and high affinity transporters whose expression is up regulated under P deficiency. Strategies for increasing nutrient uptake by over-expressing these genes are likely in situations where reasonable phosphate concentration can be maintained at the outer surface of the plasmalemma. Another possibility is that manipulating the expression of these genes might improve internal efficiency by mobilization of phosphate within the plant (Smith 2002). Exploring the rice genome for better alleles of these genes could also be useful for breeding.

Zinc deficiency
Zinc (Zn) deficiency is the most common nutrient problem for rice next to nitrogen and phosphorus, with as much as 50% of all lowland rice soils being affected (White and Zasoski 1999). Deficiency is normally

associated with continual soil wetness and occurs particularly in alkaline, organic and poorly drained soils (Yoshida et al. 1973) (Forno et al. 1975). It is also associated with high bicarbonate content and high levels of available phosphate and silica. The use of high levels of fertilizers with antagonistic effects on Zn availability and intensive cultivation of modern rice cultivars have exacerbated the deficiency problem over the past several years.

Zinc deficiency in rice occurs in the first few weeks after soil flooding. Surviving plants can then recover spontaneously within 6-8 wk, although the vegetative stage might be prolonged and yield is usually seriously affected. The mechanism of Zn solubilization in soils low in available Zn is similar to the mechanism of P solubilization, and results from acidification of the rhizosphere in the vicinity of roots by H^+ released from the roots or generated in the oxidation of iron by O_2 released by roots (Kirk and Bajita 1995).

In soils where Zn deficiency occurs as a consequence of Zn fixation rather than inherently low Zn, developing cultivars that use Zn more efficiently or possess the ability to solubilize soil bound Zn sources of low availability to plants is probably the most prudent solution, since Zn fertilizers are not widely available and expensive. Noticeable differences between rice cultivars in ability to extract Zn and grow under low Zn conditions have been observed (Neue 1994; Yang et al. 1994). There is no apparent yield cost associated with Zn-deficiency tolerance, and tolerant genotypes often show tolerance to both salinity and P deficiency, though the basis of this cross-tolerance is not known. However, despite this variability, no serious breeding programs have yet been initiated to incorporate this trait into elite breeding lines and varieties. Studies of the inheritance of this trait and unraveling of the physiological mechanisms underlying the observed genetic variation are prerequisites for a successful breeding program. Limited efforts are now underway to map this trait and to pave the way for further physiological and molecular exploration.

Conclusions and opportunities
Our knowledge of how rice genes respond to stress is increasing daily. Our understanding of how these changes translate into plant growth and crop level differences in performance under stress lags behind, and will require a focused effort at synthesis in order to convert the exciting results of genomics into tools and guidelines that plant breeders can use. The most direct application will be in marker-aided selection, though novel high-throughput trait-based screens for use in breeding programs may also emerge from this effort. Marker-assisted selection of progeny from crosses between tolerant, low-yielding cultivars and susceptible, high yield-potential lines theoretically allows for much greater efficiency in a breeding program, because extensive unreliable phenotypic screening can be eliminated, and linkage drag can be effectively reduced. In practice, the identification of suitable markers has been slowed by the low repeatability and precision of QTLs for abiotic stress tolerance. It is rare to identify a single QTL that accounts for most of the observed variation in a given cross and screening system. Nonetheless, exceptions such as *Sub1* and *Pup1* have been identified through a combination of appropriate parental crosses, careful phenotyping, and dedicated fine-mapping studies.

Future progress may be more rapid if we can effectively use advances in genomics. For example, the costly and time-consuming process of fine-mapping may be circumvented by considering the stress-responsive candidate genes that underlie a given QTL (Ishimaru et al. 2004; Wayne and McIntyre 2002). A strong putative candidate region can be used directly in breeding, however, even if final gene identity is not known, as long as its position is confirmed through association with phenotype in mapping populations (Thorup et al. 2000, Ramalingam et al. 2003). In this case, the actual identity of the candidate is not confirmed, but there a breeder may have sufficient confidence to use a tightly linked marker as a selection tool. The final validation of a specific candidate gene will require additional steps such as transformation or the evaluation of targeted knock-out mutants (Glazier et al. 2002). Knowledge of gene identity is required for the generation of gene-based molecular markers and to justify the search for allelic variants of the gene using molecular techniques or tightly targeted phenotypic screens.

One intriguing aspect of the information emerging from genomic studies of abiotic stress response is the large number of genes that respond to multiple stresses, both biotic and abiotic. These can be roughly grouped into those related to initial stress perception, those that modify processes in response to the signal, and the downstream results of that modification, with some overlap between groups (Figure 1).

Various studies have demonstrated that common genes are activated by such diverse stresses as wounding, pathogen attack, salt stress, and high temperature in both Arabidopsis (Cheong et al. 2002) and rice (Rabbani et al. 2003). Protein kinases (Agrawal et al. 2003), transcription factors (Dubouzet et al. 2003), and genes associated with hormone metabolism (Chen et al. 2002) particularly show this non-specific response. These genes may also be important in development (Cooper et al. 2003). Such genes that act early in the stress sensing and transduction processes appear to be finely tuned to allow responses to a variety of stresses, some of which act antagonistically (Xiong and Yang 2003). While it is tempting to search for superior alleles of such upstream master switches, these very upstream genes may not be appropriate targets for modification in improving abiotic stress tolerance, because they may restrict the ability of the plant to respond to other environmental challenges or to the combined stresses that characterize real environments. On the other hand, a rice cultivar tolerant to salt shock differed from its susceptible counterpart in its rapid response to stress imposition, suggesting that the immediacy of response to early upstream changes may also have adaptive significance (Kawasaki et al. 2001). Stress-responsive genes that change expression primarily as a result of damage are probably not good targets for crop improvement. The challenge before us, then, is to identify those genes underlying responses in the middle group (Figure 1) that respond to the primary stress signal with an adaptive response that is itself compatible with yield, such as the maintenance of plant hydraulic conductance and seed development under drought, sequestration of toxic ions under salt stress, or the repression of ethylene synthesis under submergence. Once such genes and processes are identified, the search for improved allelic forms among global genebanks and wild relatives can begin in earnest.

Table 1. Selected traits related to abiotic stress tolerance that have been mapped on the rice genome through QTL studies

Trait	Measurement details	Reference
Leaf rolling/drying under drought	Scored for field-grown plants at vegetative stage	Courtois et al. 2000; Price et al. 2002b
Root architecture (constitutive)	Root thickness, maximim root length, root weight or length distribution measured for plants grown in soil in containers	Courtois et al. 2003; Kamoshita et al. 2002; Price et al. 2002a; Venuprasad et al. 2002; Zheng et al. 2003
Root penetration	Percentage of roots that penetrate a physical barrier for plants grown in soil in containers	Ali et al. 2000; Ray et al. 1996; Zheng et al. 2000
Membrane stability under drought	Leaf segments collected from plants stressed to 60% RWC, membrane stability based on electrical conductance of solution	Tripathy et al. 2000
Osmotic adjustment under drought	Plants stressed to 60% RWC in soil-filled pots	Lilley et al. 1996; Robin et al. 2003
Height and heading date under drought	Measured in multiple field locations, some with drought	Li et al. 2003b
Yield and yield components under drought	Lines grown in the field with managed drought stress	Babu et al. 2003; Lafitte et al. 2004; Lanceras et al. 2004
Submergence tolerance	Seedling survival after submergence	Nandi et al. 1997; Xu et al. 2000
Salinity tolerance	Seedling growth with high salinity; ion accumulation in seedlings	Bonilla et al. 2002; Koyama et al. 2001; Lin et al. 2004; Prasad et al. 2000
Tolerance to phosphorus deficiency	Plant growth in low-P field	Wissuwa et al. 1998

Table 2. Candidate genes for drought tolerance with effect confirmed through transformation studies in rice and/or supported by coincidence with QTL region

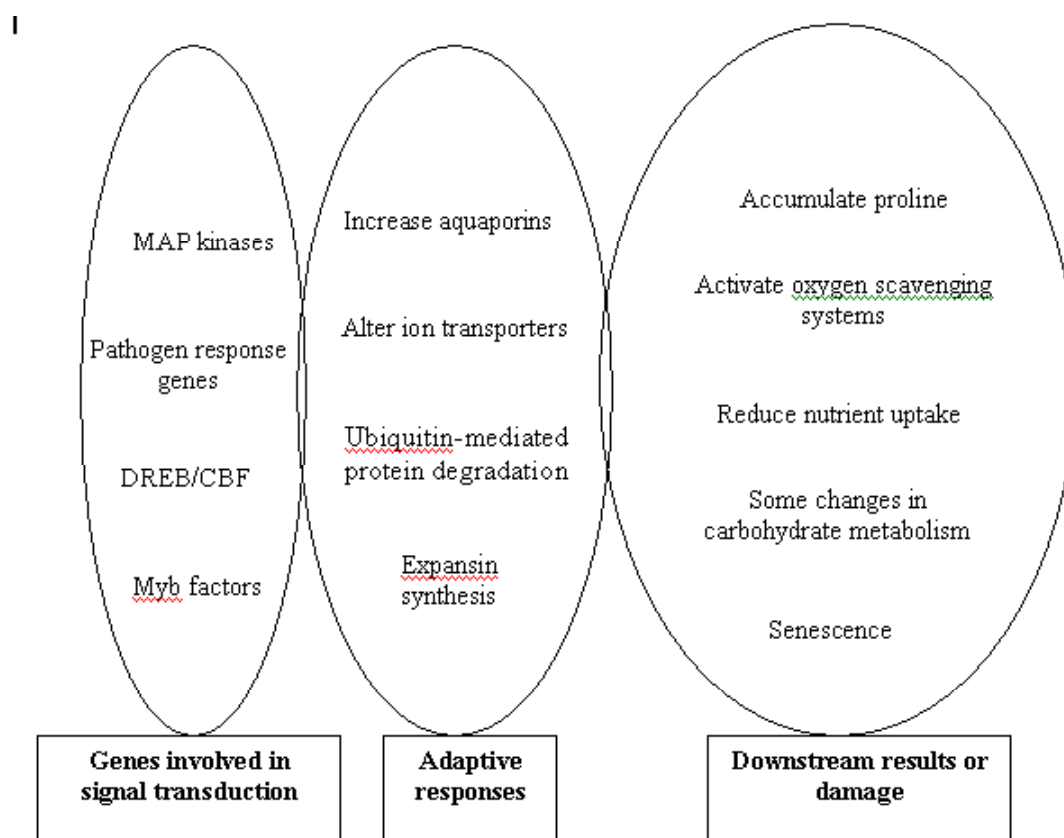| Gene	Function	Confirmation of effect in transgenic/ QTL results	Reference
Trehalose-6-phosphate synthase and phosphatase	Development and meristem growth (van Dijken et al. 2004)	Recovery of leaves after severe dehydration	Lee et al. 2003
"		Leaf wilting and growth in 5-wk old plants in pots subjected to two 4-d drying cycles	Garg et al. 2002
LEA proteins	Membrane stabilization (Koag et al. 2003)	Favorable water status and superior growth during gradual stress in soil-filled pots	Babu et al. 2004
Aquaporin	Water channel activity (Javot and Maurel 2002)	Higher hydraulic conductance and water potential in seedlings under PEG stress	Lian et al. 2004
Expansin	Regulate cell wall expansion (Li et al. 2003a)	Taller than wild type under normal conditions	Choi et al. 2003
"		OsEXP2 mapped to same interval as QTL for seminal root length	Zheng et al. 2003
Arginine decarboxylase (adc)	Produces putrescine, which may have protective role	Wilting and rolling of leaves with PEG treatment	Capell et al. 2004

Figure 1. Conceptual grouping of plant responses to stress into gene activation for upstream signal transduction, adaptive responses that allow the plant to change and moderate or survive the stress, and downstream responses that result from successful adaptation or unsuccessful responses (damage). Some examples are shown for each group. Allelic variation in genes that underlie the adaptive responses should be useful in breeding programs for improved stress tolerance.

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