

Abiotic stress tolerance in tropical rice: progress and future prospects

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ABSTRACT

Abiotic stresses limit rice production across the rainfed environments, which comprise about 45% of the global rice area. Important stresses for lowland rainfed rice include submergence and salinity, while upland rice is subject to P deficiency, and water deficit. In recent years, advances in physiology, molecular biology, and genetics have greatly improved our understanding of response of rice plant to these stresses and the basis of varietal differences to tolerance. Rice has been used as a model species in molecular and genomic studies. 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. Either molecular approaches or trait-specific physiological screenings can be used to search for these superior alleles. Marker-assisted backcrossing can then be applied to incorporate these alleles into agronomically superior germplasm. Here we review progress in applying molecular approaches to explore genetic variation in rice for tolerance to selected abiotic stresses.

Key words: *Oryza sativa*, drought, flooding, submergence, salinity, phosphorus, QTL, gene expression

Rice is an anomaly among the domesticated cereals - a tropical C₃ 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 to salinity and soil acidity, but is highly sensitive to drought and cold. Even where rice response to stress is superior to other crops, many rice-growing environments demand still greater tolerance than normally 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 salinity in coastal regions. Rice is grown in unflooded soils in the humid tropics where the crop is affected by water deficit, soil acidity, and P deficiency. 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.

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

and tolerance to abiotic stresses.

The objectives of this paper are to briefly review recent efforts to better understand the rice adaptation to abiotic stresses common in tropical rainfed rice and to highlight efforts to integrate results of advances in physiology and molecular biology into rice breeding programmes. Two stresses, drought and low P availability, are addressed with a primary focus on upland rice, while salinity and submergence are discussed with an emphasis on rain fed lowland rice areas.

Water deficit. Drought is a consistent feature across much of the 63.5 million hectares of rain fed 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 for centuries, and there is a wealth of genetic variation for response to water deficit among traditional cultivars (Mackill *et al.*, 1996). There are a 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 degree of 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).

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 and Xu 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 Comtois, 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 (lowland) 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 osmotic adjustment and 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, partly 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 of root system architecture and its associated tillering habit that allows maintenance of more favourable 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.*, 2004). The mechanisms underlying genetic variation in both constitutive and adaptive root distribution may be sensitive 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 days before heading, flowering is slowed mainly due to slower elongation of the panicle and supporting tissues. Genetic variation in delayed flowering 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 heading, resulting in inviable pollen and poor anther dehiscence. Genetic variation for pollen formation has been observed for this process independent of plant water status (Liu *et al.*, 2005). 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 which maintain better shoot water potential have an advantage.

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 (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). To date, 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 (Li *et al.*, 2005). Those studies indicate that while some QTL appear to be important for performance under drought in both upland and lowland environments, others are specific to one or the other.

Many studies reported changes in the expression of individual genes when rice is exposed to 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 (Martre *et al.*, 2002), signaling (Saijo *et al.*, 2001; Liu *et al.*, 2003), membrane integrity (Xu *et al.*, 1996; Rohila *et al.*, 2002; Babu *et al.*, 2004), carbohydrate metabolism and a transcription factor (Oh *et al.*, 2005).

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 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.*, 2005). In addition to direct effects of drought on gene expression, water deficit affects post-translational modifications such as protein phosphorylation. Advances in proteomics and metabolomics provide opportunities to follow these changes as well (Koller *et al.*, 2002; Ellis *et al.*, 2002; Nikiforova *et al.*, 2005).

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

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

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

Phosphorus deficiency. Insufficient available soil phosphorus is a major constraint for rice production. This is particularly apparent under upland conditions, those 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 soil under intensive rice production (De Datta *et al.*, 1990). Some rice soils can quickly fix up to 90% of the added P fertilizer into less soluble forms. 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, 2001a) 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 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 Fe²⁺ by root-released O₂ (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 Ae, 2001a) indicated the need for such breeding programmes 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).

Attempts have been made to detect QTLs controlling P-deficiency tolerance in rice, and four QTLs were 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 Ae, 2001 b). 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. This QTL appears to have limited effect in flooded soils. The availability of 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.

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 was 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.

Excess water. 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⁻¹ (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 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 days; however, a few tolerant cultivars can withstand complete submergence for 10 to 14 days, such as 'FRI3A', 'FRI3B', '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 check in screening trials. Breeding to further improve tolerance to submergence in rice along with yield potential has been going on 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 and Xu, 1996; Mackill *et al.*, 1993), and some breeding lines such as 'TR49830-T 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 ('TR4093 1-26') and a susceptible *japonica* line (P1543851) 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 'FR 13A' 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 to 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 appear. 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. 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.*, 2005). 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 (Panda *et al.*, 2006). The potential of these traits to provide a reliable phenotyping 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 O₂ supply and access to CO₂ 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).

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 elongates only with rising water and yield more.

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 relatively sensitive to salt stress, 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 to 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 regulation 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). 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 F₂ 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 '*Saitol*' 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 (*AtNHXI*) from Arabidopsis 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 Arabidopsis (*AtHKT 1*) 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 cOITelated 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).

Candidate genes for stress tolerance. 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 pervasiveness 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 Sub 1 and Pup 1 have been identified through a combination of appropriate parental crosses, careful phenotyping, and dedicated fine-mapping studies. Such reliable QTLs can be used directly in breeding, even if final gene identity is not known, as long as a tightly linked marker is available (Thorup *et al.*, 2000; Ramalingam *et al.*, 2003).

Future progress may be more rapid if we can effectively use advances in genomics to identify the genes that underlie QTLs. For example, the costly and time-consuming process of fine-mapping may be circumvented by identifying stress-responsive candidate genes (Ishimaru *et al.*, 2004; Wayne and McIntyre, 2002). The 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 (Fig.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 (Rabhani *et al.*, 2003; Koag, Dohi *et al.*, 2004). 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. 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 (Fig. 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 prolonged submergence. In order to shift through the many stress-responsive genes in this category to identify those where allelic variation is

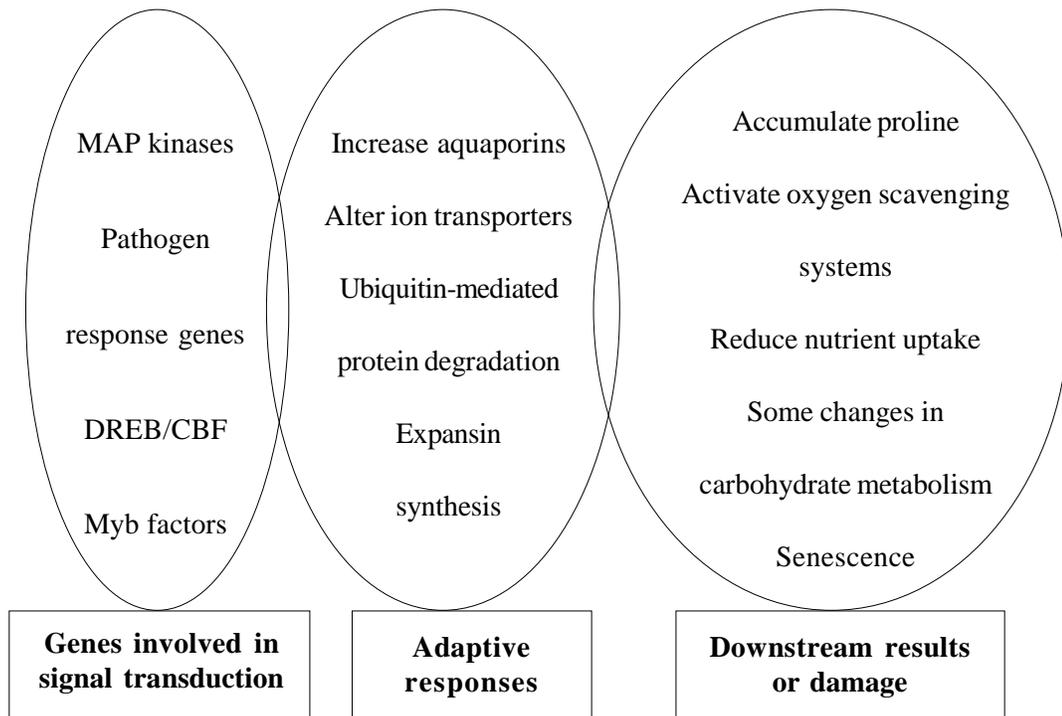


Fig. 1. Conceptual grouping of plant stress response into gene activation.

directly related to variation in performance, the wealth of existing QTL data from abiotic stress experiments can be combined with positional information from the rice physical sequence, as has been successfully undertaken for disease response (Ramalingam *et al.*, 2003). Once such genes and processes are identified, the search for improved allelic forms among global gene banks and wild relatives can begin in earnest.

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