REVIEW ARTICLE

Physiological bases of tolerance of abiotic stresses in rice and mechanisms of adaptation

Abdelbagi M Ismail, John Damien Platten and Berta Miro
International Rice Research Institute, DAPO 7777, Metro Manila, Philippines
Email: a.ismail@irri.org

ABSTRACT

Rice is grown over a wide range of climatic and edaphic conditions. The large gap in the productivity of rainfed ecosystems compared with irrigated ecosystem is largely caused by abiotic stresses such as drought, floods, and poor soils, coupled with the lack of well-adapted high-yielding varieties. Substantial progress has been made in unravelling the physiological and molecular bases of tolerance, however, progress in developing high yielding, tolerant varieties has been slow because of the undesirable phenotypes of the tolerant donors and the complexity of the tolerance mechanisms. Flash-floods normally affect rice during vegetative stage, however, in areas where direct seeding is practiced, heavy rains immediately after seeding cause severe damage to germinating seeds, resulting in poor crop stand. Complete submergence annually affects about 16 million ha of rice in South and Southeast Asia, and about one third of the total rice growing area in Africa. Salinity is particularly troubling in coastal regions in the tropics because of marine influence and build up of salt in some inlands as a consequence of excessive irrigation with improper drainage. Mechanism of adaptation of rice to flooded condition at different stages of crop growth and adaptation to salt stress have been reviewed in the present paper.

***Key words:** rice, abiotic stress, tolerance, mechanism*

INTRODUCTION

Rice is the staple food for more than half of the world population and is grown over a wide range of climatic and edaphic conditions. Its geographical distribution is largely determined by temperature and water availability. Rice is now grown on over 144 million hectares worldwide, from 50°N in northern China to 35°S in Australia, and in Argentina. It is also grown from 3 m below sea level in Kerala, India, to as high as 3000 m in Nepal and Bhutan. Rice is also grown on a variety of soils and under variable water regimes and hydrologic conditions, ranging from aerobic soils in uplands, to flooded soils in irrigated and rainfed lowlands, and to long-duration inundated conditions in flood-prone areas (Maclean *et al*., 2002). This enormous plasticity in adaptation provided rich genetic resources for improvements to cope with different weather and soil adversities. However, this diversity is mainly limited to old varieties and landraces being selected by farmers for over centuries, making them adaptable to various ecologies and less favourable growing conditions. Unfortunately, this diversity and plastic adaptability had not been sufficiently bread into modern high yielding rice varieties, largely limiting their distribution to more favourable areas because of their sensitivity to various abiotic stresses.

The large gap in the productivity of rainfed ecosystems compared with irrigated ecosystem is largely caused by abiotic stresses such as drought, floods, and poor soils, coupled with the lack of well-adapted high-yielding varieties. Efforts to identify landraces with sufficient tolerance of these abiotic stresses were initiated by IRRI and a few national programs in Asia during the 1960s and 1970s. As a result of these efforts, genotypes with considerable tolerance of major abiotic stresses were identified. Substantial progress was also made in unravelling the physiological and molecular bases of tolerance, however, progress in developing high yielding, tolerant varieties has been slow because of the undesirable phenotypes of the tolerant donors and the complexity of the tolerance mechanisms. Fortunately, major QTLs associated with tolerance were recently identified for most of the major abiotic stresses and some of them were cloned and deployed through molecular breeding (Thomson *et al*., 2010; Mackill *et al*., 2012; Ismail *et al*., 2013). Transferring tolerance traits into high-yield backgrounds will help boost and sustain the productivity in these unfavourable areas, and this is becoming more feasible with the recent availability of effective genomics and molecular breeding tools that are being used to dissect and transfer favourable alleles, providing opportunities to develop resilient varieties for less productive environments, as those affected by major stresses like drought, flooding and salinity. Varieties suited to these areas will also provide opportunities for better land productivity by being more responsive to inputs and adjustments in cropping systems, helping farmers to cope with current problems and future challenges caused by climate change. Here we summarize major adaptive traits for flood-prone and salt affected areas.

Adaptation of rice to flooded conditions

Rainfed lowland and flood-prone rice areas in Asia cover about 47 million ha or about 35% of the total global rice area. These areas usually experience variable types of excess water stress caused by various factors such as direct heavy rains and flooding from adjacent rivers, resulting in drastic reductions in productivity, usually with yields ranging between 0.5 to 2.0 t ha^{-1}, and are unstable. Flash floods for relatively short durations, commonly ranging from a few days to over two weeks are frequent. Besides, longer-term stagnant floods (SF, medium-deep or semi-deep) of 30–50-cm water depth can occur through most of the season, sometimes preceding or succeeding a flash flood where its impacts are usual more devastating. In deepwater areas, water at depth of over 50 cm to a few meters stagnates in the field, mostly for few months. The depth of water in some of these areas can exceed 4 m as in floating-rice areas. Apparently each of these types of floods requires specific adaptive traits, which necessitates the development of unique varieties (Lafitte *et al*., 2006; Sarkar *et al*., 2006).

Submergence during germination and early seedling stage

Flash-floods normally affect rice during vegetative stage, however, in areas where direct seeding is practiced, heavy rains immediately after seeding cause severe damage to germinating seeds, resulting in poor crop stand. This submergence during germination (also called anaerobic germination, AG) can occur in both rainfed and irrigated lowlands. Damage to direct seeded rice in irrigated areas can happen when lands are not well levelled, or when floodwater is used for weed suppression. In all cases, poor crop establishment results in severe yield losses. Rice varieties tolerant of flooding during germination have been identified and characterized (Angaji *et al*., 2010, Ismail *et al*., 2009). Some of the characteristics associated with tolerance include the ability to maintain carbohydrate catabolism, coleoptile elongation, anaerobic respiration and maintenance of cellular extensibility of the growing embryo in flooded soils (Ismail *et al*., 2009). As the seedling elongates to more aerated zones, it develops aerenchyma tissue or lacunae through which oxygen is provided to submerged plant parts including roots. Aeration of submerged parts also allows progressive detoxification of oxygen radicals in seeds and other toxins that develop in anoxic soils, preventing further injury.

Tolerant rice varieties like Khao Hlan On, Ma Zhan Red and Khaiyan were identified and QTLs conferring tolerance to germination in anaerobic soils were mapped. These tolerant varieties maintain higher á-amylase activity and higher soluble sugars, and deplete stored starch faster (Ismail *et al*., 2009). *RAmy3D* was found to be upregulated in tolerant varieties during germination in flooded soil. This gene is involved in degrading complex oligosaccharides and its regulation is independent of *RAmy1* and *RAmy2* gene families that are active under normoxia, but is activated under hypoxia (Ismail *et al*., 2012). *RAmy3D* together with other active amylases help maintain the supply of fermentable carbohydrates to provide energy for the growing embryo. Tolerant genotypes also show higher upregulation of the alcoholic fermentation pathway and its enzymes. The major enzymes are pyruvate decarboxylase (PDC), aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH), mostly involved in the quick detoxification of products such as acetaldehyde and ethanol. Differential upregulation of ALDH in tolerant genotypes is probably related to ethanol conversion to acetate to avoid the accumulation of acetaldehyde during reaeration (Meguro *et al.*, 2006). Moreover, the synthesis of acetate would also involve the recycling of carbon and its subsequent use in other pathways to maintain metabolism and energy generation to maintain growth under anaerobic conditions.

Submergence during vegetative stage

Complete submergence annually affects about 16 million ha of rice in South and Southeast Asia, and about one third of the total rice growing area in Africa. The effect of damage caused by transient submergence is dependent on the characteristics of flood waters, including temperature, turbidity, concentration of dissolved gases, and extent of light penetration (Das *et al.*, 2009). The main traits associated with tolerance of submergence are maintenance of high stem carbohydrates, optimum rates of alcoholic fermentation, aerenchyma formation, energy supply through underwater photosynthesis, and root aeration (Sarkar *et al.*, 2006; Colmer *et al.*, 2013; Winkel *et al.*, 2013). Major genetic improvements have taken place in recent years, after cloning and characterization of the *SUB1-A* gene from FR13A, an Indian variety (Xu *et al.*, 2006; Bailey-Serres *et al.*, 2010). This gene has subsequently been introgressed into several popular varieties with a reported yield increase of 1 to 3.5 t ha^{-1} over non-tolerant varieties following submergence for 4-18 days (Mackill *et al.*, 2012, Ismail *et al.*, 2013). *SUB1* confers a state of quiescence, thus avoiding excessive stem elongation, decreasing carbohydrate depletion and halting chlorophyll degradation (Colmer *et al.*, 2013). The whole mechanism seems to be regulated uniquely by the *SUB1A* gene, an ERF factor, which is expressed only under submergence, causing reduction in ethylene synthesis and sensitivity, suppressing gibberellic, acid synthesis, and halting elongation. Moreover, only the *SUB1A-1* allele present in few indica landraces can confer tolerance, whereas varieties containing the S*UB1A-2* allele or lacking the *SUB1A* gene altogether, are sensitive as in most *indicas*, all japonicas and all rice wild relatives tested so far (Fukao *et al.* 2006; Xu *et al.* 2006; Bailey-Serres *et al.* 2010).

Mechanisms that rice adopts after desubmergence are also important, but not clear whether these are also regulated by the *SUB1A-1* allele. Post-submergence injury involves water deficits, where leaves of sensitive genotypes wilt immediately after submergence (Setter *et al.*, 2010). Besides, high concentrations of reactive oxygen species (ROS) and toxic oxidative products are usually generated when leaves are aerated just after submergence, which could result in substantial damage to leaves causing them to wither and die. Ability to detoxify these products or halt their synthesis is therefore, essential for survival and recovery, and tolerant genotypes were found to upregulate their scavenging mechanisms during, and immediately after submergence (reviewed in Colmer *et al.*, 2013). Complete submergence is worsening, with floods lasting over 20 days in some cases, and also increasing in frequency in recent years. More efforts are needed to characterize additional sources of tolerance to identify novel genes that can add to the tolerance provided by the *SUB1A* gene, to develop more resilient varieties.

Medium-deep and deepwater rice

Medium-deep (stagnant flooding, 25-50 cm) and deepwater (50 cm to over 1 m) flooding that persists over several weeks to few months also predominate in flood-prone areas, severely hindering rice productivity. Stagnant floods reduce tillering, decrease vegetative growth, and reduce grain yield and quality. Traditional varieties adapted to these areas usually survive stagnant floods and deepwater, due to a snorkel effect, which involves internode, and leaf sheath elongation to reach water surface, and circulate oxygen to aerate submerged leaves, and roots through aerenchyma tissue. Other important characteristics of rice varieties tolerant of partial flooding involve maintenance of photosynthesis underwater, extensive aerenchymatic system, and anoxia tolerance in roots (Colmer *et al.*, 2013). Varieties adapted to deepwater areas elongate fast to keep some of their leaves above water. Two genes, *SNORKEL1* and *SNORKEL2* have been identified to be responsible for internode elongation under submerged conditions (Hattori *et al.*, 2009). Internode elongation is finely regulated by ethylene and gibberellic acid in a manner opposite to that of the *SUB1A* gene. Tolerant genotypes also maintain high soluble sugar concentrations in their vegetative tissue. Apparently, carbohydrate metabolism seems to play important roles in tolerance of all types of floods in rice.

Adaptation of rice to salt stress

Salt stress is a major constraint for rice production because modern rice varieties are highly sensitive. Salinity is particularly troubling in coastal regions in the tropics because of marine influence, but also a worrisome in some inlands because of the build up of salt as a consequence of excessive irrigation with improper drainage or use of poor quality irrigation water (Ismail *et al*., 2008). Despite its high sensitivity to salt stress, rice is one of the few crops that can thrive on salt-affected soils because of its ability to grow well in standing water that can help leach salts from topsoil (Ismail *et al*., 2007). Farmers in salt affected areas still grow traditional landraces in spite of their low yield, long duration and poor grain quality. This is because some of these landraces possess remarkable tolerance to salt stress through complex physiological mechanisms, involving sodium exclusion, higher tissue tolerance, effective sequestration of toxic salts into older tissues and in roots, stomatal regulation, and effective antioxidant scavenging system, beside others (Ismail *et al*., 2007; Moradi and Ismail, 2007). Salt tolerance during seedling stage in rice do not correlate well with tolerance during reproductive stage, suggesting different sets of traits and genes are probably involved at each stage (Moradi *et al*., 2003). Despite this complexity, most salt-tolerant landraces posses a unique set of small number of mechanisms, suggesting the possibility of developing highly tolerant rice varieties through combining superior alleles of genes controlling most of the important traits.

Apparently, salinity is a deceptively simple trait. There are, at heart, just 3 possible causes of stress in plants under hyper-saline conditions: Na^+ toxicity, Cl^- toxicity and osmotic stress. Moreover, in the majority of plant species, salinity effects can be attributed primarily to Na^+ toxicity, with a secondary effect of osmotic stress. Cl^- toxicity effects do seem to play a role in some species, though less is known of this process (Tavakkoli *et al*., 2010; 2011), and some studies also implicated nutritional imbalances as one of the consequences of salt stress. Thus it would seem that dealing with salinity stress should be straightforward, both physiologically and genetically. Indeed, known physiological tolerance mechanisms directly address these two stress conditions; Na^+ toxicity and hyper-osmotic stress. However the means by which these different mechanisms were addressed varies widely, and no single mechanism appears to be effective in conferring a high level of tolerance in any single genetic background.

Direct effects of salinity stress are related to excesses of Na^+ ions, which result in a variety of toxicity effects. Many of these are probably related to its effect on protein structure. Na^+ is chemically very similar to K^+, but its smaller radius makes its charge density significantly higher. Enzymes in cells have evolved under particular ionic conditions, in which K^+ is the predominant monovalent cation; Na^+ concentrations are typically rather low in plant tissues. If Na^+ concentrations rise, Na^+ ions replace K^+ in complexes with amino acids in protein chains. However, the higher charge density on Na^+ pulls its interacting groups closer than is typical for K^+; this potentially alters the secondary and tertiary structures of the protein, altering its native function. Proteins can evolve to be active under higher Na^+ concentrations – but this would require alteration of most genes in the genome, and thus this biochemical tolerance is not practically achievable for most species. This tolerance is typically only seen in species adapted to, and living permanently in extremely highly saline conditions, such as the case with halophytic microbes (e.g. Tadeo *et al*., 2009). It is perhaps worth noting that many of these species then *require* high levels of Na^+ to grow, suggesting that Na^+ has become an essential nutrient for them, possibly by partially replacing K^+ in the cytosol.

In the absence of this true biochemical tolerance, plants must limit the amount of Na^+ that accumulates in the cytoplasm, thus protecting the majority of their metabolic processes. This is typically achieved in one of three ways (Table 1): i) uptake of Na^+ is restricted in some fashion, either at the root level or from active tissue, often called Na^+ exclusion; ii) Na^+ entering the cell at relatively higher concentration is tolerated (tissue tolerance); or iii) responses to salinity stress are altered (growth and developmental mechanisms). Na^+ exclusion mechanisms appear to be the most commonly encountered. These can be subdivided into two major categories: restriction of Na^+ uptake into roots, through either passive blocking of Na^+ uptake, and/or active extrusion of Na^+ from the roots, and exclusion of Na^+ from tissues in which it is most damaging, as in actively growing or functioning leaves and reproductive tissue. Na^+ uptake into the plant

Table 1. Major salinity tolerance mechanisms in plants

Category	Mechanism	Primary site of action	Advantages	Disadvantages
Na^+ exclusion	Block of Na^+ uptake	Root	Passive, highly effective	Cannot entirely prevent Na^+ entry
	Extrusion of Na^+ from roots	Root	Not directly saturable. directly addresses Na^+ toxicity throughout the plant.	Could lead to progressively worse osmotic stress under water-limited soil conditions. Energetically costly?
	Exclusion of Na^+ from leaves	Root, stem, sheath	Protects most sensitive tissue (leaves). Does not require extensive adaptation to saline conditions.	Easily saturated. Does not address toxicity in storage organs. Na remains in storage organs even if salinity stress is no longer perceived.
	Leaf-to-leaf compartmentation	Older leaves, stem	Protects youngest (photosynthetically active) leaves.	Older leaves gets saturated first and subsequently shed
Tissue tolerance	Vacuolar compartmentation	All tissues	Genetically simple. Potentially larger reservoir than Na^+ exclusion. Na^+ can be used as an osmoticum in the vacuole. stress is no longer perceived.	Saturable. Requires constant active pumping to maintain, even if salinity stress is no longer perceived.
Growth and developmental adaptations	Vigorous growth	Meristem, leaves		Effectiveness limited to low $[Na^+]$.
	Hormonal responses	Varies, may involve root/shoot communication	Quick response to sudden increase in salt stress	Does not directly produce tolerance; effectiveness is short-term.
	Oxidative stress tolerance	All tissues	Genetically simple. Direct protection from ROS generated by Na^+ toxicity.	Does not address other aspects of Na^+ toxicity.
Osmotic adaptation	Compatible solutes	Root, blade	Also effective in drought tolerance	Does not address Na^+ toxicity.

predominantly occurs passively, as may be expected, and is first regulated at the level of entry into the stele. The presence of the Casparian strip forces water uptake through symplastic channels, restricting passive entry of Na^+. This mechanism appears to be highly effective: estimates suggest over 90% of Na^+ in the root environment is prevented from entering the plant in this manner, even in species and varieties sensitive to salt (Munns, 2005). However, blocking Na^+ uptake also involves restricting the rate of water flow into the plant, which may possibly inhibit growth, photosynthesis and the uptake of nutrients. This is seen in some tolerant genotypes that close their stomata fairly quickly when exposed to high salt stress, but reopen them within 2-3 days (A. M. Ismail, unpublished)

Despite the effectiveness of the block imposed by the Casparian strip, a fraction of the water entering the plant is taken up through other pathways – possibly around areas that have breaks in the endodermis, such as root hairs, areas around emerging lateral roots and the root tip. This apoplastic bypass flow allows sufficient Na^+ to accumulate in plant tissues to concentrations that cause Na^+ toxicity. Perhaps due to its effectiveness, the Casparian block is not known to show significant variation within a species, and so other mechanisms are required to deal with the remaining Na^+ influx. One that has received a lot of attention is re-export of Na^+ that enters the roots. This is mediated fundamentally by the *SOS1* Na^+/H^+ antiporter (a member of the NHX family, see below), along with other members of its activation pathway (e.g. Zhu, 2003). Transgenic plants from a variety of species overexpressing *SOS1* genes display enhanced salinity tolerance (Fukuda *et al*., 2004, Wu *et al*., 2004, He *et al*., 2005, Wu *et al*., 2005, Rodriguez *et al*., 2008, Bayat *et al*., 2011). This mechanism has the advantages that it directly addresses the problem of Na^+ toxicity, it does so throughout the plant, and is not directly saturable; it is related to the level of *SOS1* activity, and not to the capacity of any storage compartment. However, this mechanism could potentially lead to progressively worse osmotic and salinity problems in soils where water is limiting, and is potentially energetically costly. To date no naturally-occurring variation has been attributed to homologues of *SOS1* or members of its signalling pathway, but like the block of apoplastic flow, this may indicate the importance of the pathway such that mutations adversely affecting it are not viable in natural populations.

In contrast to the situation with the mechanisms blocking Na^+ uptake, considerable natural variation has been identified in mechanisms that exclude Na^+ from the leaves or reproductive organs. This has principally been observed for the classical "Na^+ exclusion" trait, in both surveys of natural variation (Platten *et al*., 2013) and QTL studies (Husain *et al*., 2003, Ren *et al*., 2005); in both cases, variation has been attributed to differences in the expression or activity of the *HKT1;5* or *HKT1;4* genes. In both wheat and rice, these genes are expressed in xylem parenchyma cells, primarily in the root and stem (Ren *et al*., 2005, Platten *et al*., unpubl.). These genes encode plasma membrane-localised Na^+ transporters that are hypothesised to facilitate the uptake of Na^+ from the xylem fluid into xylem parenchyma cells, where it is stored. By so doing, they reduce the amount of Na^+ that reaches the leaves, leading to the Na^+ exclusion trait. Na^+ exclusion relies on root, stem, leaf sheath and to a lesser extent, leaf blade vascular parenchyma tissue, as a storage reservoir. Indeed root tissue of varieties that express this trait often have higher Na^+ concentrations than corresponding varieties that don't express the trait (Platten *et al*., 2013). Thus, while naturally-occurring variation in the trait identifies its importance, it nonetheless suffers the dual disadvantages that it is saturable under long-term stress, as these tissues cannot hold an infinite quantity of Na^+; and that Na^+ taken up is retained in the plant, so even if the externally-applied stress is transient, internal tissue stress continues.

Like the classical Na^+ exclusion trait, leaf-to-leaf compartmentation results in less Na^+ accumulating in the leaves, particularly in young and active tissue. In many species older leaves are shed as the plant ages, so sequestering Na^+ in these can protect the most valuable and active tissue with comparatively little cost. A large portion of this trait could be conferred by the same mechanism that confers classical Na^+ exclusion. For example, as *HKT1;5* sequesters Na^+ along the stem, the amount of Na^+ that reaches each successive leaf is progressively lower, leading to the observed phenotype of progressively lower Na^+ concentrations in younger leaves. Indeed, in a survey of naturally-occurring variation, the extent of leaf-to-leaf partitioning of Na^+ was significantly correlated with both the overall level of Na^+ in the leaves (Na^+ exclusion), and with allelic variation in *OsHKT1;5* (Platten *et al*., 2013). However the correlation was not complete, and there were lines that appeared to show significant levels of leaf-to-leaf compartmentation that could not be adequately explained by *OsHKT1;5* variation. There may thus be distinct genetic mechanisms that contribute to this phenotype, and it can be considered as a separate trait. As mentioned, natural variation seems to exist for this trait within the genus *Oryza*, but QTLs controlling it have yet to be identified.

Tissue tolerance traits are generally considered as distinct from Na^+ exclusion, and indeed whereas Na^+ exclusion traits aim to prevent Na^+ from entering specific tissues, tissue tolerance traits increase the ability of tissues to tolerate higher Na^+ concentrations. As mentioned, no non-halophytic species is known to have a true biochemical tolerance of high cytosolic Na^+ concentrations. Rather, plants sequester Na^+ in the vacuole, preventing its toxic effects on cytosolic metabolism. Sequestration is achieved through the activity of vacuolar Na^+/H^+ antiporters, the NHX family. Like *SOS1*, these antiporters utilise the H^+ concentration (pH) gradient across the vacuolar membrane to catalyse the active extrusion of Na^+ from the cytoplasm into the vacuole. The pH gradient is regenerated by the activity of membrane H^+ pumps such as the vacuolar H^+-pyrophosphatase AVP1 and the membrane H^+-ATPases (e.g. Blumwald *et al*., 2000, Gaxiola *et al*., 2002). Also like *SOS1*, the involvement of these antiporters in salinity tolerance is well supported through mutagenesis and transgenic experiments and is thought to be a prime contributing factor to tolerance in some species such as barley, but this have yet to be demonstrated genetically through QTL studies. Tissue tolerance traits potentially have a larger reservoir for sequestration of Na^+ compared to Na^+ exclusion traits, depending on the expression of the antiporters involved, but like Na^+ exclusion the trait is saturable, and might not result in alleviation of stress if the external stress is removed.

Growth and development tolerance traits do not specifically control Na^+ movements *per se*. Rather, they aim to prevent the adverse effects of Na^+ toxicity. Mechanisms in this category are diverse. For example, vigorous growth is thought to dilute incoming Na^+ by spreading it through a larger tissue volume, keeping concentrations within the physiologically-acceptable range (Yeo *et al*., 1990). Changes in hormonal responses may delay senescence, prolonging the period available for stress to be alleviated and allowing the plant to recover. Furthermore, enzymatic processes may alleviate some of the toxic effects of Na^+, such as the accumulation of reactive oxygen species. These mechanisms do not specifically confer tolerance of Na^+, but they may help in prolonging the length of time the plant survives salt stress, giving a greater chance of recovery if the stress is transient. These processes are mostly known from physiological studies of adaptation to salinity stress, but some examples of increased tolerance have also been shown in transgenic studies (Lu *et al*., 2007; Moriwaki *et al*., 2008; Luo *et al*., 2013). Their effectiveness seems mostly limited to short-term and/or low levels of salt stress, but it seems likely they could play an important role in enhancing the effectiveness of the primary tolerance mechanisms outlined above.

Thus even a cursory perusal of the salinity literature shows a wide variety of physiological and genetic mechanisms contributing to tolerance. No one mechanism seems to be a "magic bullet" able to combat long-term high levels of Na^+ influx, while maintaining high growth rate. It seems intuitively obvious that the above-mentioned mechanisms are not mutually exclusive, and in fact that they could display additive or even synergistic effects. This is an area that has received little attention, either from a physiological or a breeding perspective. However, it is worthy of note that highly tolerant varieties of crops such as rice invariably possess multiple QTLs for salinity tolerance, with 6 – 8 being found in some rice varieties such as Pokkali and Nona Bokra (e.g. Bonilla *et al*,. 2002; Lin *et al*., 2004; Thomson *et al*., 2010). It therefore, seems likely that while a single QTL/physiological mechanism is not sufficient to achieve high tolerance, combining multiple mechanisms may be well effective. Efforts to significantly improve salinity tolerance should therefore, aim to identify QTLs from donors displaying not just one physiological mechanism, but to identify multiple donors with contrasting and complementary mechanisms. Genetic studies have supported this contention and it appears possible to drive tolerance significantly beyond what is seen in any existing landrace (Platten *et al*., 2013; and personal observations).

REFERENCES

Angaji S, Septiningsih EM, Mackill DJ, Ismail AM 2010. QTLs associated with tolerance of anaerobic conditions during germination in rice (*Oryza sativa* L.). Euphytica 172: 159-168.

Bailey-Serres J, Fukao T, Ismail AM, Heuer S, Mackill DJ 2010. Submergence tolerant rice: SUB1's journey from landrace to modern cultivar. Rice 3: 138-147.

Bayat F, Shiran B, Belyaev DV 2011. Overexpression of *HvNHX2*, a vacuolar Na^+/H^+ antiporter gene from barley, improves salt tolerance in *Arabidopsis thaliana*. Australian Journal of Crop Science 5: 428–432.

Blumwald E, Aharon GS, Apse MP 2000. Sodium transport in plant cells. Biochimica et Biophysica Acta (BBA) - Biomembranes 1465: 140–151.

Bonilla P, Dvorak J, MacKill D, Deal K and Gregorio G 2002. RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. The Philippine Agricultural Scientist 85: 68–76.

Colmer TD, Armstrong W, Greenway H, Ismail AM, Kirk GJD, Atwell BJ 2013. Physiological mechanisms in flooding tolerance of rice: transient complete submergence and prolonged standing water between 0.3 to over 3 m. Progress in Botany, (in press).

Das KK, Panda D, Sarkar RK, Reddy JN, Ismail AM 2009. Submergence tolerance in relation to variable floodwater conditions in rice. Environmental and Experimental Botany 66: 425-434.

Fukao T, Xu K, Ronald PC, Bailey-Serres J 2006. A variable cluster of ethylene response factor –like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell 18: 2021-2034.

Fukuda A, Nakamura A, Tagiri A, Tanaka H, Miyao A, Hirochika H, Tanaka Y 2004. Function, intracellular localization and the importance in salt tolerance of a vacuolar Na^+/H^+ antiporter from rice. Plant and Cell Physiology 45: 146–159.

Gaxiola RA, Fink GR, Hirschi KD 2002. Genetic manipulation of vacuolar proton pumps and transporters. Plant Physiology 129: 967–973.

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu JZ, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M 2009. The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. Nature 460: 1026-1030.

He C, Yan J, Shen G, Fu L, Holaday AS, Auld D, Blumwald E, Zhang H 2005. Expression of an Arabidopsis vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field. Plant and Cell Physiology 46: 1848–1854.

Husain S, Munns R, Condon AG 2003. Effect of sodium exclusion trait on chlorophyll retention and growth of durum wheat in saline soil. Australian Journal of Agricultural Research 54: 589–597.

Ismail AM, Ella ES, Vergara GV, Mackill DJ 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). Annals of Botany 103: 197-209.

Ismail AM, Heuer S, Thomson MJ, Wissuwa M 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Molecular Biology 65: 547-570.

Ismail AM, Johnson DE, Ella ES, Vergara GV, Baltazar A M 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. Annals of Botany-PLANTS, pls019 Special Issue: Flooding Stress.

Ismail AM, Singh US, Singh S, Dar MB, Mackill DJ 2013. The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. Field Crops Res (in press) http://dx.doi.org/10.1016/j.fcr.2013.01.007.

Ismail AM, Thomson MJ, Singh RK, Gregorio GB, Mackill DJ 2008. Designing Rice Varieties Adapted to Coastal Areas of South and Southeast Asia. Journal of the Indian Society of Coastal Agricultural Research 26: 69-73.

Lafitte RH, Ismail AM, Bennett J 2006. Abiotic stress tolerance in tropical rice: progress and the future. Oryza 43: 171-186.

Lin HX, Zhu MZ, Yano M, Gao JP, Liang ZW, Su WA, Hu XH, Ren ZH, Chao DY 2004. QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theoretische Und Angewandte Genetik 108: 253–260.

Lu ZQ, Liu DL, Liu SK 2007. Two rice cytosolic ascorbate peroxidases differentially improve salt tolerance in transgenic Arabidopsis. Plant Cell Reports 26: 1909–1917.

Luo X, Wu J, Li Y, Nan Z, Guo X, Wang Y, Zhang A, Wang Z, Xia G, Tian Y 2013. Synergistic effects of *GhSOD1* and *GhCAT1* overexpression in cotton chloroplasts on enhancing tolerance to methyl viologen and salt stresses. PLoS ONE 8: e54002.

Maclean J L, Dawe DC, Hardy B, Hettel GP 2002. Rice Almanac. Los Banos (Philippines): International Rice Research Institute, pp 16-24.

Mackill DJ, Ismail AM, Singh US, Labios RV, Paris TR 2012. Development and rapid adoption of submergence-tolerant (Sub1) rice cultivars. Advances in Agron 115: 299-352.

Meguro N, Tsuji H, Tsutsumi N, Nakazono M and Hirai A 2006. Involvement of aldehyde dehydrogenase in alleviation of post-anoxic injury in rice. In: Abiotic Stress Tolerance in Plants, 111-119. Eds. Ashwani K. Rai and Teruhiro Takabe. Springer, Netherlands.

Moradi F, Ismail AM, Gregorio GB, Egdane JA 2003. Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. Indian Journal of Plant Physiology 8: 105-116.

Moriwaki T, Yamamoto Y, Aida T, Funahashi T, Shishido T, Asada M, Prodhan S, Komamine A, Motohashi T 2008. Overexpression of the *Escherichia coli* catalase gene, *katE*, enhances tolerance to salinity stress in the transgenic indica rice cultivar, BR5. Plant Biotechnology Reports 2: 41–46.

Munns R 2005. Genes and salt tolerance: bringing them together. New Phytologist 167: 645–663.

Platten JD, Egdane J, Ismail AM 2013. Salinity tolerance, Na^+ exclusion and allele mining of *HKT1;5* in *Oryza sativa* and *O. glaberrima*: many sources, many genes, one mechanism? BMC Plant Biology (in press).

Ren Z-H, Gao J-P, Li L-G, Cai X-L, Huang W, Chao D-Y, Zhu M-Z, Wang Z-Y, Luan S, Lin H-X 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genetics 37: 1141–1146.

Rodriguez-Rosales MP, Jiang X, Galvez FJ, Aranda MN, Cubero B, Venema K 2008. Overexpression of the tomato K^+/H^+ antiporter *LeNHX2* confers salt tolerance by improving potassium compartmentalization. New Phytologist 179: 366–377.

Sarkar RK, Reddy JN, Sharma SG, Ismail AM 2006. Physiological basis of submergence tolerance in rice and implications for crop improvement. Current Sciences. 91: 899-906.

Setter TL, Bhekasut P, Greenway H 2010. Desiccation of leaves after de-submergence is one cause for intolerance to complete submergence of the rice cultivar IR 42. Functional Plant Biology 37:1096-1104

Tadeo X, López-Méndez B, Trigueros T, Laín A, Castaño D, Millet O 2009. Structural basis for the amino acid composition of proteins from halophilic archea. PLoS Biology 7: e1000257.

Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald GK 2011. Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. Journal of Experimental Botany 62: 2189–2203.

Tavakkoli E, Rengasamy P, McDonald GK 2010. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. Journal of Experimental Botany 61: 4449–4459.

Thomson MJ, de Ocampo M, Egdane J, Rahman MA, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumwald E, Seraj ZI, Singh RK, Gregorio GB, Ismail AM 2010. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice 3: 148–160.

Winkel A, Colmer TD, Ismail AM, Pedersen O 2013. Internal aeration of paddy field rice (*Oryza sativa*) during complete submergence - importance of light and floodwater O_2. New Phytologist 197: 1193–1203.

Wu CA, Yang GD, Meng QW, Zheng CC 2004. The cotton GhNHX1 gene encoding a novel putative tonoplast Na^+/H^+ antiporter plays an important role in salt stress. Plant and Cell Physiology 45: 600–607.

Wu YY, Chen Q-J, Chen M, Chen J, Wang X-C 2005. Salt-tolerant transgenic perennial ryegrass (*Lolium perenne* L.) obtained by *Agrobacterium tumefaciens*-mediated transformation of the vacuolar Na^+/H^+ antiporter gene. Plant Science 169: 65–73.

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey Serres J, Ronald PC, Mackill DJ 2006. Sub1A is an ethylene-responsive –factor-like gene that confers submergence tolerance to rice. Nature 442: 705-708.

Yeo AR, Yeo ME, Flowers SA, Flowers TJ 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. Theoretische Und Angewandte Genetik 79: 377–384.

Zhu J-K 2003. Regulation of ion homeostasis under salt stress. Current Opinion in Plant Biology 6: 441–445.