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·Invited Review·

Understanding Abiotic Stress Tolerance Mechanisms: Recent Studies on Stress Response in Rice

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Abstract

Abiotic stress is the main factor negatively affecting crop growth and productivity worldwide. The advances in physiology, genetics, and molecular biology have greatly improved our understanding of plant responses to stresses. Rice plants are sensitive to various abiotic stresses. In this short review, we present recent progresses in adaptation of rice to salinity, water deficit and submergence. Many studies show that salt tolerance is tightly associated with the ability to maintain ion homeostasis under salinity. Na^+ transporter SKC1 unloads Na^+ from xylem, plasma membrane Na^+/H^+ antiporter SOS1 excludes sodium out of cytosol and tonoplast Na^+/H^+ antiporter NHX1 sequesters Na^+ into the vacuole. Silicon deposition in exodermis and endodermis of rice root reduces sodium transport through the apoplastic pathway. A number of transcription factors regulate stress-inducible gene expression that leads to initiating stress responses and establishing plant stress tolerance. Overexpression of some transcription factors, including DREB/CBF and NAC, enhances salt, drought, and cold tolerance in rice. A variant of one of ERF family genes, *Sub1A-1*, confers immersion tolerance to lowland rice. These findings and their exploitation will hold promise for engineering breeding to protect crop plants from certain abiotic stresses.

Key words: abiotic stress; sodium transport; transcription factor; submergence tolerance; rice.

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Abiotic stresses are believed to cause major problems in agriculture by reducing crop growth and productivity. Because of their sessile nature, plants must endure adverse environmental conditions and consequently evolve a variety of responses to acclimatize to environmental stresses. During evolution, plants have developed sophisticated mechanisms to sense the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress-responsive genes and ultimately lead to changes at the physiological and biochemical levels.

Rice, a staple crop for over half of the world's population, is sensitive to a variety of abiotic stresses, including salinity, drought, submersion and cold (Lafitte et al. 2004). Researchers from all over the world have made great efforts in understanding the mechanisms of responses to abiotic stresses in rice. Here we review some recent studies of responses to abiotic stresses in rice. A greater understanding of the physiology and molecular biology of stress tolerance may provide a useful platform to improve stress-tolerant rice varieties.

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Sodium Transport and Salt Tolerance

Rice plants are relatively susceptible to soil salinity, and NaCl is a major salt that causes this problem (Flowers 2004). In general, electrical conductivity of saturated paste extract (EC_e) of 4.0 deciSiemens per meter (dS/m) is considered a lower limit to define saline soils. Rice and most grain crops are glycophytes that show stress symptoms and reduced yield even when the EC_e is lower than 4.0 dS/m (~40 mmol/L NaCl). The salinity threshold for rice is 3.0 dS/m with a 12% reduction in yield, per dS/m, beyond this threshold (Maas 1990).

High concentrations of Na^+ in soil have all kinds of adverse effects in plants, such as disruption of intracellular ion homeostasis, membrane dysfunction and inhibition of metabolic activity (Hasegawa et al. 2000), resulting in inhibition of growth and yield reduction. More knowledge about the regulation of Na^+ transport within whole plants and in specific cell types is required to fully elucidate plant salt tolerance.

Na^+ entry and the role of silicon

Sodium is a micronutrient in rice plants. Under salt stress, excessive Na^+ transport from roots to leaves affects seedling growth. Sodium in soil enters plant roots by two ways: the symplastic pathway mediated by cation channels/transporters, and the apoplastic pathway in which Na^+ enters the transpiration stream (Figure 1). The apoplastic pathway bypasses symplastic control over sodium transport by the transpirational bypass flow. Additionally, a major part of Na^+ influx into rice plants depends on leakage of the apoplastic pathway (Yadav et al. 1996; Garcia et al. 1997).

Previous research has suggested that the alleviation of salt stress by silicon in rice was due to the suppression of the apoplastic pathway (Yeo et al. 1999). Studies on salt-stressed rice plants have shown that silicon alleviates the reduction of growth and net photosynthesis, as well as reducing the Na^+ uptake and transpirational bypass flow. Since the stomatal conductance has increased, the reduction of sodium uptake could be due to a reduction in this bypass flow rather than the transpiration rate (Yeo et al. 1999).

Recently, extensive studies on the role of silicon under salt stress provided further understanding of this process (Gong et al. 2006). A supplement of 3 mmol/L silicate reduced the shoot Na^+ concentrations of rice plants treated with 50 mmol/L NaCl, resulting in improved shoot growth. In these plants, the transpirational bypass flow decreased dramatically from 4.2% to 0.8%, and the apparent Na^+ concentration in xylem decreased from 6.2 to 2.8 mmol/L, with no concomitant reduction in transpiration rate, suggesting that silicon reduced Na^+ loading to xylem. X-ray microanalysis of root transverse sections showed there was greater silicon deposition in the endodermis than in the rhizodermis and exodermis, co-localizing to sites of the silicon transporter *Lsi1* at the exodermis and endodermis (Ma et al. 2006). Silicon deposition restricted the movement of water and ions through the apoplast. Thus silicate reduced Na^+ uptake by blocking Na^+ influx through the apoplastic pathway (Gong et al. 2006).

Silicon is the second most abundant element in the Earth's crust, comprising 31% of its mass (Epstein 1999) and is also a major constituent of many plants. In rice, silicon can accumulate to levels of up to 10% of shoot dry weight, which is often several times higher than that of essential macronutrients (Savant et al. 1997). Silicon is beneficial to plant growth and helps plants to overcome biotic and abiotic stresses by improving resistance to pests and diseases, as well as salt and drought stresses (Epstein 1999; Richmond and Sussman 2003; Ma 2004). It has been reported that a role exists for silicon in the reduction of salt stress in many crop grasses including rice (Matoh et al. 1986), wheat (Ahmad et al. 1992), maize (Shu and Liu 2001) and barley (Liang et al. 2003). The identification of rice silicon transporter *Lsi1* (Ma et al. 2006) may facilitate further research aimed at elucidating the role of silicon in abiotic stresses.

Na^+ transport and K^+/Na^+ homeostasis

Sodium does not freely permeate the plasma membrane, as it enters the plant cell through transporters and nonselective cation channels. HKT (High-affinity K^+ Transporter) is a group of well-studied plant Na^+ transporters identified in several species (Platten et al. 2006). In wheat (Laurie et al. 2002), rice (Garciadeblás et al. 2003) and barley (Haro et al. 2005), it has been shown that HKT transporters are involved in root Na^+ uptake. High-affinity Na^+ uptake was found in K^+-starved seedlings of the aforementioned species. In low K^+ and Ca^{2+} concentrations, the rate of Na^+ uptake was very rapid and the K_m value was low, but high-affinity Na^+ uptake was sensitive to external K^+ (Garciadeblás et al. 2003). OsHKT2;1 (OsHKT1), a high-affinity Na^+ transporter, was localized to root epidermis and took part in rice Na^+ uptake (Horie et al. 2001; Golldack et al. 2002; Garciadeblás et al. 2003; Platten et al. 2006). In conditions of high Na^+ concentrations, Na^+ may be taken up ectopically by K^+ and other cation transporters. Nonselective cation channels are a variety of ion channels characterized by their low discrimination between cations, in particular Na^+ and K^+. They may mediate the entry of sodium into plant cells under salinity stress (Amtmann and Sanders 1999; Tester and Davenport 2003). In rice, however, the molecular identities and mechanisms of most of these transporters and channels are still unknown.

When absorbed by roots, inorganic ions are loaded into xylem vessels and transported to shoots through the transpiration stream. Any strategies to reduce shoot Na^+ content may contribute to plant salt-tolerance. When loaded into phloem

sieves via symplastic diffusion, Na^+ in shoots can return to roots (Alberts et al. 2002). This mechanism of Na^+ recirculation from shoots has been found in *Arabidopsis thaliana* (Berthomieu et al. 2003). AtHKT1;1 (AtHKT1), a Na^+ selective transporter (Uozumi et al. 2000; Mäser et al. 2002), mediates Na^+ loading into phloem sap in shoots and its unloading from phloem in roots. The defective mutant allele of *AtHKT1;1*, *sas2*, accumulates more Na^+ in shoots with lower Na^+ concentration in phloem sap, and *sas2* plants were sensitive to salt stress (Berthomieu et al. 2003). While reduction of Na^+ accumulation in leaves by phloem may be an important process in plant tolerance to salinity, no cases to date have been reported in rice.

A decrease in Na^+ transport from roots to shoots is crucial for salt tolerance. Through cloning and functional analysis of the QTL (quantitative trait locus), *SKC1* demonstrated recirculation of Na^+ by being unloaded directly from xylem sap in rice (Ren et al. 2005). SKC1, a HKT family member (OsHKT8/OsHKT1;5), was a Na^+ selective transporter identified in a salt-tolerant *indica* variety, Nona Bokra. The Na^+ transport activity of NSKC1 (from Nona Bokra) was higher than that of KSKC1 (from a salt-susceptible *japonica* variety, Koshihikari). Under salt stress, seedlings carrying NSKC1 exhibited more tolerance to salinity than those carrying KSKC1, resulting from greater Na^+ extraction from xylem sap by NSKC1. The xylem sap and shoot Na^+ content in NSKC1 seedlings was lower than that noted in KSKC1 seedlings, whereas the reverse was true for K^+ content. As a major QTL for shoot K^+ content (Lin et al. 2004), SKC1 is a key determinant to the maintenance of the K^+/Na^+ homeostasis under salt stress. Similar mechanisms were identified in *Arabidopsis* (Sunarpi et al. 2005). In *athkt1;1* loss-of-function mutants, the Na^+ content was increased in the xylem sap but reduced in the phloem sap. This suggested that in addition to the previously described function (Berthomieu et al. 2003), AtHKT1;1 also unloads Na^+ from xylem sap, similar to SKC1.

Potassium, an essential macro-element, is required for the maintenance of turgor and for enzyme activity in plant cells (Epstein 1972; Kochian and Lucas 1988). A high K^+/Na^+ ratio is important for salt tolerance. Plants absorb and transport K^+ through a large number of channels and transporters, which vary in kinetics, energy coupling and regulation (Véry and Sentenac 2003 and references therein). Some of their activities can be regulated by protein phosphorylation (Xu et al. 2006a). While 17 members of the KT-HAK-KUP transporter family have been identified in rice cultivar cv. Nipponbare (Bañuelos et al. 2002), counterparts of other families remain to be determined. Physiological research has demonstrated that K^+ transport is sensitive to Ca^{2+} (Epstein 1973; Zimmermann et al. 1999) but not to Si (Gong et al. 2006). Under salt stress, extracellular Na^+ inhibits root K^+ uptake (Epstein 1973; Fu and Luan 1998). Then distribution of K^+ in plants may be affected by ions,

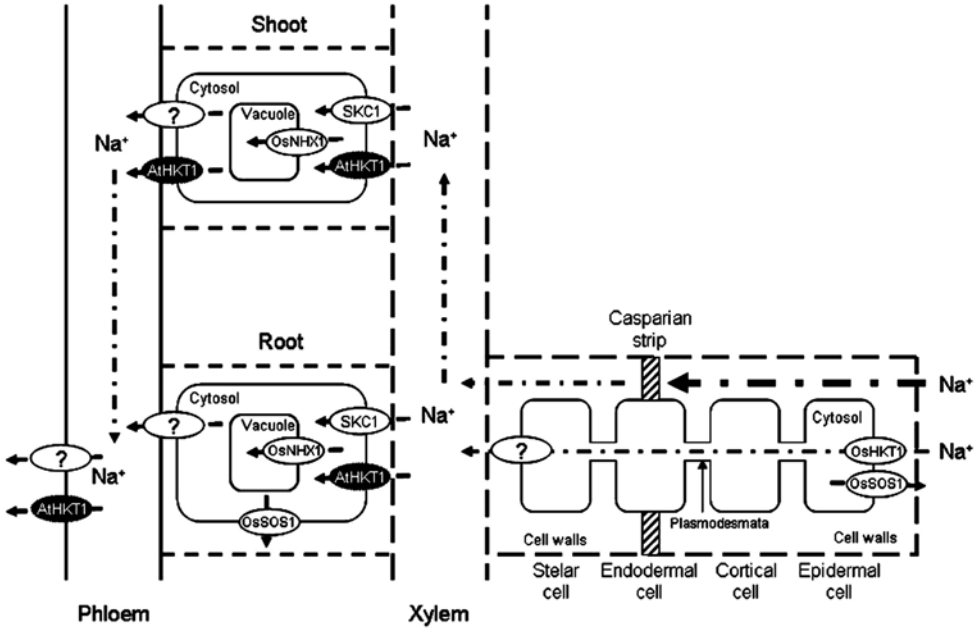

Figure 1. Model of Na^+ transport in rice plants.

Silicon deposition in the exodermis and endodermis reduces bypass flow. OsHKT2;1 (OsHKT1) mediates root Na^+ uptake. SKC1 retrieves Na^+ from xylem vessels into parenchyma cells. OsSOS1 transports Na^+ out of the cytosol and OsNHX1 sequesters Na^+ into vacuole. The roles of AtHKT1;1 (AtHKT1) (black ovals) have been combined suggesting possible roles of their counterparts in rice.

transporters and their regulators. Under salinity, SKC1 acts as the determinant that maintains shoot K^+ content in rice (Lin et al. 2004).

Na^+ efflux and compartmentation

Both Na^+ efflux and compartmentation contribute to lower cytosolic Na^+ concentrations and consequently, osmotic adjustment. In *Arabidopsis*, Na^+ efflux is mediated by SOS1, a plasma-membrane Na^+/H^+ antiporter. The SOS pathway has been extensively investigated. Perception of salt stress leads to a Ca^{2+} oscillation that activates the SOS3-SOS2 kinase complex. This activated kinase complex phosphorylates and activates SOS1 and AtNHX1, a tonoplast Na^+/H^+ antiporter (Zhu 2003 and references therein). SOS1 functions in long-distance Na^+ transport, between roots and shoots, by loading and unloading Na^+ from the xylem stream, as well as in root Na^+ efflux (Shi et al. 2002). Transgenic *Arabidopsis* plants overexpressing SOS1 have reduced Na^+ accumulation in xylem sap and shoot, and increased salt tolerance (Shi et al. 2003). Because the rice ortholog of *SOS1* has been identified (Mullan et al. 2007) and the rice SOS signaling pathway has been reconstituted in yeast (Martinez-Atienza et al. 2007), similar SOS pathway functions may exist in rice.

Vacuolar sequestration of Na^+ is catalyzed by AtNHX1 in *Arabidopsis* (Apse et al. 1999) and OsNHX1 in rice (Fukuda et al. 2004). The expression of the *NHX1* gene was up-regulated by salinity both in *Arabidopsis* (Shi and Zhu 2002) and rice (Fukuda et al. 1999). In the presence of 200 mmol/L NaCl, yield and fruit quality of transgenic tomato plants overexpressing *AtNHX1* were equivalent to wild-type plants grown in 5 mmol/L NaCl (Zhang and Blumwald 2001). The overexpression of *OsNHX1* in rice improved salt tolerance of the transgenic plants, without adverse effects on their Na^+ and K^+ contents and plant growth (Fukuda et al. 2004). This may be useful for genetic improvement of salt tolerance in rice.

Transcription Factors and Abiotic Stresses

Transcriptional regulation, also known as transcriptome reprogramming, is essential for plant adaptation to abiotic stresses. To date, multiple transcription factors required for transcriptome reprogramming under abiotic stresses have been identified and functionally analyzed. Among them, some have been well addressed in rice, for example, DREBs (dehydration-responsive element-binding protein) /CBFs (C-repeat-binding factor) and NACs (NAM, ATAF, and CUC).

DREBs/CBFs

The DRE (dehydration-responsive element)/CRT (C-Repeat) was identified as a *cis*-acting element regulating gene expression in response to dehydration (salt, drought, and cold stresses) in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki 1994). Transcription factors DREB1/CBF1-3, CBF4 and DREB2, belonging to the ERF/AP2 family, were reported to bind to DRE/CRT elements (Stockinger et al. 1997; Liu et al. 1998; Haake et al. 2002; Yamaguchi-Shinozaki and Shinozaki 2005). While three *DREB1/CBF1-3* genes, *DREB1A/CBF3, DREB1B/CBF1* and *DREB1C/CBF2,* were induced by cold, but not by drought or salt stress (Medina et al. 1999), and were consequently believed to regulate the expression of DRE/CRT genes under cold, *DREB2A* and *CBF4* were mainly responsive to drought and salt stresses (Liu et al. 1998; Haake et al. 2002). Interestingly, overexpression of *DREB1s* in *Arabidopsis* increased freezing, drought, and salt tolerance, however, overexpression of *DREB2A* in transgenic plants showed no increase in stress tolerance (Jaglo-Ottosen et al. 1998; Liu et al. 1998). These data suggest activation of DREB2A requires post-translational modification. Sakuma et al. (2006) found that the deletion of a region between residues 136 and 165 transformed DREB2A to a constitutively active form. Transgenic *Arabidopsis* plants expressing this active form exhibited improved drought tolerance, and slight freezing tolerance as well. It suggested the region between residues 136 and 165 of DREB2A is an inhibitory domain in the normal condition and is modified under salt/drought stress.

The functions of DREB orthologs have been demonstrated in rice. Dubouzet et al. (2003) identified five DREB cDNAs in rice: *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A*. Similar to their homologs in *Arabidopsis*, *OsDREB1A* and *OsDREB1B* were induced by cold, while *OsDREB2A* was regulated by salt and drought stress (Dubouzet et al. 2003). However, rice DREBs binding sites differed from their AtDREB counterparts as OsDREB1A showed much higher affinity binding to the DRE core sequence with GCCGAC than that with ACCGAC (Dubouzet et al. 2003), while AtDREBs bound equally to both sequences (Stockinger et al. 1997; Liu et al. 1998). Overexpression of OsDREB1A in *Arabidopsis* and rice induced expression of DRE/CRT genes (Dubouzet et al. 2003; Ito et al. 2006) and OsDREB1A overexpression lines showed phenotypes similar to AtDREB1A overexpression lines, with improved stress tolerance as well as growth retardation under normal growth conditions (Dubouzet et al. 2003; Ito et al. 2006). These results demonstrated that the DREB1/CBF pathway was conserved in monocotyledons and dicotyledons. Contrary to the growth inhibition observed in cv. Kita-ake and Nipponbare rice, overexpression of *Arabidopsis* DREB1A in rice cv. Nakdong enhanced tolerance to abiotic stress without inhibiting growth or causing phenotypic alterations (Oh et al. 2005). The discrepancies observed between the different studies may have resulted from genotype differences, as observed by comparative analysis of their transcriptomes (Ito et al. 2006).

NAC genes

The *NAC* gene family encodes one of the largest families of plant-specific transcription factors and has not been found in other eukaryotes. There are 75 and 105 putative *NAC* genes in rice and *Arabidopsis* genomes, respectively (Ooka et al. 2003). Genes in the *NAC* family were found mainly to be involved in regulating plant development (Olsen et al. 2005). Their roles in abiotic stresses were only discovered recently.

A salt- and drought-induced gene, *ERD1*, was regulated in an ABA-independent manner (Kiyosue et al. 1993; Nakashima et al. 1997). However, no DRE/CRT element was found in its promoter region, suggesting a novel regulatory pathway for drought and salt adaptation (Kiyosue et al. 1993; Nakashima et al. 1997). Promoter analysis showed that an MYC-like site was necessary for induction of *ERD1* (Simpson et al. 2003). The MYC-like sequence was recognized by three transcription factors of the NAC family, ANAC019, ANAC055, ANAC072, and was named NACRS (NAC recognition sequence) (Tran et al. 2004). Consistent with *ERD1* expression patterns, the three *NAC* genes were induced under salt and/or drought stress, but were not remarkably regulated by cold (Tran et al. 2004). In addition to *ERD1*, many other salt and/or drought stress-induced genes were also regulated by the three genes, and consistently, overexpression of the three genes greatly enhanced drought tolerance of *Arabidopsis* (Tran et al. 2004).

Rice may use a similar set of NAC transcription factors to regulation salt and/or drought responsive genes. Chao et al. (2005) found that multiple rice transcription factors, including a *NAC* gene, were induced in the early stage of salt stress. OsNAC6, a member of ATAF subfamily, was also induced by cold, salt, drought and abscisic acid (ABA) (Ohnishi et al. 2005). However, the precise functions of these rice NAC genes remain largely unknown. Recently, Hu et al. (2006) reported a NAC transcription factor significantly enhanced drought and salt tolerance in rice. The rice NAC gene *SNAC1* was up-regulated by drought and salt predominantly in guard cells. *SNAC1*-overexpressing rice plants showed greater sensitivity to ABA and increased stomatal closure to prevent water loss. Drought resistance in transgenic plants was significantly improved under field conditions at the stage of anthesis, without phenotypic changes or yield reduction. However, although SNAC also triggered a series of salt and/or drought responsive genes including *OsERD1*, differences were noted in the regulation controlled by SNAC compared to ANACs, as the former could not interact with NACRS in the *OsERD1* promoter region. These data, in conjunction with differences noted between DREB/CBF regulons in rice and *Arabidopsis*, suggested that stress-related regulation pathways further evolved after the divergence of monocotyledons and dicotyledons.

Other transcription factors

Although multiple transcription factors, including ICE (inducer of CBF expression), CBFs/DREBs, AREB/ABF/ABI/bZip, MYC/MYB and NACs, have been well characterized (Chinnusamy et al. 2004, 2006), we are far from fully understanding transcriptional reprogramming under salt/drought stress. It was estimated that about 8% of yeast genes were affected by salt stress (reviewed by Zhu 2002). If a similar percentage was assumed for rice, there would be about 4 000 genes responsive to salt stress. To date, hundreds of salt responsive genes have been identified in rice using high throughput technologies, such as microarray/genechip (Kawasaki et al. 2001; Rabbani et al. 2003; Chao et al. 2005; Wu et al. 2006). Although these numbers are small compared with the potential 4 000 genes, they cannot be fully explained by previously identified regulatory pathways. In *Arabidopsis*, a comparison of a transcriptome under cold and a CBF regulon revealed that only 12% of cold responsive genes were regulated by CBFs (Fowler and Thomashow 2002). In addition, even in the CBF regulon, a few of the genes did not display DRE/CRT elements in their promoter region. It has consequently been hypothesized that subregulons control those genes without a DRE/CRT element, given that some transcription factors with DRE/CRTs in their promoters, for example *RAP2.1*, were represented in the CBF regulon (Fowler and Thomashow 2002). A designed microarray was used to analyze the response of transcription factors to biotic and abiotic stress, and demonstrated that more than 28 transcription factors were induced by abiotic stress (Chen et al. 2002). In rice, many transcription factors, including zinc finger, NAC, bHLH, MYB and WRKY, were also identified to be induced by salt and drought stresses. Extensive research (Kawasaki et al. 2001; Rabbani et al. 2003; Chao et al. 2005; Wu et al. 2006) also identified multiple transcription factors that were induced by stress, and interestingly, Chao et al. (2005) found that transcription factors were rich in the earliest salt-induced genes. These data suggest that multiple regulatory pathways under salt/drought stress remain to be characterized.

Sub1 and Submergence Stress

Lowland rice grows well under waterlogged conditions because of the well-developed aerenchyma, which facilitates oxygen diffusion in roots. However, sudden and total inundation sustained for several days can be fatal. Rice production has become more susceptible to submergence since the introduction of semi-dwarfism in 1960s. The effect of complete submergence on growth and development is variable and is associated with seedling age, as younger plants are less tolerant than older plants (Adkins et al. 1990). Total immersion

causes various symptoms of injury, including leaf and stem elongation, leaf degeneration, dry mass loss and a tendency to lodging (Jackson and Ram 2003).

Submergence tolerance is defined as "the ability of a rice plant to survive 10–14 d of complete submergence and renew its growth when the water subsides" (Catling 1992). When completely submerged for more than 3 d, most existing rice cultivars are seriously injured and die within a week. Only a few tolerant cultivars, for example FR13A, Thavalu, Kurkaruppan and Goda Heenati, can withstand complete submergence for 10–14 d (Setter et al. 1997). Compared with susceptible lines, tolerant cultivars commonly suppress underwater elongation of leaves and keep a higher level of carbohydrates under submergence (Das et al. 2005).

FR13A was selected from a local *indica* var. Dhullaputia grown in Orissa, India and released in the 1940s (Mackill 1986). *Sub1*, a major QTL for submergence tolerance that accounted for 69% of the variation in tolerance was mapped in a donor line IR40931-26 derived from FR13A (Xu and Mackill 1996). Xu et al. (2006b) mapped the *Sub1* locus to the interval of 0.06 centimorgans, which contained three ethylene-response-factor (ERF) genes *Sub1A*, *Sub1B* and *Sub1C*. *Sub1B* and *Sub1C* were conserved in all analyzed rice varieties, whereas *Sub1A* was absent in some varieties such as M202 and Nipponbare. *Sub1A* has two alleles: tolerant allele *Sub1A-1* and intolerant allele *Sub1A-2*. Enhanced tolerance in *Sub1A-1* transgenic intolerant plants confirmed that *Sub1A-1* was the major genetic determinant for submergence tolerance. The *Sub1* region had been introgressed into M202 by marker-assisted selection (Xu et al. 2004). New local cultivars containing *Sub1* locus would ensure security for rice farming in rain fed lowland.

Fukao et al. (2006) demonstrated further that the *Sub1* haplotype played a central role in the regulation of acclimatizing responses to submergence, such as leaf and stem elongation, chlorophyll breakdown and carbohydrate consumption. Increased ethylene entrapped in plants by water leads to accelerated leaf elongation, but acceleration in growth is detrimental as it hastens energy depletion and increases susceptibility (Jackson and Ram 2003). Under submergence stress, ethylene accumulation causes upregulation of *Sub1A-1* and *Sub1C* transcripts, while the *Sub1C* transcript accumulation is negatively regulated by *Sub1A-1* (Fukao et al. 2006). *Sub1A-1* inhibited leaf elongation by suppressing the expression of the expansin-encoding genes, *ExpA1, 5, 6, 7,* and *16*. In addition, submergence stress induced the accumulation of ethylene and tolerant plants produced less ethylene than intolerant plants, indicating that *Sub1A-1* reduced ethylene production during submergence through feedback regulation. *Sub1A-1* also restrained carbohydrate consumption and chlorophyll breakdown to maintain the capacity of regrowth when water subsided. In *Sub1A-1* plants, the induction of α-amylose genes (*RAmy3C*, *RAmy3D, RAmy3E*) and sucrose synthase genes (*Sus1, Sus2, Sus3*) was significantly lower than in intolerant plants, leading to less carbohydrate consumption and shorter periods of energy starvation during submergence. Moreover, *Sub1A-1* plants maintained higher levels of chlorophyll than those of intolerant plants after 2 weeks of submergence, and after removal of the submergence stress, the new leaf growth rate was three times in Sub1A-1 plants compared with intolerant plants. Thus, *Sub1A* locus finely modulates acclimation responses that conferred tolerance to submergence stress in FR13A (Fukao et al. 2006).

Future Perspectives

Together with conventional plant physiology, genetics and biochemical approaches to studying plant responses to abiotic stresses began to bear fruit recently. Transcriptome analysis reveals stress-responsive genes and signal cross-talk is genome-wide. Ion transporters and a group of transcriptional factors that regulate stress responsive genes have been identified. But we are still far from having a clear picture. The foremost difficulty in putting together the puzzle is not having enough pieces. Therefore, the challenge in the future remains to identify more signaling elements. Once enough components are known, signaling specificities and cross-talks can be properly addressed.

Although there have been many transgenic plants with high stress tolerance generated, plant abiotic stress tolerance is a complex trait that involves multiple physiological and biochemical mechanisms and numerous genes. Transgenic plants with commercial value, especially transgenic rice plants, should at the same time retain relatively high productivity, and other traits important for agriculture. Moreover, genetic modification should be combined with marker-assisted breeding programs with stress-related genes and QTLs, and ultimately, the different strategies should be integrated, and genes representing distinctive approaches should be combined to substantially increase plant stress tolerance. Through more widespread application of forward and reverse genetic analyses in model plants and with the growing power of genomics and proteomics tools, progress in understanding abiotic stress signaling will certainly accelerate. With a better understanding comes more effective ways to improve plant tolerance to abiotic stress. A new world in modern agriculture is coming nearer and nearer.

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