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Review

Cellular traits for sodium tolerance in rice (*Oryza sativa* L.)

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Abstract The present review focuses on two important aspects of Na^+ toxicity in rice (*Oryza sativa* L.), (i) that Na^+ stress induces different changes in cytosolic Ca^{2+}, $[Ca^{2+}]_{cyt}$, and pH, $[pH]_{cyt}$, in tolerant and sensitive cultivars, and (ii) that cells from a tolerant cultivar can better maintain a low cytosolic Na^+ and/or Na^+/K^+ ratio. Experiments with single rice protoplasts, fluorescence microscopy and specific ion-selective dyes suggest that Na^+ must be sensed inside the cytosol, before any prolonged changes in $[Ca^{2+}]_{cyt}$ and $[pH]_{cyt}$ occur. Inhibitor analyses show that Na^+-induced increase in $[pH]_{cyt}$ in the tolerant cv. Pokkali, and a decrease in $[pH]_{cyt}$ in the sensitive cv. BRRI DHan29, likely are coupled to different H^+-ATPases. Expression analysis of OsHKT2;1 (previous name OsHKT1), OsHKT2;2 (previous name OsHKT2) and OsVHA transcripts in rice using RT-PCR and fluorescence *in situ*-PCR, shows a variable and cell- specific induction in the two rice cultivars under salt stress condition. We conclude that the transient uptake of Na^+, which occurs only in the tolerant cultivar, and the fast compartmentalization of Na^+ into the vacuole, probably are the most important cellular traits for Na^+-tolerance in rice. The low $[Na^+]_{cyt}$ in cv. Pokkali might depend on the fast down-regulation of OsHKT2;1, causing less uptake of Na^+, and fast up-regulation of the OsVHA transcript, and subsequent activation of the Na^+/H^+-anti-porter in the tonoplast. To decrease the cytosolic Na^+/K^+ ratio under Na^+ toxicity, cv. Pokkali may also induce increased uptake of K^+ through induction of OsHKT2;2, and other specific K^+-transporter genes.

Key words: Cytosolic calcium and pH, H^+-ATPase, HKT, Na^+-toxicity, *Oryza sativa*.

Salinity poses a major threat for maintaining the challenge of food supply for the ever-increasing word population, as it reduces the agricultural productivity in affected areas. Currently more than 6% of the world's land (19.5 percent of irrigated land plus 2.1 percent of dryland agriculture) is now affected by salinity of various magnitudes (http://www.plantstress.com/Articles/index.asp- 22 October 2007). Furthermore, the existing problem is getting aggravated, as sea-level rises because of global warming (that causes primary salinization) and expansion of irrigated area worldwide (that causes secondary salinization). As estimated by FAO, about 20–30 million ha of all irrigated lands were seriously damaged in 2002 due to the build-up of salts (Martinez-Beltran and Manzur 2005). Moreover, every year 0.25–0.50 million ha of irrigated lands are lost from production, due to increased salt level.

Salt stress is a worldwide problem, most acute in North and Central Asia, Australia and South America (Pessarakli 1999). Some of the most serious salt problems occur in rice growing regions like China, Bangladesh, India, Thailand, Pakistan and Egypt. Rice is one of the most sensitive plants to salt stress with critical tolerance level 40 mM (Glenn et al. 1997). There are, however, several land races of rice like Pokkali and Nona Bokra cultivated under salt stress in the coastal belt of different regions, though their yield potentiality is very low compared to that of modern high yielding rice cultivars. Rice plants, which are tolerant to salt stress to different degree, possess some facilitating mechanisms both at whole plant level (Yeo and Flowers 1982; Anil et al. 2005), and at cellular level (Golldack et al. 2003; Kader and Lindberg 2005; Kader et al. 2006; Anil et al. 2007). At the cellular level they exhibit an ability to restrict the entry of Na^+ into the cell cytosol, compartmentalize cytosolic Na^+ into vacuole, or exclude cytosolic Na^+ to the apoplast or to the environment. Salt tolerance at cellular level involves several hundred of stress-responsive genes for ionic homeostasis as well as osmotic homeostasis (Bartels and Sunkar 2005; Chen and Zhu 2005; Sreenivasulu et al. 2007). This review aims to highlight the fundamental cellular mechanisms of Na^+-tolerance in rice, including the salt-stress signaling response by changes of cytosolic Ca^{2+} and pH, reported so far.

Salinity causes two types of stress

Salinity stress causes both osmotic stress and ionic toxicity due to high level of Na^+ and/or Cl^- ions and can elicit many different physiological responses (Greenway and Munns 1980; Hasegawa et al. 2000; Zhu 2001). For

the Gramineaceous crop rice, however, Na^+ toxicity is the principal reason for causing damage (Tester and Davenport 2003). Under non-saline conditions, cytosolic Na^+ in higher plants remains 1 to 10 mM (Taiz and Zeiger 2002). The potassium ion (K^+), on the other hand, is one of the essential and most abundant monovalent cations in cells. This ion needs to be maintained within 100–200 mM range in the cytosol for efficient metabolic functioning (Walker et al. 1996; Taiz and Zeiger 2002; Cuin et al. 2003). As a co-factor in the cytosol, K^+ activates more than 50 enzymes, which are very susceptible to high cytosolic Na^+ and high Na^+/K^+ ratios (Munns et al. 2006). Therefore, apart from low cytosolic Na^+, maintenance of a low cytosolic Na^+/K^+ ratio is also critical for the function of cells (Rubio et al. 1995; Zhu et al. 1998).

At saline conditions, Na^+ competes with K^+ for uptake through common transport systems, since Na^+ and K^+ are physico-chemically similar monovalent cations. Thus, elevated levels of cytosolic Na^+, or in other way high Na^+/K^+ ratios, exert metabolic toxicity by a competition between Na^+ and K^+ for the binding sites of many enzymes (Bhandal and Malik 1988; Tester and Davenport 2003). Moreover, at high concentration, Na^+ can displace Ca^{2+} from the plasma membrane, resulting in a change in plasma membrane permeability. This can be reflected by a leakage of K^+ from the cells (Cramer et al. 1989). A high uptake of Na^+ and leakage of K^+ result in an imbalance of the Na^+/K^+ ratio in the cytosol, which, in turn, leads to many imbalances in enzymatic reactions of the cell.

Changes in cytosolic calcium and pH are involved in salt stress signaling

Calcium has a central role in signaling

In both yeast and plants several osmo-sensors, e.g. receptor-like kinases are suggested to be involved in osmotic stress signaling (Reiser et al. 2003; Tamura et al. 2003; Buoudsocq and Lauriere 2005). Plant mitogen-activated protein kinases (MAPKs) are considered important participants in osmoregulation in plants, yeast and animal cells. Osmotic stress also activates a number of phospholipid systems, generating a wide array of messenger molecules (Zhu 2002; Boudsocq and Lauriere 2005). How plants sense Na^+, and if it is sensed inside or outside the plasma membrane are still unknown. It has been proposed that the SOS- (Salt-Overly-Sensitive) pathway is involved in the sensing of Na^+ by the SOS1 protein, a plasma membrane Na^+/H^+ anti-porter, which has a long C-terminal tail, probably resided in the cytoplasm (Zhu 2003; Zhang et al. 2004; Shabala et al. 2005).

In a majority of the mentioned processes, calcium has a central role as a transducer and/or regulator of signals. Moreover, the cytosolic calcium and pH homeostasis in cells are closely linked (Bush 1995). Transient shifts in intracellular and apoplastic pH are essential steps in several transduction processes. The changes in pH are involved in stress signaling either directly, or in cross talk with plant hormones or Ca^{2+} (Gilroy and Trewavas 1994; Ward et al. 1995; Blatt and Grabov1997; Roos 2000; Felle 2001; Gao et al. 2004).

Calcium signaling in different parts of the plant cell

An early response of plant cells to many types of stresses, including salt stress, is an increase in cytosolic calcium concentration, $[Ca^{2+}]_{cyt}$ (Lynch et al. 1989; Bush 1995; Knight et al. 1997; Halfter et al. 2000; Knight 2000; Hasegawa et al. 2000; Gao et al. 2004). Kader et al. (2007) evidenced changes in $[Ca^{2+}]_{cyt}$ dynamics in rice cells upon NaCl stress. Upon addition of 100 mM NaCl a transient increase in $[Ca^{2+}]_{cyt}$ in rice cvs. BRRI Dhan29 and Pokkali was obtained in the presence of 0.1 mM extra cellular Ca^{2+}. Moreover, a change in calcium level can also occur in the apoplast (Gao et al. 2004). It was suggested by Han (2003) that extra cellular calcium in guard cells also may act as a physiological signal in plants, and that increases in cytosolic Ca^{2+} arise in response to activation of a cell surface receptor. Organelles, such as mitochondria, chloroplasts and nuclei also have possibilities to generate their own calcium signals (Xiong et al. 2006). Changes in free calcium concentration in an organelle may favor the re-localization of proteins and regulatory components and, thus, have an important influence on the integrated functioning of the cell. Plant cells, therefore, have a high flexibility to respond to different types of environmental changes.

Increase in $[Ca^{2+}_{cyt}]$ depends on extra-cellular influx of Ca^{2+} and/or release from intracellular stores into the cytosol (Sanders et al. 2002). However, in some cases also a high salinity can cause a decrease in $[Ca^{2+}_{cyt}]$ (Cramer and Jones 1996; Halperin et al. 2003). Thus, the change in calcium is not uniform and may vary with species, cell type or tissue type (Cramer and Jones 1996).

Alteration in $[Ca^{2+}_{cyt}]$ causes a signal transduction via calcium-dependent protein kinases, inositol phosphate, calmodulin and other Ca^{2+}-controlled proteins (Knight 2000). The downstream responses lead to protection and acclimation of the plant to the stress condition. Besides a signaling function, calcium at higher external concentration than 0.5 mM has a direct effect by inhibiting the uptake of Na^+ into the cell by non-selective cation channels (Amtmann and Sanders 1999, Schachtman and Liu 1999, Demidchik and Tester 2002). Also cGMP, produced in response to salt and osmotic stress may down-regulate the Na^+-influx by voltage-independent channels (Don-

aldson et al. 2004).

Calcium changes induced by ionic toxicity and osmotic stress are different

Addition of 100 mM NaCl to intact roots of transgenic *Arabidopsis* induced both increases in apoplastic concentration of Ca^{2+}, $[Ca^{2+}]_{apo}$, and $[Ca^{2+}]_{cyt}$ (Gao et al. 2004). Repeated periods of NaCl treatment induced drastic transients and prolonged alteration in $[Ca^{2+}]_{cyt}$. Unlike the addition of mannitol, addition of NaCl gradually increased the resting level of $[Ca^{2+}]_{cyt}$. The finding that $[Ca^{2+}]_{cyt}$ increases during NaCl stress is in line with earlier observations using maize root protoplasts (Lynch et al. 1989) and with experiments using rice protoplasts (Kader et al. 2007). In the experiments with rice protoplasts $[Ca^{2+}]_{cyt}$ dynamics were detected by use of fluorescence microscopy and the calcium sensitive dye Fura 2-AM. Different reactions were obtained in the salt-tolerant and -sensitive rice cultivars and with different external calcium concentrations (Kader et al. 2007). Addition of NaCl in the presence of low external Ca^{2+} concentration (0.1 mM) induced a higher increase in $[Ca^{2+}]_{cyt}$ in the salt-tolerant cv. Pokkali than in the sensitive cv. BRRI Dhan29. However, in the presence of 1 mM external Ca^{2+} concentration there was an increase in $[Ca^{2+}{}_{cyt}]$ only in cv. BRRI Dhan29, not in cv. Pokkali (Kader et al. 2007). The reason for this can be explained by the fact that Na^+ does not enter into cells of cv. Pokkali in the presence of high Ca^{2+} concentration (Kader and Lindberg 2005). Therefore, it is likely that Na^+ must enter into the cytosol to elicit a $[Ca^{2+}]_{cyt}$ signal.

From inhibitor analyses it was concluded that internal stores for calcium appear to be major source for increase in $[Ca^{2+}]_{cyt}$ in cv. Pokkali, although the apoplast, or external medium, is more important in cv. BRRI Dhan29 (Kader et al. 2007). Some investigations show that salt stress induces a rapid increase in inositol-(1,4,5)-triphospate (IP_3) and that this compound can further increase $[Ca^{2+}{}_{cyt}]$, by opening of IP_3-regulated channels in the tonoplast (Sanders et al. 2002). It is likely that prolonged elevation of $[Ca^{2+}]_{cyt}$ depends on the activation of these channels. Also in experiments with single protoplasts from the extremely salt-tolerant quince, *Cydonia oblonga* cv. Mill, NaCl addition caused transient increases in $[Ca^{2+}]_{cyt}$, of which amplitude increased with added NaCl concentration (unpublished results by C. D'Onofrio and S. Lindberg). The sharp spikes in $[Ca^{2+}]_{cyt}$ obtained in experiments with intact plants have not been found in measurement with a single protoplast. One reason may be the lack of cell walls. A specific reaction in the apoplast might be necessary for the spikes to emerge in the cytosol. On the other hand, the *prolonged and early* $[Ca^{2+}]_{cyt}$ elevation found in protoplasts of quince and the salt-tolerant rice cv. Pokkali, is probably necessary for activation of adaptive responses.

The osmotic effect, mimicked by addition of sorbitol, caused a different reaction in rice protoplasts than addition of NaCl. In both the salt-sensitive cv. BRRI Dhan29 and the tolerant cv. Pokkali, $[Ca^{2+}]_{cyt}$ decreased upon addition of 200 mM sorbitol (Kader et al. 2007). On the other hand, addition of sorbitol to protoplasts from the halo-tolerant species quince did not affect $[Ca^{2+}]_{cyt}$ (unpublished results by C. D'Onofrio and S. Lindberg). Moreover, a low concentration of sorbitol (100 mM) did not have an effect on $[Ca^{2+}]_{cyt}$ levels in *Arabidopsis*, although iso-osmotic concentration of Na^+ did so (Donaldson et al. 2004). It can be concluded that $[Ca^{2+}]_{cyt}$ changes induced by osmotic stress are different from those induced by Na^+. They do not only depend on species, cell type or tissue type (Cramer and Jones 1996), but also on pre-exposure to stress (Knight et al. 1997) and rate of stress development (Plieth et al. 1999).

Ionic toxicity and osmotic stress also induce different pH-changes

Upon a change in $[Ca^{2+}{}_{cyt}]$, cells are challenged also with the excess of monovalent ions, like H^+ in the cytosol (Plieth et al. 1997, 1999; Gao et al. 2004). Transient shifts in both intracellular and apoplastic pH are suggested to be essential steps in several signal transduction processes (Gilroy and Trewavas 1994; Ward et al. 1995; Blatt and Grabov 1997; Roos 2000; Felle 2001; Gao et al. 2004). The changes in intracellular pH due to salt stress were reported in many plant species.

Rice protoplasts from the salt-tolerant and -sensitive cultivars of rice, loaded with the pH-senstive dye BCECF-AM, responded differently upon NaCl addition (Kader et al. 2007). In protoplasts from the sensitive cv. BRRI Dhan29, $[pH]_{cyt}$ *decreased*, similar to intact *Arabidopsis*, but in protoplasts from the tolerant cv. Pokkali, $[pH]_{cyt}$ *increased* (Gao et al. 2004; Kader et al. 2007). By use of the vacuole-specific dye 6-CFDA it was shown that the pH in vacuoles of cv. Pokkali decreased after 5–10 min, although there was a slight increase in cv. BRRI dhan29 (Kader at al. 2007). The results suggest that salt addition causes transport of H^+ from cytosol into the vacuole in cv. Pokkali, probably as a result of activated H^+-ATPase. This was also confirmed by expression analyses of the tonoplast H^+-ATPase (Kader et al 2006, 2007). When protoplasts from cv. Pokkali were pretreated with NH_4NO_3, an inhibitor of vacuolar H^+-ATPase, before NaCl addition, the increase in $[pH]_{cyt}$ was less than for control protoplasts. On the other hand, NH_4NO_3 did not affect the $[pH]_{cyt}$ change in cv. BRRI Dhan29, but the NaCl-induced decrease in $[pH]_{cyt}$ was very low when the protoplasts were pretreated with NH_4VO_3, an inhibitor of the plasma membrane H^+-ATPase. It is, therefore, likely that proton movement instead occurs mainly within the apoplast and cytosol in cv.

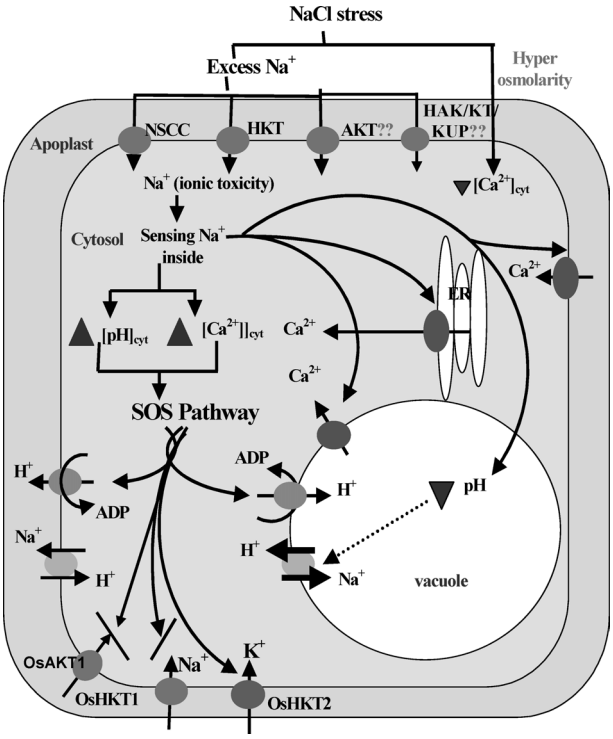

Figure 1. A model for perception of Na^+ stress and subsequent changes in $[Ca^{2+}]_{cyt}$ and $[pH]_{cyt}$ and vacuolar pH for the adaptation of Na^+ homeostasis in cytosol of rice cv. Pokkali. As shown in Kader et al (2006), Na^+ is possibly sensed inside the cytosol and the sensing alters cytosolic Ca^{2+} and pH and vacuolar pH to activate the SOS pathway. The SOS pathway then may down-regulate K^+ selective channels and transporters like OsHKT1 (Horie et al. 2001, 2007; Golldack et al. 2002; Kader et al. 2006) and OsAKT1 (Golldack et al. 2003) to restrict Na^+ entry into the cytosol. For maintaining cytosolic Na^+ homeostasis, the SOS pathway may also induce OsHKT2 (Kader et al. 2006) for increased K^+ uptake and OsNHX1 (Fukuda et al. 2004; Chen et al. 2007) for vacuolar compartmentalization of cytosolic Na^+.

BRRI Dhan29 (Figure 1; Kader et al. 2007).

In yeast cells (*Saccharomyces cerevisiae*) cytosolic acidification caused by salt-stress is proposed to be involved in activation of the Na^+/H^+ antiporter in the plasma membrane (Kinclova et al. 2001). Therefore, it is likely that the cytosolic acidification in BRRIDhan29, in a similar way, could cause an activation of the plasma membrane Na^+/H^+ antiporter (Kader et al. 2007). On the other hand, an increase in $[pH]_{cyt}$ in the salt-tolerant cv. Pokkali is consistent with salt-induced cytosolic alkalinization in *F. oxysporum*, where cytosolic alkalinity activates PacC, a transcription factor for Na^+-ATPase (Caracuel et al. 2003). It is, therefore, likely that the contemporary increases in $[Ca^{2+}{}_{cyt}]$ and $[pH]_{cyt}$, and decrease in vacuolar pH, are important mechanisms to induce adaptive mechanisms in the salt-tolerant rice Pokkali.

In intact *Arabidopsis* roots, mannitol neither affected $[pH]_{cyt}$ nor apoplastic pH, although addition of NaCl to the same roots caused a decline in both pHs (Gao et al. 2004). Moreover, in protoplasts from both rice and quince, sorbitiol did not change pH_{cyt} (Kader et al. 2007, unpublished results C. D'Onofrio and S. Lindberg). Thus, also the pH-changes are different when induced by Na^+ or osmotic stress.

Adaptive mechanisms for Na^+ and K^+ homeostasis

A lower Na^+-influx into the cytosol correlates with salt tolerance level

Under saline condition, the ability to keep a low cytosolic Na^+-concentration appears to be an important trait of salt-tolerant plants (Maathuis and Sanders 2001; Flowers and Hajibagheri 2001; Carden et al. 2003; Golldack et al. 2003; Kader and Lindberg 2005; Anil et al. 2007). Plant cells can maintain a low cytosolic Na^+-concentration, either by restricting Na^+ influx into the cell, or by extruding cytosolic Na^+ into the apoplast/vacuole, or by both. Although the mechanisms by which rice roots takes up Na^+ is not yet clarified, there are several studies showing that the accumulation of Na^+ in the cytosol differs between salt-sensitive and salt-tolerant rice cultivars. The salt-tolerant rice cv. Pokkali can maintain a low cytosolic Na^+ by reducing Na^+-uptake into the cytosol, and also by extruding Na^+ from the cytosol into the vacuole. This cultivar only transiently takes up Na^+ into the cytosol, and takes up less Na^+ than the salt-sensitive cvs. IR29 or BRRI Dhan29 (Golldack et al. 2003; Kader and Lindberg 2005). Anil et al. (2007) reported less permeability of plasma membrane to Na^+ in the salt-tolerant rice cv. Pokkali compared to that in salt-sensitive rice cv. Jaya.

Transport proteins mediate Na^+ influx into the cytosol in different ways in sensitive and tolerant plant species and cultivars

Sodium ion, like any other mineral nutrients to be taken up by cells, may pass the plasma membrane either in the root epidermal cells or cortical cells. In plant cells, plasma membrane potential is negative inside (~ -120 to -200 mV). Therefore, an increase in positively charged extra cellular Na^+ develops a large electrochemical potential gradient for Na^+ that favours Na^+ to be transported passively from the environment into the cytosol. Transport proteins involved in mediating passive Na^+-influx include high-affinity potassium transporter (HKT), low-affinity cation transporter (LCT1) and non-selective cation channels (NSCCs).

Non-selective cation channels (NSCCs) are proposed to be the dominant pathways for Na^+-influx into many plant species including rice (Davenport and Tester 2000; Demidchik and Tester 2002; Demidchik et al. 2002; Kader and Lindberg 2005), though their molecular identity is still elusive. Using inhibitors for NSCCs and K^+-selective channels and transporters we showed that

NSCCs mediate Na-influx in both salt-sensitive rice cv. BRRI Dhan29 as well as salt-tolerant rice cv. Pokkali (Kader and Lindberg 2005). However, K^+-selective channels and transporters play differential roles in these two rice cultivars with varying salt-tolerance capacity. Along with NSCCs, K^+-selective channels and transporters contribute to the total Na^+-influx in cv. BRRI Dhan29, but not in cv. Pokkali. Consistently we also have found that OsHKT2;1 is down-regulated in the salt-tolerant rice cv. Pokkali under salt stress but not in the sensitive cv. BRRI Dhan29 (Kader et al. 2006). In response to salt stress a down-regulation of OsHKT2;1was also shown by Horie et al. (2001; 2007) and Golldack et al. (2002) and of OsAKT expression in cv. Pokkali by Golldack et al. (2003).

High-affinity potassium transporters (HKTs) are suggested to mediate a substantial Na^+-influx in many species (Uozumi et al. 2000; Horie et al. 2001; Golldack et al. 2002; Mäser et al. 2002; Gárciadeblás et al. 2003). In rice, nine HKT homologues are identified (Gárciadeblás et al. 2003). Except one, they encode proteins with distinct transport activities, which might be expressed in various tissues and/or organs. Horie et al. (2001) suggested that OsHKT2;1 encodes a Na^+-transporter and OsHKT2;2 a Na^+/K^+-coupled transporter. Garciadeblas et al. (2003) showed that OsHKT2;1 could be a high-affinity Na^+-transporter and OsHKT1;1 (previous name OsHKT4) a low-affinity Na^+-transporter. OsHKT1;5 (previous name OsHKT8) has recently been shown to be a Na^+-transporter, but contributing to the increased ability of salt-tolerance through re-circulating of Na^+ from xylem sap, and thus, by maintaining shoot K^+ homeostasis under salt stress (Ren et al. 2005; Rus et al. 2005). The Na^+-transport activity of this protein was found higher in the salt-tolerant cultivar Nona Bokra than in salt-sensitive japonica rice variety Koshihikari (Lin et al. 2004; Gao et al. 2007). This is analogous to the function of AtHKT1 gene in *Arabidopsis*, which is a Na^+-transporter, and interestingly, plays a very important role in controlling the cytosolic Na^+ detoxification (Berthomieu et al. 2003; Rus et al. 2004; Sunarpi et al. 2005). AtHKT1;1 (previous name AtHKT1) functions in mediating tolerance to salt stress by unloading Na^+ from xylem vessels to xylem parenchyma cells and thus, protecting the plant leaves from salt stress (Sunarpi et al. 2005). This transporter might also be responsible for unloading of Na^+ from the phloem (Berthomieu et al. 2003). Therefore, it is very likely that the HKT gene family in rice has an important role for plant ion homeostasis, even though some of the members evidently transport Na^+. Apart from these NSCCs and HKTs, other transport proteins that might be involved in mediating Na^+ transport under salinity stress are HAK/KT/KUP-type transporters, inward-rectifying potassium channels, and low-affinity cation transporters of the LCT-1 type (Schachtman et al. 1992, 1997; Maathuis et al. 1997; Amtmann and Sanders 1999; Golldack et al. 2003).

Excess cytosolic Na^+ is differentially dealt with in salt-sensitive and -tolerant rice cultivars

Like other plants, rice can deal with the internal Na^+ by sequestering it into the apoplast or vacuole to maintain a low level of cytosolic Na^+. Salt-tolerant rice cv. Pokkali takes up Na^+ into the cytosol only transiently and compartmentalizes it into the vacuole immediately (Kader and Lindberg 2005). Recently Anil et al. (2007) also reported effective sequestration of cytosolic Na^+ in the intracellular compartments in the salt-tolerant rice cv. Pokkali. OsNHX1, a tonoplast Na^+/H^+ antiporter in rice, plays an important role in compartmentalization of cytosolic Na^+ into the vacuole, and its over-expression improves the salt tolerance of transgenic rice (Fukuda et al. 2004; Chen et al. 2007). An induction of OsVHA, an energizer for OsNHX1, in the salt-tolerant cv. Pokkali correlates with its ability to compartmentalize cytosolic Na^+ into the vacuole (Kader et al. 2006). Vacuolar compartmentalization of cytosolic Na^+ is very low in the salt-sensitive rice cv. BRRI Dhan29, instead this cultivar sequesters a portion of cytosolic Na^+ back into the apoplast. Apoplastic sequestration of cytosolic Na^+ is, however, not an efficient strategy for salt tolerance in rice, since it is shown previously, that most of the Na^+ in rice leaves comes through apoplastic streaming (Yeo et al. 1999). Kawasaki et al. (2001) reported a large-scale gene expression profile in salt-tolerant rice cv. Pokkali as well as in salt-sensitive cv. IR29 under controlled high-salt conditions. Under salt treatment many transcripts that were up-regulated in the tolerant cultivar responded more slowly in the sensitive cultivar.

At salt stress, the ratio of cytosolic Na^+/K^+ is disrupted in many higher plants, since the concentration of Na^+ is much higher than at normal condition. Apart from the high cytosolic Na^+, plants also suffer from high cytosolic Na^+/K^+ ratio. Therefore, apart from dealing with excess cytosolic Na^+, plant cells can also handle this unfavourable high Na^+/K^+ ratio by increasing the concentration of cytosolic K^+-level. However, the cytosolic K^+-level may also be harmful for cells when it exceeds the normal range (Greenway and Osmond 1972). We recently suggested that OsHKT2;2, the only HKT member in rice supposed to be involved with K^+ transport, plays an important role in the salt tolerance in the salt-tolerant cv. Pokkali by increasing cytosolic K^+ level (Kader et al. 2006). We found a substantial induction of OsHKT2;2 in shoots of salt-tolerant cv. Pokkali, and to a lesser extent in roots of the same cultivar, but not in the salt-sensitive cv. BRRI Dhan29. Although OsHKT2;2 (K^+-Na^+ coupled transporter) does not mediate K^+-influx from a high K^+ solution in the absence of

Na^+, it confers tolerance to salinity under high Na^+, probably by increased ability of K^+-uptake, as shown in *S. cerevisiae* (Horie et al. 2001). The induction of OsHKT2;2 in epidermis, exodermis and vascular tissue in roots in our study might indicate its involvement in K^+-uptake. Furthermore, the expression of OsHKT2;2 in the phloem and the transition from phloem to mesophyll cells, along with mesophyll cells, may indicate its involvement in the recirculation of K^+ within the mesophyll cells through the phloem.

In addition to metabolites such as sugars, minerals and salts, such as phosphate, also can use the phloem pathway to be redistributed from old source leaves towards young and expanding sink leaves (Sondergaard et al. 2004). Ren et al. (2005) proposed a model showing redistribution of cytosolic Na^+ from mesophyll cells to phloem as an adaptation to maintain cellular K^+ nutrient status. Thus, the induction of OsHKT2;2 in the salt-tolerant cv. Pokkali might confer salt tolerance by increasing its expression in leaves, through contributing to a low cytosolic Na^+/K^+ ratio, as suggested by Horie et al. (2001).

The up-regulation of K^+-transporter genes upon salt stress possibly reflects the plants ability to maintain certain cytosolic K^+ levels to survive under salt stress (Su et al. 2001, 2002; Pilot et al. 2003; Maathuis, 2006). Since K^+, at a high concentration, also is inhibitory for enzymatic functions in the cytosol (Greenway and Osmond 1972), the induction of OsHKT2;2 in Pokkali shoot decreased after some stress period. In a recent study Obata et al. (2007) reported enhanced salt tolerance and increased cellular K^+ content in rice (cv. 'Nipponbare') cells over-expressing OsKAT1, which encodes a K^+ channel protein, and suggested that OsKAT1 is involved in salt tolerance of rice by participating in maintenance of cytosolic cation homeostasis during salt stress.

Conclusion

Salt-tolerant cultivars of rice, like halophytic plants, are able to maintain a low cytosolic Na^+ or/and a low cytosolic Na^+/K^+ ratio under excess of external Na^+. As shown in the model in Figure 1, restricting Na^+ entry into the cytosol by down-regulation of some of the Na^+-transporters and compartmentalization of cytosolic Na^+ into the vacuole by the induction of tonoplast Na^+/H^+ antiporter play a very vital role in this process. An induction of some of the K^+-transporters may also help maintain correct cytosolic Na^+/K^+ ratio. At laboratory conditions salt-tolerant transgenic crops with an over-expression of the tonoplast Na^+/H^+-antiporter were reported for many plants like *Arabidopsis* (Apse et al. 1999), tomato (Zhang and Blumwald 2001), wheat (Xue et al. 2004), maize (Yin et al. 2004) and rice (Fukuda et al. 2004; Chen et al. 2007). However, tolerance for environmental stresses in plants is controlled at the transcriptional level by a complicated gene regulatory network (Chen and Zhu 2005; Sreenivasulu et al. 2007). Since salt-tolerance in plants is a multigenic trait with many quantitative trait loci (QTLs), plants need to possess a wide range of adaptations for osmotic stress, as well as ionic toxicity (for Na, Cl etc.), to be tolerant under high salt at field condition. This multigenic trait of salt tolerance, in turn, makes plant breeder's job challenging. That's why the effort from last ten year's research using transgenic plants to improve salt-tolerance has not yet been established in the field (Flowers 2004). Nevertheless, a very important goal for salt stress research in rice over the years is to understand how plants sense salt stress, and how cellular and physiological changes allow plants to be adept at dealing salt stress. It's very important that different components of salt stress adaptations in rice like sensing the stress, signaling cascade, downstream responses for ionic homeostasis and osmotic adjustments and the components for cross talk with other stresses etc. are becoming available. These components will obviously deliver the most significant platform to materialize plant breeder's challenge to develop salt-tolerant rice cultivars of high yield potentiality.

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