Differentially expressed membrane transporters in rice roots may contribute to cultivar dependent salt tolerance

Prasad Senadheera1, R. K. Singh3 and Frans J. M. Maathuis2,*

1 Department of Plant Science, University of Colombo, Colombo, Sri Lanka
2 Biology Department, Area 9, University of York, York YO10 5DD, UK
3 International Rice Research Institute, Los Baños, Philippines

Received 11 December 2008; Revised 3 March 2009; Accepted 6 March 2009

Abstract

Salinity tolerance in rice, like in other glycophytes, is a function of cellular ion homeostasis. The large divergence in ion homeostasis between the salt-tolerant FL478 and salt-sensitive IR29 rice varieties can be exploited to understand mechanisms of salinity tolerance. Physiological studies indicate that FL478 shows a lower Na+ influx, a reduced Na+ translocation to the shoot, and maintains a lower Na+:K+ ratio. To understand the basis of these differences, a comparative investigation of transcript regulation in roots of the two cultivars was undertaken. This analysis revealed that genes encoding aquaporins, a silicon transporter, and N transporters are induced in both cultivars. However, transcripts for cation transport proteins including OsCHX11, OsCNGC1, OsCAX, and OsTPC1 showed differential regulation between the cultivars. The encoded proteins are likely to participate in reducing Na+ influx, lowering the tissue Na+:K+ ratio and limiting the apoplastic bypass flow in roots of FL478 and are therefore important new targets to improve salt tolerance in rice.

Key words: Monovalent ion uptake, rice, root membrane transporters, salinity tolerance, silicon accumulation-transciptomics.

Introduction

Soil salinity is one of the major abiotic stresses limiting agricultural production in many areas of the world. In plants, salinity severely decreases growth through osmotic stress, large increases in cellular Na+ and Cl− contents and negative effects on K+, Ca++, and NO3− nutrition (Flowers and Colmer, 2008). These deleterious effects occur at both the cellular and the whole tissue level.

Salt tolerance in glycophytes is predominantly associated with the restriction of toxic ion absorption at the root level, extrusion of ions into the apoplast, and sequestration of excess ions into intracellular compartments and tissues that are less sensitive (Blumwald, 2000; Flowers and Colmer, 2008). Plants face dilemmas in executing these strategies. For example, where the osmotic imbalance is concerned, ion uptake is beneficial to lower the osmotic potential but excess ion uptake, particularly of Na+, may be toxic. Increased uptake capacity for the less toxic cation K+ may be problematic due to the physicochemical similarity between Na+ and K+ that leads to a lack of discrimination between these cations at the transport sites (Maathuis and Amtmann, 1999). Salt-tolerant species are more capable of achieving this delicate balance and, in glycophytes, this is often exemplified by lower levels of Na+ accumulation in tolerant species and ecotypes (Munns, 2002).

Amongst the major crops, rice is highly sensitive to salt stress and generally only tolerates salinities ranging between 1.9–3 dS m−1 (Grattan et al., 2002), which is comparable to a concentration of around 20–30 mM NaCl. Rice sensitivity to salinity is dependent on growth stage with most stress symptoms being displayed in the seedling and panicle induction stages (Akbar et al., 1972). Although some non-symplastic uptake of ions occurs in most plants, rice is unique in the sense that its apoplastic pathway has considerable conductance for Na+ (Yeo et al., 1987; Garcia et al., 1997). This so-called ‘bypass flow’ is increased by an
inadequate silicon supply (Gong et al., 2006) and possibly depends on Ca$^{2+}$ homeostasis (White, 2001).

Rice salt sensitivity varies considerably across cultivars, a phenomenon that can potentially be exploited to discover genes and proteins that contribute to tolerance. The overall mechanisms of salt tolerance in rice, as in other crops, depend on the control of salt uptake at the root level, regulation of influx into cells, control over long-distance transport, and compartmentation at the cellular and tissue levels. Tolerance also relies on maintaining a high cytosolic K$^{+}$/Na$^{+}$ ratio and a reduction of cytosolic Na$^{+}$ load (Maathuis and Amtmann, 1999). However, the implementation of these strategies may be significantly different in tolerant and sensitive cultivars, both at the cellular and whole tissue levels. For example, short-term measurements using the Na$^{+}$ sensitive dye SBFI showed that cells derived from tolerant cultivars show a much smaller Na$^{+}$ conductance in their plasma membrane (Kader and Lindberg, 2005; Anil et al., 2007) which could underlie the often observed lower tissue [Na$^{+}$] in tolerant varieties.

Transcriptomics approaches have been applied to identify differentially regulated rice genes in response to salt stress for shoots (Chao et al., 2005; Zhou et al., 2007) and roots (Kawasaki et al., 2001) and also comparing rice with other cereals (Ueda et al., 2006). However, such approaches are likely to identify many false positives due to unwanted side-effects of the treatment and/or to the use of different species. To minimize these risks, the use of cultivars with different salt sensitivity for comparative transcriptomics studies is far more preferable.

An extensive transcriptomics study comparing cultivars with varying salt tolerance was carried out on shoot tissues derived from plants at the seedling or panicle initiation stages (Walia et al., 2005, 2007). Genes from many functional classes were found to be differentially regulated in shoots of the tolerant cultivar and included those encoding transcription factors, signal transduction components, cell wall components, and membrane transporters. In the latter group, differential regulation was observed for genes encoding carriers and channels involved in transporting cations ($\textit{HKT, HAK, KAT, CNGC, GLR}$), anions ($\textit{CLC}$) and organic substrates (sugar transporters).

Root tissue forms the initial defence barrier against salt stress and many functions in roots, particularly related to ion transport, are specific for this organ. Therefore, the physiological and morphological properties and transcriptional regulation in root tissue of the well-characterized sensitive and tolerant cultivars IR29 and FL478 has been investigated. Since significant differences in tissue [K$^{+}$] and [Na$^{+}$] is one of the most obvious manifestations of variation between FL and IR, our focus was on the role of membrane proteins that are responsible for Na$^{+}$ and K$^{+}$ homeostasis. Our analyses yielded a number of root-specific transcripts that are likely to contribute to salt tolerance and a further number of transcripts that were specifically regulated in the tolerant variety.

Materials and methods

Plant growth and salt treatment

Seeds of two rice cultivars, FL478 (FL) and IR29 (IR), were obtained from the International Rice Research Institute (Los Baños, Philippines). Seeds were germinated and seedlings were transferred to hydroponic medium (1.25 mM KNO$_3$, 0.5 mM Ca(NO)$_3$_2$\cdot$4H$_2$O, 0.5 mM MgSO$_4$$\cdot$7H$_2$O, 42.5 $\mu$M FeNaEDTA, 0.625 mM KH$_2$PO$_4$, and 1.0$\times$10$^{-2}$ $\mu$M Cu$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, B$^{3+}$, Mo$^{6+}$, and Co$^{2+}$) (Arteca and Arteca, 2000) 10 d after sowing (DAS) and grown under controlled conditions at 22/19 °C day/night temperatures, 100 $\mu$mol m$^{-2}$ s$^{-1}$ of irradiance for 16 h d$^{-1}$, and 40% relative humidity. Seedlings were exposed to salinity stress by adding 50 mM or 100 mM NaCl to the hydroponic solution at 15 DAS. Hydroponic solution was renewed every 2 d. Plants were harvested at different time intervals for analyses. For monovalent cation analysis tissue was collected at various time points as indicated in the figure legends. Total RNA extraction for transcriptome analysis, and measurement of long-term accumulation of Na$^{+}$, K$^{+}$, and Ca$^{2+}$ and recording photosynthetic rates were done on the 12th day of the treatment (27 DAS). Seeds of the $\textit{isl1}$ mutants were received from Professor Jian Feng Ma. They were cultivated and treated as described above except for the addition of 3 mM Si in the form of Na$_2$O$_7$SiO$_3$ to the hydroponic medium 3 d prior to the salinity treatment.

Photosynthesis, stomatal conductance, and relative growth rate measurements

Net photosynthesis per unit leaf area and stomatal conductance of the youngest fully expanded leaf were determined at the 12th day of salt treatment using a Li-Cor 6400 infrared gas analyser (Li-Cor Biosciences, Nebraska, USA). Measurements were made at 500 $\mu$mol m$^{-2}$ s$^{-1}$ of photosynthetic active radiation, 400 $\mu$mol mol$^{-1}$ of chamber CO$_2$ concentration, 24 °C and 42% relative humidity in the leaf chamber.

To measure the relative growth rates of plants, a minimum of three plants from three independent replicates was randomly selected from three treatments (control, 50 mM NaCl, and 100 mM NaCl) at the beginning and end of the treatment. $\textit{RGR}$ was determined using the equation as described by Poorter and Garnier (1999).

Tissue cation and N analysis

Both long- and short-term Na$^{+}$, K$^{+}$, and Ca$^{2+}$ content measurements of leaves, culms, and roots were measured using flame photometry. Harvested tissues were washed with cold 20 mM LaCl$_3$ solution for 10 min. Fresh weights of the sample were noted and samples were subsequently dried at 80 °C for 3 d. Dried samples were incubated in 5 ml of 20 mM LaCl$_3$ for 24 h and measurements were recorded using a flame photometer (Sherwood flame photometer-410 Cambridge, UK). For N analysis, dried plant material was wrapped in aluminium foil prior to
loading into a CHNOS elemental analyser ‘vario Micro’ (Elementar, Hanau, Germany).

**RNA isolation and microarray hybridization**

Root RNA was isolated from control and salt-treated (50 mM) FL and IR plants using Trizol® reagent. RNA was purified by RNAeasy spin columns (Qiagen, London, UK). RNA was pooled from 3–4 independent sets of 6–8 plants for each experiment. This procedure was repeated three times for each cultivar and treatment, i.e. a total of 12 RNA samples was collected.

The 12 samples were sent to the Arizona microarray facility (http://ag.arizona.edu/microarray) where cDNA synthesis, Cy3 and Cy5 labelling and hybridization was carried out on NSF 45K 70-mer oligo microarrays. The arrays contain around 45 000 elements, representing all known ORFs present in the rice genome. For each cultivar, in one out of three replicate hybridizations, Cy3 and Cy5 dye labelling was swapped between treatment and control.

**Microarray data analysis**

All raw fluorescence data for both Cy3 and Cy5 labelled probes can be found for each biological replicate in the supplementary data. Data were analysed using SNOMAD software (available at http://pevsnerlab.kennedykrieger.org/snomadinput.html) for lowess signal correction, and spreadsheet software for other manipulations such as background subtraction, global mean normalization, calculation of average signals, and standard deviations as previously described (Maathuis, 2006; Moscatiello et al., 2006). Transcripts were included for further analysis when the following criteria were met for signal (S), background signal (BS), signal ratio (SR), and standard deviation of average SR (SD): (i) S-BS ≥100 and S/BS ≥1.5 in at least one channel and this fulfilment would assign a ‘present’ label; (ii) a ‘present’ signal in all three replicas; (iii) if criteria (i) and (ii) were met, SR/SD ≥1.5. Although the background signal intensity can have a large impact on the interpretation of microarray data (Pan et al., 2005), a doubling or halving of the chosen value (100) did not significantly change the general analysis outcome.

Transcripts were annotated as significantly regulated when, in addition to the above criteria, (i) the average of SR values for treated and control transcripts differed more than 2-fold and (ii) a t test of significance at P <0.05 was met. The fold-change cut-off criterion (2-fold) was based on the distribution of fold-changes observed in normalized control data. These provide a measurement for inherent variability and thus an estimate for the proportion of false positives that can be expected (Maathuis, 2006). A cut-off criterion of 2-fold should yield a false positives rate of less than 10%.

Prediction of transmembrane domains (TMDs) was carried out using the bulk sequence data retrieval from TIGR at: http://www.tigr.org/tdb/e2k1/osa1/batch_download.shtml and subsequent sequence analysis according to TMHMM at http://www.cbs.dtu.dk/services/TMHMM/.

**RT-PCR validation of microarray data**

Semi-quantitative RT-PCR was carried out on selected genes to confirm microarray data. A total of 2 μg RNA, isolated from the roots of treated and control samples, was used to synthesize first strand cDNA using the transcriptor high-fidelity cDNA synthesis kit (Roche, Mannheim, Germany). PCR was applied to 1 μl of cDNA with gene-specific primers against, OsHAK1 (Os04g32920), OsCAX (Os02g04630), OsCHX11 (Os05g31730), OsTPC1 (Os01g48680), and a high affinity nitrate transporter (Os02g02170). A list of primer sequences used in this analysis is given in the Supplementary data at JXB online. The house-keeping gene, tubulin α-1 (Os07g38730) was used as the control. PCR consisted of 35 cycles of 45 s at 56 °C, 1 min 30 s at 72 °C, and 30 s at 95 °C. For HAK, CHX, and the high affinity nitrate transporter, PCR was optimized for 35–45 cycles.

**Statistical analysis**

All data shown were derived from experiments carried out across a minimum of three biological replications. Growth, ion content, and microarray results were subjected to unpaired two-tailed t tests to identify significance at the P<0.05 level.

**Results and discussion**

**Physiological responses of FL478 and IR29 to salinity stress**

Previous studies (Walia et al., 2005, 2007) and our current results show that FL478 (FL) and IR29 (IR) exhibit discrete differences in many physiological parameters when exposed to salt stress. The most obvious is the significantly greater relative growth rate of FL (Fig. 1) which is particularly apparent in FL roots. Elongation of roots in the presence of salinity as seen in FL may be a stress-avoidance strategy where roots grow away from surface layers that tend to accumulate high levels of salts. Other parameters such as relative growth and photosynthetic rates, although negatively affected in both cultivars, remained higher in FL (Fig. 1) while maintaining a relatively low stomatal conductance compared to IR.

One potentially important tolerance mechanism is the restriction of Na⁺ into the plant, particularly into shoot tissues (Moradi et al., 2003), whereas an associated function is that of maintaining adequate nutrition of other minerals such as K⁺ and Ca²⁺ (Maathuis and Amtmann, 1999; Davenport and Tester, 2000). Earlier work showed that long-term (7–10 d) Na⁺ accumulation is lower in FL whereas that of K⁺ is higher (Walia et al., 2005), an observation that was repeated by us over a period of 12 d: after exposure to either 50 or 100 mM NaCl, FL maintained significantly lower [Na⁺], especially in leaf tissue, compared
to IR (Fig. 2A). By contrast, concentrations of K\(^+\), which are comparable in control conditions, were significantly higher in leaves of the tolerant FL, culminating in a notably lower Na\(^+\):K\(^+\) ratio in FL leaves (Fig. 2C, D). Similarly, Na\(^+\):K\(^+\) ratios in FL roots were two times lower than in IR (Fig. 2D) indicating a larger capacity in this cultivar to maintain adequate K\(^+\) nutrition in both roots and shoots.

Short-term Na\(^+\) uptake experiments (3 h) were carried out to see if the trends discussed above also pertain to shorter periods. Figure 3 shows that a 3 h exposure to 50 mM NaCl resulted in a different pattern of tissue Na\(^+\) in the two cultivars, indicating that FL and IR may also employ varying strategies to maintain Na\(^+\) homeostasis during relatively short periods of exposure to salt. Na\(^+\) accumulation increased in both cultivars but a clear difference was already apparent after 50 min uptake with higher Na\(^+\) concentrations in IR leaf and culm tissues. More importantly, the leaf [Na\(^+\)] in FL quickly stabilizes and even reduces slightly after 3 h, whereas that of IR leaves continues to increase. Thus, after 3 h, IR actually contained higher [Na\(^+\)] in its leaf tissue than in its roots.

The findings from these short-term uptake experiments agree with those made in protoplasts and suspension culture cells where [Na\(^+\)] was measured with the Na\(^+\) reporting dye SBFI (Kader and Lindbergh, 2005; Anil et al., 2007). These studies reported a much smaller Na\(^+\) influx in the tolerant cultivar Pokkali compared to the sensitive BRRI Dhan29 or Jaya variety and the main conclusion was that plasma membranes from Pokkali have a considerably lower Na\(^+\) conductance. Our short-term net Na\(^+\) uptake data appear to confirm this model and show a 2–3-fold larger Na\(^+\) uptake in the sensitive cultivar.
Ca\(^{2+}\) has multiple effects on plant salinity and in rice, as in many other species, ameliorates stress symptoms (Pua et al., 2001; Shah et al., 2002). In many plants Ca\(^{2+}\) translocation to the shoot is compromised during salinity, due to the combination of reduced transpiration and the symplastic immobility of Ca\(^{2+}\) (Lynch and Lauchli, 1985). However, shoot Ca\(^{2+}\) deficiency does not appear to occur in rice: in both varieties, shoot Ca\(^{2+}\) levels remained unchanged or even increased in the presence of NaCl (Fig. 2C), particularly in the sensitive IR. The substantial apoplastic conductance of rice roots, or ‘bypass flow’ (Garcia et al., 1997), compared to other plants may explain why Ca\(^{2+}\) supply to the leaves is not affected by salinity. Given that substantial amounts of Ca\(^{2+}\) are transported via the apoplastic route (White, 2001), the higher accumulation of leaf Ca\(^{2+}\) in IR suggests the conductance of this pathway is larger in this cultivar compared to FL.

In combination, the short and long exposure data show that (i) net Na\(^{+}\) influx is considerably lower in FL than in IR, both during short- and long-term exposure to salt, (ii) Na\(^{+}\) translocation to the shoot in FL is limited, and (iii) in both cultivars [K\(^{+}\)] decreases but FL maintains a higher concentration in its leaves. This variation could significantly contribute to the higher level of tolerance in FL and must be related to transport functions, particularly of Na\(^{+}\) and K\(^{+}\) in roots and their translocation to the shoots. To investigate further which molecular mechanisms could underlie these observed differences, whole genome transcriptomics were used to assess the transcriptional regulation of genes that encode root membrane transporter proteins and those that are potentially involved in the above phenomena were identified. To validate microarray data, RT-PCR was carried out on a selection of transcripts that ranged from around 0.3–14-fold changes and Fig. 4 shows excellent agreement between the two types of analysis.

**Transporter genes that may contribute to salinity tolerance in both cultivars**

In both cultivars (Tables 1, 2), genes encoding nitrogen transporters are significantly up-regulated although not necessarily for the same isoform. Many other studies showed that salinity affects transcript levels of nitrogen transporters (Maathuis et al., 2003; Walia et al., 2007) which may point to generic nutrient deficiency during stress. In addition, high Cl\(^{-}\) concentrations can inhibit NO\(_3\)/CO\(_3\) transport and thus up-regulation of nitrate and ammonium uptake would be beneficial to withstand salt stress (Ehlting et al., 2007). Ambient NaCl will also depolarize root cells and, therefore, reduce ammonium uptake which partly occurs through membrane potential-driven uniport (Ludewig et al., 2002). Analysis of N content in roots and shoots of both cultivars showed that %N is not significantly affected in FL by salt treatment. However, there is a significant (t test, P <0.05) decrease in the N content of NaCl treated

---

**Fig. 4.** Semi-quantitative RT-PCR analysis of selected genes to validate microarray data. Genes were selected to represent the different regulatory responses recorded in transcriptomics. Differential expression values are shown and calculated using densitometry for NaCl-treated roots (FL50 and IR50) relative to the control condition (FL0 and IR0) for both the transcriptomics analysis (T) and semi-quantitative RT-PCR analysis (RT). (*–*, No detectable expression.)
IR leaves (Table 3). Collectively, these results suggest that nitrogen nutrition becomes compromised in the sensitive cultivar IR, whereas the tolerant FL is capable of maintaining its N homeostasis, possibly by up-regulating relevant root N transporters.

The root-specific aquaglyceroporin, OsNIP2;1 has been shown to be an important constituent of silicon uptake in rice (Ma et al., 2006). Rice is a typical silicon accumulator and has a large requirement for this element which is believed to play a multitude of roles, including resistance to abiotic and biotic stress (Liang et al., 2003; Zhu et al., 2004). Maintaining the integrity of the casparian strip in root exo- and endodermis is an important role of silicon which restricts apoplastic entry of ions such as Na⁺ (Pary and Soni, 1972; Savant et al., 1997). Thus, silicon reduces the bypass flow and limits Na⁺ translocation to the shoot, whereas it does not affect K⁺ uptake and distribution (Yeo et al., 1999; Gong et al., 2006). Salinity-induced up-regulation of OsNIP2;1 would improve silicon uptake and help restrict bypass-mediated Na⁺ translocation from the root to the shoot.

The relevance of OsNIP2;1 in salt tolerance was studied further using a loss of function mutant. The nip2;1 or lsi1 mutant is considerably more salt-sensitive than the wild type, even in the presence of 3 mM silicon (see Supplementary Fig. S1 at JXB online). As expected in a mutant where the bypass pathway is not, or less, reduced by silicon addition, cation tissue analysis in lsi1 and the wild type shows a considerably higher Na⁺ concentration in lsi1 leaves (see Supplementary Fig. S2A at JXB online).

### Table 1. Membrane protein transcripts regulated in roots of the salt-tolerant rice cultivar FL478

Plants were grown in hydroponics for 3 weeks and subsequently treated with 50 mM NaCl for 12 d. Membrane protein transcripts that changed by >2-fold are listed with the corresponding change in the same transcript from the salt-sensitive cultivar IR29. Open space denotes the absence of signal. TMD: number of predicted transmembrane domains.

| Locus       | Annotation                                                   | Degree of regulation | TMD | Ortho/homologues in Arabidopsis thaliana |
|-------------|--------------------------------------------------------------|----------------------|-----|------------------------------------------|
|             |                                                              | FL 478               | IR 29 | FL/IR |                                         |
| **Water transport** |                                                              |                      |      |                                            |
| Os03g05290  | Aquaporin TIP1.1                                             | 2.39                 | 2.32 | 0.97  | At2g36830 (AtTIP1.1)                     |
|             |                                                              |                      |      |                                            |
| **Nitrate and ammonium transport** |                                                              |                      |      |                                            |
| Os04g43070  | Ammonium transporter (OsAMT1.1)                              | 2.08                 | 0.98 | 2.12  | At9g24290 (AtAMT1.5)                     |
| Os02g40730  | Ammonium transporter (OsAMT1.3)                              | 3.03                 | 1.2  | 2.53  | At4g13510 (AtAMT1.1)                     |
| Os02g02170  | High affinity nitrate transporter                            | 5.14                 |      |      | At5g60770 (AtNRT2.4)                     |
|             |                                                              |                      |      |                                            |
| **Amino acid transport** |                                                              |                      |      |                                            |
| Os02g44980  | Amino acid transport protein, putative, amino acid/auxin permease | 2.20                | 1.35 | 1.63  | At2g42005 Amino acid transporter         |
| Os08g03350  | Plasma-membrane localized histidine transporter (OsHT1)      | 2.94                 | 1.56 | 1.88  | At5g40780 (AtLHT1)                      |
| Os01g40410  | Amino acid transporter family protein                        | 0.37                 | 1.39 | 3.76  | At3g28960 Amino acid transporter         |
|             |                                                              |                      |      |                                            |
| **Mono- and divalent cation transport** |                                                              |                      |      |                                            |
| Os05g31730  | Putative cation-proton exchanger (OsCHX1)                    | 14.9                 | 2.49 | 5.99  | At4g23700 (AtCHX17)                     |
| Os07g15370  | Metal cation transporter Nramp1                              | 2.04                 | 1.06 | 1.92  | At1g80830 (AtNRM1)                      |
| Os01g56420  | Ctr copper transporter family protein, expressed             | 2.28                 | 1.00 | 2.28  | At5g59030 (AtCOP1)                      |
| Os04g36720  | Ferric-chelate reductase (OsFRO1)                            | 0.50                 | 1.14 | 0.44  | At5g49730 (AtFRO6)                      |
| Os02g04030  | CAX-type proton/calcium exchanger protein                    | 0.48                 | 1.31 | 0.37  | At1g55730 (AtCAX5)                      |
| Os07g47350  | Potassium transporter (OsHAK7)                               | 0.41                 | 0.36 | 0.30  | At5g20050 (AtKUP3/AtKT4)                |
|             |                                                              |                      |      |                                            |
| **Cation channels** |                                                              |                      |      |                                            |
| Os06g33600  | Cyclic nucleotide-gated ion channel 1                       | 0.44                 | 1.38 | 0.32  | At5g53130 (ACNGC1)                      |
|             |                                                              |                      |      |                                            |
| **Sugar transport** |                                                              |                      |      |                                            |
| Os09g249240 | Sugar/sugar alcohol proton symporter                        | 0.40                 | 1.57 | 0.25  | At4g02050 (AtSTP7)                      |
| Os10g42830  | Sugar/sugar alcohol proton symporter                        | 0.45                 | 1.23 | 0.37  | At5g17010 xylose transporter             |
|             |                                                              |                      |      |                                            |
| **Other transporters** |                                                              |                      |      |                                            |
| Os02g51110  | Silicon influx transporter (OsLsi1/OsSIT1/OsNIP2.1)          | 2.12                 | 1.82 | 1.16  | At5g37820 (AtNIP4.2/AtNLMS)             |
| Os06g38950  | ABC transporter family protein, 2.76                        | 1.89                 | 1.46 | 5     | At9g47790 (AtATH7)                      |
|             |                                                              |                      |      |                                            |
| **Other transporters** |                                                              |                      |      |                                            |
| Os06g38950  | ABC transporter family protein, 2.76                        | 1.89                 | 1.46 | 5     | At9g47790 (AtATH7)                      |
| Os03g09070  | Sulphate transporter 1.2                                    | 2.03                 | 0.70 | 2.86  | At1g78000 (AtSulf1.2)                   |
| Os02g21750  | ABC transporter family protein, 0.44                        | 1.68                 | 0.26 | 4     | At1g25520 (AtPSP1/AtMDR8)               |
| Os08g43120  | ABC transporter, putative, pleiotropic drug resistance ABC transporter (OsPDR1) | 0.48 | 1.28 | 0.37 | At2g36380 (AtPDR6)                      |
| Os06g19110  | Cadmium tolerance factor                                    | 0.47                 |      | 20    |                                            |
| Os11g04830  | Cadmium tolerance factor                                    | 0.44                 | 1.27 | 0.34  | 20                                         |
| Os01g19290  | Nodulin like protein                                        | 0.44                 | 1.46 | 0.30  | 20                                         |
| Os11g04830  | Cadmium tolerance factor                                    | 0.44                 | 1.27 | 0.34  | 20                                         |
However, Ca$^{2+}$ levels were also considerably higher in *lsi1* mutants (see Supplementary Fig. S2B at *JXB* online).

**OsTIP1;1** is a tonoplast-expressed aquaporin found in both root and leaf tissue and is up-regulated in both FL and IR (Tables 1, 2). In roots, it is predominantly found in the rhizodermis and exodermis. TIPs are generally contributing to osmotic and turgor homeostasis (Maurel *et al.*, 1993) and several have been recorded to be up-regulated in *Arabidopsis* roots in response to salt stress (Maathuis *et al.*, 2003). No significant regulation of *OsTIP1;1* occurs in shoot tissue (Walia *et al.*, 2005) and no other aquaporins were identified as being regulated more than 2-fold in roots. This may suggest that *OsTIP1;1* contributes to osmotic homeostasis and salt tolerance in rice roots.

**Table 2.** Membrane protein transcripts regulated in roots of the salt-sensitive rice cultivar IR29

| Locus            | Annotation/putative function                                      | Degree of regulation | TMD | Ortho/homologues in *Arabidopsis thaliana* |
|------------------|-------------------------------------------------------------------|----------------------|-----|------------------------------------------|
| Water transport  |                                                                   |                      |     |                                          |
| Os03g05290       | Aquaporin TIP1.1                                                   | 2.39  2.32  0.97     | 6   | At2g36830 (AtTIP1.1)                     |
| Nitrate and ammonium transport |                                                               |                      |     |                                          |
| Os01g050820      | Nitrate transporter                                                | 2.10  1.87  0.89     | 10  | At1g12940 (AtNRT2.5)                     |
| Os12g29960       | Nitrate transporter, putative, nodulin family protein              | 2.58  1.87  0.89     | 12  | At2g39210 nodulin-type channel           |
| Os11g23890       | Low-affinity nitrate transporter                                    | 0.46  0.62  1.35     | 11  | At3g16180 proton-dependent oligopeptide or low-affinity nitrate transporter |
| Amino acid transport |                                                               |                      |     |                                          |
| Os02g09810       | Amino acid transporter family protein                               | 2.11  1.21  0.57     | 10  | At3g30390 Amino acid transporter         |
| Os01g40380       | Amino acid transporter                                             | 2.13  1.21  0.57     | 4   | At2g41190 Amino acid transporter         |
| Mono- and divalent cation transport |                                                               |                      |     |                                          |
| Os05g31730       | Cation-proton exchanger (*OsCHX1.1*)                               | 2.49  1.49  0.59     | 12  | At4g23700 (AtCHX17)                     |
| Os12g03830       | Major facilitator superfamily antiporter                           | 0.44  1.19  2.68     | 9   | At5g13740 (AtZIF1)                      |
| Os11g04020       | Major facilitator superfamily antiporter                           | 0.39  0.62  1.58     | 12  | At5g13750 (AtZIFL1)                     |
| Cation channels  |                                                                   |                      |     |                                          |
| Os01g48680       | Voltage-gated Ca$^{2+}$-permeable channel (*OsTPC1*)               | 2.19  0.53  0.24     | 11  | At4g03560 (AtTPC1)                      |
| Other transporters|                                                                  |                      |     |                                          |
| Os08g44750       | Nodulin MtN21 family protein                                       | 0.45  0.79  1.76     | 10  | At1g75500                               |

**Table 3.** Nitrogen content in shoot (S) and root (R) tissues of FL478 and IR29 rice cultivars grown without (control) and with 50 mM NaCl

At 15 DAS, plants were exposed to salt by adding NaCl to the hydroponic medium to a concentration of 50 mM. After 12 d of exposure to salt, percentage N on a dry weight basis in shoot (S) and root (R) of the tolerant FL478 and sensitive IR29 was determined. Results are mean values of three replicates (±SD) comprising three plants per replicate.

| Cultivar | Percentage nitrogen content |
|----------|-----------------------------|
|          | Control  | 50 mM NaCl                  |
| FL478    | S        | 4.39±0.16                   |
|          | R        | 2.46±0.25                   |
| IR29     | S        | 4.30±0.12                   |
|          | R        | 2.61±0.13                   |

However, Ca$^{2+}$ levels were also considerably higher in *lsi1* mutants (see Supplementary Fig. S2B at *JXB* online).

**OsTIP1;1** is a tonoplast-expressed aquaporin found in both root and leaf tissue and is up-regulated in both FL and IR (Tables 1, 2). In roots, it is predominantly found in the rhizodermis and exodermis. TIPs are generally contributing to osmotic and turgor homeostasis (Maurel *et al.*, 1993) and several have been recorded to be up-regulated in *Arabidopsis* roots in response to salt stress (Maathuis *et al.*, 2003). No significant regulation of *OsTIP1;1* occurs in shoot tissue (Walia *et al.*, 2005) and no other aquaporins were identified as being regulated more than 2-fold in roots. This may suggest that *OsTIP1;1* contributes to osmotic homeostasis and salt tolerance in rice roots.

Amongst membrane protein transcripts without functional annotation, Os02g37380 is up-regulated in both cultivars by 3.2-fold and 12.3-fold in FL and IR, respectively (see Supplementary data FL Up Down and IR Up Down at *JXB* online). This one transmembrane span protein does not show homology to any annotated genes, is expressed exclusively in roots (e.g. http://mpss.udel.edu/rice/) and the large increase in its transcript number in response to 50 mM NaCl suggests a function in root salt tolerance.

**Transporter genes that may contribute to reduced root Na$^+$ influx in FL**

A major proportion of Na$^+$ that gets into plant roots is likely to be transported through non-selective cation channels (Demidchik and Maathuis, 2007) although more recent work suggests that, at least in halophytes, K$^+$ channels may also mediate Na$^+$ uptake (Wang *et al.*, 2007). The molecular identity of channels that mediate Na$^+$ influx is largely unknown. In *Arabidopsis*, a member of the cyclic nucleotide gated channel (CNGC) family, AtCNGC3, was shown to have a moderate effect on Na$^+$ uptake (Gobert *et al.*, 2006). Thus, down-regulation of OsCNGC1 in FL roots (Table 1), which showed more than 2-fold expression compared with IR in control conditions (Table 4), may similarly contribute to restricting Na$^+$ entry. OsCNGC1

However, Ca$^{2+}$ levels were also considerably higher in *lsi1* mutants (see Supplementary Fig. S2B at *JXB* online).

**OsTIP1;1** is a tonoplast-expressed aquaporin found in both root and leaf tissue and is up-regulated in both FL and IR (Tables 1, 2). In roots, it is predominantly found in the rhizodermis and exodermis. TIPs are generally contributing to osmotic and turgor homeostasis (Maurel *et al.*, 1993) and several have been recorded to be up-regulated in *Arabidopsis* roots in response to salt stress (Maathuis *et al.*, 2003). No significant regulation of *OsTIP1;1* occurs in shoot tissue (Walia *et al.*, 2005) and no other aquaporins were identified as being regulated more than 2-fold in roots. This may suggest that *OsTIP1;1* contributes to osmotic homeostasis and salt tolerance in rice roots.

Amongst membrane protein transcripts without functional annotation, Os02g37380 is up-regulated in both cultivars by 3.2-fold and 12.3-fold in FL and IR, respectively (see Supplementary data FL Up Down and IR Up Down at *JXB* online). This one transmembrane span protein does not show homology to any annotated genes, is expressed exclusively in roots (e.g. http://mpss.udel.edu/rice/) and the large increase in its transcript number in response to 50 mM NaCl suggests a function in root salt tolerance.

**Table 3.** Nitrogen content in shoot (S) and root (R) tissues of FL478 and IR29 rice cultivars grown without (control) and with 50 mM NaCl

At 15 DAS, plants were exposed to salt by adding NaCl to the hydroponic medium to a concentration of 50 mM. After 12 d of exposure to salt, percentage N on a dry weight basis in shoot (S) and root (R) of the tolerant FL478 and sensitive IR29 was determined. Results are mean values of three replicates (±SD) comprising three plants per replicate.

| Cultivar | Percentage nitrogen content |
|----------|-----------------------------|
|          | Control  | 50 mM NaCl                  |
| FL478    | S        | 4.39±0.16                   |
|          | R        | 2.46±0.25                   |
| IR29     | S        | 4.30±0.12                   |
|          | R        | 2.61±0.13                   |
transcript level in shoots is not significantly affected by salinity in either cultivar (Walia et al., 2005).

Although all HAK high affinity K+ transporters are competitively inhibited by Na+, some may also transport this ion, as was shown for barley HvHAK1 (Santa-Maria et al., 1997). If similar properties pertain to OsHAK7, its down-regulation in FL roots may prevent Na+ leak into the root symplast and, consequently, the overall Na+ load.

Transporter genes that may contribute to reduced Na+ translocation to FL shoots

As mentioned above, Ca2+ translocation to the shoot is often compromised during salinity, due to a combination of reduced transpiration and the symplastic immobility of Ca2+. Ca2+ nutrition in the root may also impact on salinity via limiting the bypass flow (Anil et al., 2005). OsACA4 is a P-type 2B Ca2+ ATPase located at the plasma membrane and involved in Ca2+ extrusion into the apoplast (Geisler et al., 2000). In control conditions (Table 4), expression of OsACA4 was around 5-fold higher in FL compared to IR which may point to a greater release of Ca2+ into the FL root apoplast. This mechanism may be further augmented during salinity stress by the reduced vacuolar Ca2+ deposition in FL roots resulting from the down-regulation of the CAX-type antiporter Os02g04630 whose transcript level was not affected in IR by salinity. Augmented levels of apoplastic Ca2+ have been proposed to limit the bypass flow by reducing the ‘leakiness’ of endodermal junctions (Anil et al., 2005) and thus would restrict Na+ translocation to the shoot.

In Arabidopsis roots, CAX transcript levels also responded to salinity stress (Maathuis, 2006) and several CAX isoforms were shown to be important in salt-related Ca2+ translocation and signalling (Zhao et al., 2008). The Ca2+ permeable channel OsTPC1 has also been shown to impact on rice Ca2+ nutrition and sensitivity to environmental stress where overexpression resulted in reduced growth in plants (Kurusu et al., 2004). Its down-regulation in FL and up-regulation in IR as shown in both microarray and RT-PCR data (Table 1; Fig. 4) may point to differential Ca2+ homeostasis that impacts on salt tolerance.

Transporter genes that may contribute to a reduced Na+:K+ ratio in FL

K+ nutrition and homeostasis can be negatively affected during salt stress and salinity-induced transcriptional regulation of K+ transporters has been observed before in rice (Bañuelos et al., 2002; Walia et al., 2005) and Arabidopsis (Maathuis et al., 2003). On the basis of microarray data, the Os05g31730 transcript level is considerably higher after salinity treatment, in both FL and IR (Table 1).
Os05g31730 encodes the putative monovalent cation exchanger CHX11. The closest homologue to OsCHX11 in Arabidopsis is AtCHX17. AtCHX17 is primarily expressed in the epidermal and cortical root tissues (Cellier et al., 2004). In Arabidopsis, CHX17 expression also rapidly increased in response to salinity. In addition, it increased after ABA treatment or K⁺ deprivation. A loss of function mutant accumulated less K⁺ in roots in response to salt stress or K⁺ shortage (Cellier et al., 2004). These findings indicate that AtCHX17 helps maintain K⁺ homeostasis by providing extra K⁺ acquisition capacity, for example, to compensate for the loss of K⁺ uptake through HAK/KUP-type systems which are sensitive to Na⁺ inhibition (Santa-Maria et al., 1997). It is tempting to envisage a similar role for OsCHX11 in rice and it would be interesting to see if ABA and K⁺ deficiency induce OsCHX11 transcription. Its far greater up-regulation in FL could explain this cultivar’s ability to maintain a significantly higher K⁺ concentration in its tissues compared with IR.

In FL roots, OsHAK7 showed significantly decreased transcription. The primary high affinity K⁺ uptake mechanism in rice root is believed to be OsHAK1 (Grabov, 2008), but no functional data are available for OsHAK7. Its closest Arabidopsis homologue is AtKUP4 which has been shown to be involved in root hair growth (Rigas et al., 2001) and is probably located in the vacuole (Whiteman et al., 2008). If OsHAK7 is similarly expressed at the tonoplast, as was also shown for other members of the HAK family (Bañuelos et al., 2002), its down-regulation by approximately 2.4-fold in FL might signify retention of vacuole sequestered root K⁺.

Concluding remarks

Improvement of rice salt tolerance is urgently required, but necessitates a detailed understanding of the processes, genes, and proteins involved. Comparing physiological and transcriptional parameters in cultivars with divergent levels of sensitivity could provide an excellent basis to increase our knowledge in this respect. For rice cultivars FL478 and IR29, such a study was carried out for root tissue and membrane transporters were identified that may contribute to the difference in tolerance between FL and IR such as OsCHX11 and OsCNGC1. Our data show that transcripts of specific membrane proteins in roots, for example, OsCHX11, OsTIP1;1, Lsi1, and Os02g37380, may form important targets for tolerance that were not identified in shoot tissue. Vice versa, putative target membrane proteins previously detected in shoot tissue (Walia et al., 2005, 2007) show little overlap with those found in roots, emphasizing the existence of different mechanisms in shoots and roots and the necessity to carry out analyses in multiple tissues.

Supplementary data

Supplementary data are available at *JXB* online and comprise the following figures and data files.

**Supplementary Fig. S1.** Effect of increasing salinity on relative growth rates for the rice mutant *lsil* and wild-type cultivar *Nipponbare*.

**Supplementary Fig. S2.** Na⁺ and Ca²⁺ ion concentrations in different tissues of rice mutant *lsil* and wild-type cultivar *Nipponbare* after exposure to salinity stress in the presence of Si.

**Supplementary data.** Gene expression data of the transcriptome assay of the roots of FL478 and IR29 in response to salinity stress and sequences of the primers used in validating microarray data analysis. Files contain: a ‘Leg-’ describing various data sets; ‘Raw data’ consisting of signal and background fluorescence intensities of each wavelength for treatment and control for FL and IR; ‘Normalized data’ for each element; ‘Primers’ used for control RT-PCR analyses.

Acknowledgements

We thank Professor Jian Feng Ma (Okayama University) for providing seeds of *lsil* rice mutants and Dr Leon van den Berg (University of York) for assisting in N analysis. This study was partly funded by a Commonwealth Commission of the UK grant to PS.

References

Akbar M, Yabuno T, Nakao S. 1972. Breeding for saline resistant varieties of rice. I. Variability for salt tolerance among some rice varieties. *Japan Journal of Breeding* 22, 277–284.

Anil VS, Krishnamurthy P, Kuruvilla S, Sucharitha K, Thomas J, Mathew MK. 2005. Regulation of the uptake and distribution of Na⁺ in shoots of rice (*Oryza sativa L.*) variety Pokkali: role of Ca²⁺ in salt tolerance response. *Physiologia Plantarum* 124, 451–464.

Anil VS, Krishnamurthy H, Mathew MK. 2007. Limiting cytosolic Na⁺ confers salt tolerance to rice cells in culture: a two-photon microscopy study of SBFI-loaded cells. *Physiologia Plantarum* 129, 607–621.

Arteca R, Arteca JM. 2000. A novel method for growing Arabidopsis thaliana plants hydroponically. *Physiologia Plantarum* 108, 188–193.

Bañuelos MA, Garcia-deblas B, Cubero B, Rodriguez-Navarro A. 2002. Inventory and functional characterization of the HAK potassium transporters of rice. *Plant Physiology* 130, 784–795.

Blumwald E. 2000. Sodium transport and salt tolerance in plants. *Current Opinion in Cell Biology* 12, 431–434.

Cellier F, Coméjéro G, Ricaud L, Luu DT, Lepetit M, Gosti F, Casse F. 2004. Characterization of AtCHX17, a member of the cation/H⁺ exchangers, CHX family, from Arabidopsis thaliana suggests a role in K⁺ homeostasis. *The Plant Journal* 39, 834–846.
Chao DY, Luo YH, Shi M, Luo D, Lin HX. 2005. Salt-responsive genes in rice revealed by cDNA microarray analysis. Cell Research 15, 796–810.

Davenport RJ, Tester M. 2000. A weakly voltage-dependant, non-selective cation channel mediates toxic sodium influx in wheat. Plant Physiology 122, 823–834.

Demidchik V, Maathuis FJM. 2007. Physiological roles of non-selective cation channels in plants: from salt stress to signalling and development. New Phytologist 18, 387–404.

Ehlting B, Dluzniewska P, Dietrich H, et al. 2007. Interaction of nitrogen nutrition and salinity in Grey poplar (Populus tremula×alba). Plant, Cell and Environment 30, 796–811.

Flowers TJ, Colmer TD. 2008. Salinity tolerance in halophytes. New Phytologist 179, 945–963.

Garcia A, Rizzo CA, Ud-Din J, Bartos SL, Senadhiria D, Flowers TJ, Yeo AR. 1997. Sodium and potassium transport to the xylem are inherited independently in rice, and the mechanism of sodium:potassium selectivity differs between rice and wheat. Plant, Cell and Environment 20, 1167–1174.

Gobert A, Park G, Amtmann A, Sanders D, Maathuis FJM. 2006. Arabidopsis thaliana cyclic nucleotide gated channel 3 forms a non-selective ion transporter involved in germination and cation transport. Journal of Experimental Botany 57, 791–800.

Gong HJ, Randall DP, Flowers TJ. 2006. Silicon deposition in the root reduces sodium uptake in rice (Oryza sativa L.) seedlings by reducing bypass flow. Plant, Cell and Environment 29, 1970–1979.

Grabov A. 2008. Plant KT/KUP/HAK potassium transporters: single-family–multiple families. Annals of Botany 99, 1035–1041.

Geisler M, Axelsen KB, Harper JF, Palmgren MG. 2000. Molecular aspects of higher plant P-type Ca2+-ATPases. Biochimica et Biophysica Acta 1465, 52–78.

Gratton SR, Zeng L, Shannon MC, Roberts SR. 2002. Rice is more sensitive to salinity than previously thought. California Agriculture 56, 189–195.

Kader MA, Lindberg S. 2005. Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, Oryza sativa L. determined by the fluorescent dye SBFI. Journal of Experimental Botany 56, 3149–3158.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ. 2001. Gene expression profiles during the initial phase of salt stress in rice. The Plant Cell 13, 889–905.

Kurusu T, Sakurai Y, Miyao A, Hirochika H, Kuchitsu K. 2004. Identification of a putative voltage-gated Ca2+-permeable channel (OsTPC1) involved in Ca2+ influx and regulation of growth and development in rice. Plant and Cell Physiology 45, 693–702.

Liang Y, Chen Q, Liu Q, Zhang W, Ding R. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (Hordeum vulgare L.). Journal of Plant Physiology 160, 1157–1164.

Ludwig U, von Wiren N, Frommer WB. 2002. Unipo of NH3 by the root hair plasma membrane ammonium transporter LeAMT1;1. Journal of Biological Chemistry 277, 13548–13555.

Lynch J, Lauchli A. 1985. Salt stress disturbs the calcium nutrition of barley (Hordeum vulgare L.). New Phytologist 99, 345–354.

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. 2006. A silicon transporter in rice. Nature 440, 688–691.

Maathuis FJM. 2006. The role of monovalent cation transporters in plant responses to salinity. Journal of Experimental Botany 57, 1137–1147.

Maathuis FJM, Amtmann AA. 1999. Nutrition and Na+ toxicity; the basis of cellular K+/Na+ ratios. Annals of Botany 84, 123–133.

Maathuis FJM, Filatov V, Herzyk P, et al. 2003. Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. The Plant Journal 35, 675–692.

Maurel C, Reizer J, Schroeder JI, Chrispeels MJ. 1993. The vacular membrane protein γ-TIP creates water specific channels in Xenopus oocytes. EMBO Journal 12, 2241–2247.

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.

Moscatiello R, Mariani P, Sanders D, Maathuis FJM. 2006. Transcriptional analysis of calcium-dependent and calcium-independent signalling pathways induced by oligogalacturonides. Journal of Experimental Botany 57, 2847–2865.

Munns R. 2002. Comparative physiology of salt and water stress. Plant, Cell and Environment 25, 239–250.

Pan KH, Lih CJ Cohen SN. 2005. Effects of threshold choice on biological conclusions reached during analysis of gene expression by DNA microarrays. Proceedings of the National Academy of Sciences, USA 102, 8961–8965.

Pary DW, Soni SL. 1972. Electronic- probe micro analysis of the silicon in the roots of Oryza sativa L. Annals of Botany 36, 781–783.

Poorter H, Garnier E. 1999. Ecological significance in inherent variation in relative growth rate and its components. In: Pugnaire FI, Vallades F, eds. Handbook of functional ecology. New York: Marcel Dekker Inc, 81–120.

Pua ARM, Rivera GC, Bonilla PS. 2001. Interactive effects of calcium and salinity on the seedling growth and photosynthesis of salt-sensitive and salt-tolerant varieties of rice (Oryza sativa L.). Philippine Journal of Science 130, 63–70.

Rigas S, Debrosses G, Haralampidis K, Vicente-Aguillo F, Feldmann K, Grabov A, Dolan L, Hatzopoulos P. 2001. Trh1 encodes a potassium transporter required for tip growth in Arabidopsis root hairs. The Plant Cell 13, 139–151.

Santa-Maria GE, Rubio F, Dubcovsky J, Rodriguez-Navarro A. 1997. The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. The Plant Cell 9, 2281–2289.

Savant NK, Snyder GH, Datnoff LE. 1997. Silicon management and sustainable rice production. Advances in Agronomy 8, 151–199.

Shah A, Imamu Huq SM, Kawai S, Islam A. 2002. Effects of applying calcium salts to coastal saline soil and growth and mineral nutrition of rice varieties. Journal of Plant Nutrition 25, 561–576.
Ueda A, Kathiresan A, Bennett J, Takabe T. 2006. Comparative transcriptome analyses of barley and rice under salt stress. *Theoretical and Applied Genetics* 112, 1286–1294.

Walia H, Wilson C, Condamine P, et al. 2005. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiology* 139, 822–835.

Walia H, Wilson C, Zeng L, Ismail AM, Condamine P, Close TJ. 2007. Genome-wide transcriptional analysis of salinity-stressed japonica and indica rice genotypes during panicle initiation stage. *Plant Molecular Biology* 63, 609–623.

Wang SM, Zhang JL, Flowers TJ. 2007. Low-affinity Na⁺ uptake in the halophyte *Suaeda maritima*. *Plant Physiology* 145, 559–571.

White PJ. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* 52, 891–889.

Whiteman S, Serazetdinova L, Jones A, Sanders D, Peck S, Maathuis FJM. 2008. Identification of novel proteins and phosphorylation sites in the vacuolar membrane of *Arabidopsis thaliana*. *Proteomics* 8, 3536–3547.

Yeo AR, Flowers A, Rao G, Welfare K, Senanayake N, Flowers TJ. 1999. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in transpirational bypass flow. *Plant, Cell and Environment* 22, 559–565.

Yeo MR, Yeo ME, Flowers TJ. 1987. Contribution of an apoplastic pathway to sodium uptake by rice roots in saline condition. *Journal of Experimental Botany* 38, 1141–1153.

Zhao J, Barkla B, Marshall J, Pittman JK, Hirschi KD. 2008. The *Arabidopsis* cax3 mutants display altered salt tolerance, pH sensitivity and reduced plasma membrane H⁺-ATPase. *Planta* 227, 659–669.

Zhou J, Wang X, Jiao Y, Qin Y, Liu X, He K, Chen C, Ma L, Wang J. 2007. Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot, flag leaf, and panicle. *Plant Molecular Biology* 63, 591–608.

Zhu Z, Li G, Qian Q, Yu J. 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science* 167, 527–533.