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Minireview

Na^+ transport in plants

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Abstract The ability of plants to grow in high NaCl concentrations is associated with the ability of the plants to transport, compartmentalize, extrude, and mobilize Na^+ ions. While the influx and efflux at the roots establish the steady state rate of entry of Na^+ into the plant, the compartmentation of Na^+ into the cell vacuoles and the radial transport of Na^+ to the stele and its loading into the xylem establish the homeostatic control of Na^+ in the cytosol of the root cells. Removal of Na^+ from the transpirational stream, its distribution within the plant and its progressive accumulation in the leaf vacuoles, will determine the ability to deal with the toxic effects of Na^+. The aim of this review is to highlight and discuss the recent progress in understanding of Na^+ transport in plants.

Keywords: Sodium; Transport; Plants; NHX; HKT; Ion channels

1. Introduction

The aim of this review is to highlight the recent progress in understanding of Na^+ transport in plants. Several recent reviews of this area of study have been made [1–3] and these describe, in more detail, and in a more eloquent way than we present here, the transport of Na^+ from the growing medium into the roots, the Na^+ loading into and unloading from the xylem, and its (re)distribution within the plant. There have been several important publications that made incremental contributions to this field, and we seek to highlight those in context. And there are a few areas of debate that deserve comment and continued reexamination to which we seek to draw your attention. Because of space constraints, we were unable to reference many of important contributions of our colleagues and we regret omissions. In this review, we use the nomenclature for HKT transporters suggested by Platten et al. [4].

2. The influx of Na^+ into the plants

The greatest proportion of the root surface area is that presented by root hairs, and it is across the plasma membrane of these epidermal cells that the greatest portion of ion uptake occurs. Early studies of Na^+ uptake with whole plants and excised roots led to the notion that two modes of Na^+ uptake were in operation in the roots, a rapidly saturating system that showed high affinity transport for both Na^+ and K^+, and a non-saturating low affinity transport system [5]. Even the simplest model for root sodium transport must account for multiple compartments: the epidermal layer and cortex as one compartment (with vacuoles) are separated from the stele by the Casparian band, stelar cells (with vacuoles) adjacent to either the xylem (xylem parenchyma), or phloem (phloem companion cells) (Fig. 1). In this paper, we will discuss the influx of Na^+ into the roots as the entry of Na^+ into the first plant compartment from the external medium. Under typical physiological conditions, plants maintain a high K^+/Na^+ ratio in their cytosol with relatively high K^+ (100–200 mM) and low Na^+ concentrations (1–10 mM) [6]. Given the negative electrical membrane potential difference at the plasma membrane (−140 mV) [6], a rise in extracellular Na^+ concentrations will establish a large Na^+ electrochemical potential gradient that will favor the passive transport of sodium from the environment into the cytosol. Influx measurements of Na^+ into whole root/excised roots show a very high rate during the time period in which efflux of the radioactive tracers is minimal [1]. The high rate component (initially linear) of Na^+ influx is reduced within ten minutes, presumably by Na^+ efflux from the root epidermis and cortex. These high fluxes have been recorded also in barley [7]. This high rate of influx has been highlighted as a potential confounding phenomenon in the assessment of low affinity transport kinetics [7]. Tester and Davenport [1] also pointed out that the high rates of influx measured with tracers match well with the magnitude of sodium inward currents measured in protoplasts isolated from roots.

As stated earlier, Na^+ entry into the cells of the root is passive; uniporter or ion channel type transporters are the likely candidates for sodium influx. These include HKT, LCT1, NSCC (including CNGCs and GLRs). Although the role of each type of transporter can vary within species and growth conditions, evidence would suggest that the different transporters could work in parallel, mediating Na^+ uptake into the roots.

LCT1, a low affinity cation transporter, was isolated from wheat using a screen for complementation of K^+ uptake mutant of yeast [8]. In yeast, LCT1 catalyzed uptake of many

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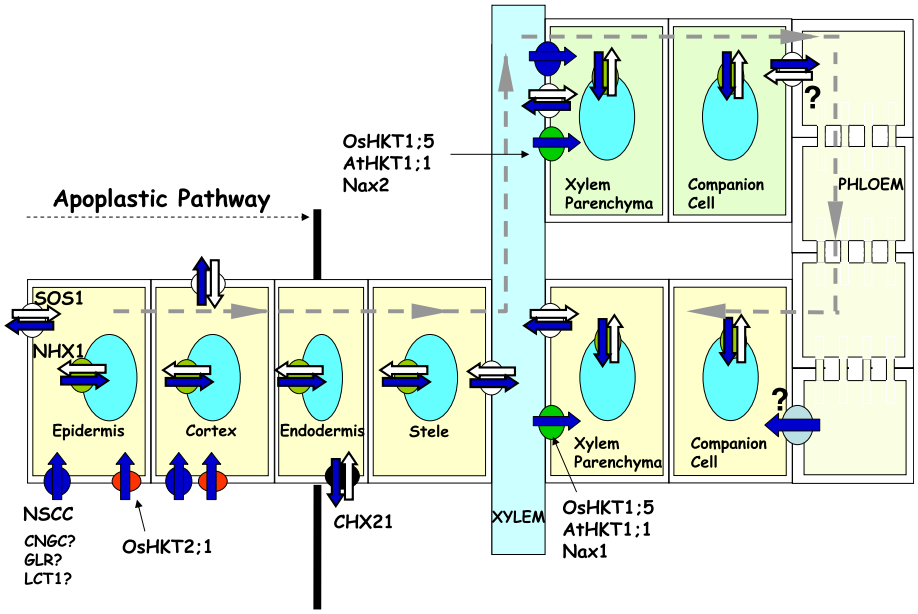

Fig. 1. Movement of Na^+ in the plant. Schematic diagram showing radial transport and longitudinal movement of Na^+ ions from the soil through the roots, up the xylem to the shoots, and recirculation through the plant. Na^+ enters the root cytosol via NSCC (non-selective cation channels) and possibly other HKT-like transporters (including OsHKT2;1). Low cytosolic Na^+ concentrations are maintained by the action of plasma membrane-bound (SOS1) and vacuolar-bound (NHX1) Na^+/H^+ antiporters. A CHX-like cation/H^+ antiporter (CHX21) contributes to Na^+ movement from the endodermal cells into the apoplast of the stele. In the stele, Na^+ is loaded into the xylem by a plasma membrane-bound Na^+/H^+ antiporter (SOS1). In the root, Na^+ can be unloaded from the xylem into the root via Na^+-selective uniporters (AtHKT1;1, OsHKT1;5; Nax1). In the leaves, Na^+ can be unloaded by Na^+-selective uniporters (OsHKT1;5, AtHKT1;1 and Nax2), and NSCC channels. Leaf cytosolic Na^+ concentrations are maintained by the action of both SOS1- like and NHX1-like Na^+/H^+ antiporters. Mechanisms for phloem loading and unloading and recirculation of Na^+ via the phloem remain to be determined.

cations, including Na^+, Rb^+, and Ca^{2+}. While causing hypersensitivity to sodium in yeast, which was mitigated by the addition of high (20 mM), but not low (2 mM) Ca^{2+} to the medium [9], LCT1 also increased uptake of Li^+ and Cs+. It was suggested that LCT1 may be a component of the Ca^{2+}-insensitive Na^+ influx seen in wheat roots, as it was not saturated at low external Na^+ (7 mM), a property attributed to NSCCs [9]. As yet, the only in planta studies with LCT1 have been made in the context of heavy metal uptake and detoxification in relation to external calcium [10], and although a similar protein (CAJ76575) appears in GenBank, sequences of significant identity are not present in rice or Arabidopsis; LCT1 may be unique to wheat.

Ion channel transporters are likely candidates for mediating the passive transport of Na^+ into the cells. In the last few years, evidence has been presented supporting the existence of weakly voltage-dependent non-selective cation channels (NSCC) that are the main pathway for Na^+ entry into the roots, at high soil NaCl concentrations [1,2]. Although there are many candidate genes in the databases that could encode these NSCC channels, their identity remains elusive. Two families of non-selective cation channels, CNGCs (cyclicnucleotide-gated channels) [11], and GLRs (glutamate-activated channels) [12] have been suggested to be candidate NSCC channels [1]. The inhibition of Na^+ influx and NSCC currents upon addition of membrane-permeant cyclic nucleotide analogues provided correlative evidence for the operation of CNGCs in plants [13], a family of plant channels that in *Arabidopsis* comprises 20 members [14]. To date, five AtCNGCs have been characterized (AtCNGC1, 2, 3, 4, and 10) [11,15–17]. Electrophysiological studies have suggested that AtCNGC1 and AtCNGC4 are equally permeable to K^+ and Na^+ and when expressed in *Xenopus* oocytes, they displayed activation by cyclic nucleotides [11,15]. AtCNGC2 appears to be selective for K^+ and to discriminate against Na^+ [11]. AtCNGC10 rescued K^+ transport defective mutants of *E. coli*, yeast and *Arabidopsis akt1-1*, suggesting that AtCNGC10 mediates the transport of K^+ into the roots [16]. AtCNGC3 was recently characterized by functional complementation of yeast and by characterization of *Arabidopsis* T-DNA knockout mutants [17]. AtCNGC3 was primarily expressed in the cortical and epidermal root cells. Heterologous expression in yeast indicated that AtCNGC3 could mediate the uptake of Na^+ and K^+, and the growth of the mutant seedlings in toxic NaCl (and KCl) concentrations was improved, suggesting a restricted ion influx in the mutant plants [17]. Ionotropic glutamate receptors (GLRs) are proteins that interact with glutamate and form cation channels with a wide range of permeabilities. In *Arabidopsis* the family of putative GLRs comprises 20 members [12]. Glutamate-activated Na^+ and Ca^{2+} voltage-independent currents were characterized in *Arabidopsis* roots. Demidchik et al. [12] noted that although the effects of environmental factors on apoplastic glutamate remains unclear, the concentrations of glutamate required for half-activation of these channels correlated well with the range of apoplastic glutamate concentrations reported (0.2–0.5 mM), suggesting a role of these channels in Na^+ uptake.

High affinity potassium transporters (HKT) have been shown to function as Na^+/K^+ symporters and as Na^+-selective uniporters [2,18]. In *Arabidopsis*, there is only a single member of the HKT family. In the different plant tissues that have been examined, AtHKT1;1 has been found in different cell types, but in the root, AtHKT1;1 is localized in the stele. Na^+ influx remained unchanged in a *AtHKT1;1* T-DNA insertion knock-

out mutant, thus suggesting that in *Arabidopsis* AtHKT1;1 does not mediate significant Na^+ influx [19,20]. However, in rice, the expression of some of the nine members comprising the rice HKT family is consistent with a potential role in root influx. Both OsHKT2;1 and OsHKT2;2 transcripts were detected in both leaves and roots from the salt-tolerant Pokkali and the salt-sensitive BRRI Dhan29 rice varieties [21]. OsHKT2;1 has been shown to mediate Na^+ influx (and not K^+) [22], when the plants are K^+-deficient [18]. OsHKT2;2 was shown to catalyze Na^+-dependent K^+ uptake [22]. During salt-stress the expression of OsHKT2;1 was down-regulated in the salt tolerant variety, suggesting a reduction in Na^+ influx in the salt-tolerant variety [21]. On the other hand, the initial expression of OsHKT2;2 was reduced in the roots of the salt-sensitive variety [21] and strongly enhanced in the leaf mesophyll cells of the salt tolerant variety and the connecting area between the phloem and mesophyll cells. The wheat TaHKT2;1 functions as a Na^+/K^+ symporter [23] and its down-regulation in planta reduced the accumulation of Na^+ in the roots and improved salt-tolerance [24].

3. Efflux of Na^+ from the root

The dramatic unidirectional influx measured in intact and excised roots is counteracted by a large efflux, at steady state Na^+ concentrations. It is the net of these fluxes that contributes to the accumulation of sodium in the root and the rest of the plant.

In the presence of elevated levels of Na^+ outside the cell, the electrochemical gradients that make sodium uptake into the root (and cells) passive make the efflux of Na^+ from the cell an active process. While sodium extrusion in animals and microorganisms (including yeast) is directly energized by ATP hydrolysis (Na^+-ATPases), these Na^+ pumps are absent from higher plants [2]. Secondary active transport (Na^+/H^+ antiporters) at the plasma membrane facilitates Na^+ efflux. Plasma membrane Na^+/H^+ antiporter activity has been measured in membranes isolated from both root and shoot tissues. This electroneutral exchange of sodium for protons to facilitate efflux is the only mode of transport that has been measured for efflux under physiological conditions. In Arabidopsis, the gene product SOS1 has been identified to mediate, at least partially, cellular sodium efflux [25]. Recently, its rice ortholog was shown to functionally complement an *Arabidopsis* mutant [26]. SOS1 was first identified by mapping of a "salt oversensitive phenotype" identified in a screen for altered root growth on saline media [27]. SOS2 and SOS3, genes found in the same root-bending screen, regulate SOS1 activity: SOS3 is a myristoylated calcium binding protein that recruits SOS2 to the plasma membrane; SOS2, a serine/threonine protein kinase, activates SOS1 by phosphorylation and dramatically increases Na^+/H^+ exchange activity in isolated plasma membrane vesicles [28].

A comparison of Na^+/H^+ exchange activities in plasma membrane vesicles isolated from *Arabidopsis* roots of wild-type and *sos1* mutant plants clearly shows a diminished activity in membranes isolated from the *sos1* mutant [28]. The membranes of the mutant retained some Na^+/H^+ exchange activity, and this was attributed to other possible exchangers that might be present. The CHX family of cation/proton exchangers in *Arabidopsis* comprises 28 members [29] and may contain suitable isoforms that account for this activity, although the membranes used in these studies were isolated from leaves, and the 'residual' activity seen in these may not be the same as in roots.

Na^+/H^+ exchange activity has not been reported for plasma membranes isolated from roots of Arabidopsis, although SOS1 antibodies detected a band of the appropriate size in the plasma membrane fraction of root microsomal membranes [28]. Shabala et al. [30] used vibrating microelectrodes to compare fluxes in excised roots of Arabidopsis wild-type and mutants of the SOS pathway. Differences in K^+ and H^+ fluxes between wild-type and *sos1* mutants were qualitatively different in the root apex and mature root zones on addition of 50 mM NaCl. As transport effects were also different among the *sos* mutants, the authors concluded that SOS1 may act both dependent and independent of SOS2/SOS3 regulation [30]. Certainly other antiporters might be expressed in the apex and mature zones of the root (i.e. CHXs), and SOS1 expression is also induced by NaCl [25]. Thus, the exposure to NaCl of the excised roots may not have been sufficiently long for the expression of SOS1 and its activation. The potential for CAX1 regulation by SOS2, shown by Cheng et al. [31] is also worth consideration; as cytosolic Ca^{2+} regulation itself may influence a broad range of ion fluxes. Certainly the differences in activity attributed to SOS1 and/or its regulatory pathway might be different in vivo or in vitro [30], but experimental treatments would also strongly affect the interpretation of phenotype.

SOS1 function in whole plant Na^+ transport is an area requiring some resolution, as tissue localization of SOS1 expression is ambiguous and the potential interaction of the protein with other cellular targets contributing to Na^+ transport complicates the ability to make direct attributions of function. SOS1 promoter–GUS fusions show expression in root epidermis at the root cap and also in vascular tissues, though particularly in close association with the xylem [25]. Expression in the shoot also indicated a predominantly vascular localization, but the plants grown for these assays were grown in the absence of salinity stress. In leaves, SOS1 activity was assayed after salinity treatment of the plants, presumably because mRNA levels are dramatically lower before NaCl treatment, both in roots and shoots [25]. 35S-SOS1 transgenic plants show a dramatic increase in SOS1 expression, relative to wild-type, only under salt-stress treatment, suggesting that *SOS1* mRNA stability may be regulated as well [32]. A more detailed characterization of SOS1 expression, perhaps with the native promoter–SOS1–GFP fusions that could be crossed into the different *sos* mutant backgrounds, might be helpful. Nonetheless, tissue Na^+ accumulation and its dynamics clearly point to the importance of SOS1 in sodium efflux in the root, as *sos1* mutant plants (and also *sos2* and *sos3* mutants) displayed a higher root Na^+ concentration relative to wild-type [25,33]. Conversely, SOS1 overexpressing plants show reduced Na^+ accumulation in the shoot and reduced Na^+ concentration in the xylem [32]. While Na^+ efflux has not been directly measured in *Arabidopsis sos* mutant plants, the increase in root Na^+ concentration (and also shoot Na^+ concentration) must be due to SOS1-dependent (if not mediated) efflux, as Na^+ influx in *sos1* plants was the same as that of wild-type plants [20]. The relative contribution of Na^+ efflux and influx in the root to net Na^+ root and shoot accumulation has been compared in *Arabidopsis* and *Thellungiella halophila*, its salt-tolerant relative [34]. Net root accumulation was similar in the both species, but higher shoot Na^+ accumulation in *Arabidopsis* was

attributed to lower rates of unidirectional Na^+ influx in *T. halophila* roots, while rates of root unidirectional efflux were similar in the two plants [34]. This reduced rate of influx in *T. halophila* was found to be related to the lower conductance to Na^+ (and higher K^+ selectivity) of *T. halophila* root cell membranes, which was consistent also with less depolarization of the plasma membrane electrical potential difference of *T. halophila* cells on exposure to Na^+ [35]. Tester and Davenport [1] and Kronzucker et al. [36] raised the question of what adaptive significance the high rate of cycling of Na^+ at the plasma membrane might have. This remains an open question, but the systematic application of these kinetic measurements to mutants in *Arabidopsis*, and also those that should be forthcoming in rice as well as *T. halophila* (perhaps a TILLING mutant population for forward and reverse screens?), may provide more information.

4. Root radial transport

Transport of Na^+ across the root and into the xylem occurs along symplastic and apoplastic pathways from the epidermis to the xylem. However, with the deposition of apoplastic barriers (suberin lamellae/Casparian band), a higher selectivity for ion movement (both in and out of the stele) is facilitated. Restriction of xylem loading can be accomplished, in part, by the restriction of Na^+ entry from the cortical cells into the stele, by an effective Casparian band and efficient sodium efflux from the cells at this border [37]. The contribution of a physical barrier to the restriction of Na^+ radial transport was evident in the comparison to the salt-sensitive *Populus tomentosa* to the salt tolerant *Populus euphratica* [38]. The latter had lower Na^+ in the stele and higher Na^+ in the cortex after 20 days exposure to high salinity; net uptake of sodium was significantly less in *P. euphratica* [38]. Salinity stress can induce a more rapid maturation of the endodermal Casparian bands in young cotton roots ([39], and references therein) and conversely, growth in high humidity/low transpiration can lead to the formation of more permeable endodermis [40]. An *Arabidopsis* mutant that overaccumulated Na^+ in the shoot, *sas1*, was found to be defective in root radial transport of Na^+, as the root Na^+ concentration was the same as wild-type and shoot Na^+ concentration was two–sevenfold higher than wild-type plants [41]. The xylem was found to have 5.5-fold higher Na^+ concentrations. Although this mutation has yet to be associated with a gene, it has been localized to a small region at the bottom of chromosome III [41]. AtCHX21 expression in the plasma membrane of the endodermal cells is strongly suggestive of a role for the protein in ion selectivity at the endodermis [42]. The *chx21* mutant plants had lower xylem Na^+ concentrations and displayed lower Na^+ accumulation in the leaves as compared to wild-type plants, leading to the hypothesis that AtCHX21 contributed to Na^+ efflux from the endodermal cells into the stele [42]. It remains to be seen whether the knockout is more tolerant to higher concentrations of Na^+ in the growth medium.

5. Xylem loading/unloading

As much as 10–15% of the surface area of the xylem tracheids is in contact with the xylem parenchyma at structures called bordered pits [1]. This large surface area can easily accommodate the large quantities of ions and water that must be passed from the root to the shoot. Tester and Davenport [1] and deBoer and Volkov [43] pointed out that this interface can be quite plastic, with large increases in this surface area occurring, via the proliferation of transfer cells at certain loci along the xylem, upon the exposure of some plants to salinity. Export of Na^+ to the xylem is assumed to be active, given that the xylem parenchyma electrical membrane potential difference has been measured at between 60 and 100 mV negative inside the xylem parenchyma cell (XPC) (reviewed in [43]). The electrochemical potential for Na^+ may be lower than that facing the root epidermis under high salinity, as xylem sap concentrations have been measured in the range of 1–20 mM [22]. Higher xylem Na^+ concentrations, particularly under conditions of significant transpiration, would lead to the untenable accumulation of Na^+ in the shoot. Under conditions of moderate salinity in *Arabidopsis*, SOS1 was proposed to mediate the loading of Na^+ into the xylem, as the *sos1* mutant plants had less accumulation of Na^+ [25]. Unloading of Na^+ from the xylem is likewise assumed to be passive, mediated by Na^+ permeable channels.

At higher salinity, xylem loading of Na^+ is possibly passive, as higher XPC cytosolic Na^+ and a relatively depolarized plasma membrane would favor Na^+ movement in to the xylem, as is the case for K^+ loading via SKOR channels [44]. The role for a SOS1 antiporter in loading of the xylem is supported by experiments in which acidification of the xylem in *Plantago maritima* and barley resulted in an increase in the Na^+ concentration of the xylem sap [43].

Removal of sodium from xylem has been observed along the basal portions of the root and shoot of corn, bean and soybean [1,43]. Under normal conditions, the cellular uptake of sodium is passive, owing to the electrical potential difference at the plasma membrane, and it is therefore likely to be mediated by channel- or uniporter-type transporters. However, Lacan and Durand [45] proposed a model for Na^+ retrieval from the xylem in which a plasma membrane-bound Na^+/H^+ antiporter could operate "in reverse", using the Na^+ gradient across the XPC plasma membrane (high Na^+ in the xylem, low Na^+ in the cytosol) to facilitate H^+ extrusion and Na^+ uptake by the XPC. This model required first, a large Na^+ gradient, and second, a decrease in the pH difference between the xylem and the XPC cytosol (either by alkalinization of the xylem or acidification of the cytosol). The assumptions of this model are faulty as its theoretical basis. Biological membranes lack passive H^+ conductances [46]. On the other hand, both passive and active (albeit secondary) pathways exist for Na^+ transport. It is hard to conceive a Na^+/H^+ antiporter driven by the Na^+ gradient across the membrane when passive conductances (HKTs, NSCCs) in the same membrane will dissipate any Na^+ electrochemical potential difference across the XPC. The acidification of the XPC cytosol with respect to the xylem is unlikely (reviewed in [43]). Also, Na^+ contents in xylem sap of wild-type *Arabidopsis* have been reported in the range of 9 mM [47] and 5 mM [48] when the plants were grown at 100 mM NaCl for two days, suggesting the lack of large Na^+ concentration gradients (larger in the xylem with respect to XPC cytosol). The apparent increase of shoot Na^+ concentrations in *Arabidopsis sos1* mutant plants grown at high salinity (100 mM) treatment—in contrast to the reduced shoot sodium observed at moderate salinity stress owing to reduced xylem loading via

SOS1—prompted Shi et al. [47] to adopt the model proposed by Lacan and Durand [45] notwithstanding a simpler proposed model in which excessive net influx of Na^+ into the root (owing to reduced efflux in the *sos1* mutant plants) would lead to greater loading of the xylem. In addition to preferential expression of SOS1 in vascular tissues (discussed earlier), subsequent publications have contributed interpretations that continue to favor the model. While some part of this might be due to ambiguous phenotypes, the clear demonstration of the role for AtHKT1;1 in xylem unloading (in addition to the strong evidence for similar mechanisms in rice and wheat), which is consistent with experimentally determined parameters for the xylem, will make the operation in of the Na^+/H^+ antiport "in reverse", that is, driven by the Na^+ gradient thermodynamically not favorable. Clearly, due to the existence of passive pathways (i.e. AtHKT1;1 and possible cation channels), any Na^+ gradient would dissipate passively. The elements discussed above precludes the operation of a Na^+/H^+ antiporter in xylem unloading and until direct evidence shows otherwise, this notion should be put to rest.

A convincing body of evidence has been accumulated that supports the role for AtHKT1;1 in xylem unloading. AtHKT1;1 was first proposed to be a pathway for sodium influx owing to the rescue of the salt sensitivity of mutants of the SOS pathway by a *hkt1* knockout [49,50]. But influx of Na^+ in the *hkt1* mutant plants was not different from the wild-type [19,20]. HKT1 promoter activity was also inconsistent with an influx role, as it was shown to be active in the stele of the root and vascular tissue in the shoot [51]. Subsequently it was shown that AtHKT1;1 localized to the plasma membrane of XPC [52]. This contrasted with the phloem-associated signal from AtHKT1;1 promoter–GUS fusions [19]. Over accumulation of Na^+ in the shoots and reduced Na^+ accumulation in roots, with an increase in the concentration of Na^+ in the xylem of athkt1;1 mutants pointed to a similar role for AtHKT1;1 in Arabidopsis as for HKT-type proteins in rice and wheat [48]. Davenport et al. [33] reported kinetics of Na^+ uptake and translocation in WT, *athkt1*, and *sos1* mutants that are consistent with previous observations of alterations in shoot and root ion content changes in *athkt1* mutants, and support the idea that AtHKT1;1 mediates retrieval of Na^+ from the xylem in Arabidopsis roots. While Na^+ translocation to the shoot was increased in the *hkt1* mutant plants, only a small reduced recirculation, not sufficient to account for differences in shoot Na^+ accumulation, was observed in relative to the wild-type plants [33]. If there is a role for AtHKT1;1 in phloem recirculation (see below) it may be small. Two natural variants of HKT1 in the Ts-1 and Tsu-1 Arabidopsis accessions appear to have a reduced expression level in the root, relative to the Col-0 ecotype [53]. While a number of differences exist between Col-0 and Ts/Tsu HKT1 coding regions that may also contribute to altered activity, the HKT1 variants in the Ts-1 and Tsu-1 accessions are correlated to higher shoot Na^+ concentrations [53]. And despite the higher shoot Na^+ concentrations in these accessions, they appear to be more salt tolerant [53], which is in stark contrast to the sensitivity shown in the athkt1 mutations isolated by Gong et al. [54], where a recessive mutation with a deletion in transmembrane domains of AtHKT1;1 function resulted in increased shoot Na^+ and increased sensitivity. This interesting phenotype, and its suggested relationship to the variant HKT1 locus in the Tsu-1 accession, requires further investigation.

Two recent reports in rice and wheat are consistent with HKT-type proteins mediating xylem unloading of Na^+. Ren et al. [55] cloned a salinity tolerance QTL from rice, named SKC1, which is expressed in vascular tissue preferentially in XPC. The xylem Na^+ concentration was much lower in the tolerant variety (Koshihikari), compared to its NIL (SKC1) line and shoot accumulation of Na^+ was also lower in the tolerant variety [55]. SKC1 is the same as OsHKT1;5 (formerly OsHKT8). In wheat, two loci have been characterized that also appear to encode HKT-type proteins that reduce Na^+ content by xylem unloading [56]. Nax2 appeared to function in xylem unloading of Na^+ in the root only, but Nax1 appeared to function in the root and leaf, contributing to a reduction in Na^+ concentration along the length of the leaf sheath [56].

A recent report using wheat-*L. elongatum* group 3 disomic substitution and addition lines is suggestive of one or more alleles from *L. elongatum* that reduce leaf Na^+ accumulation relative to the salt-sensitive variety, Chinese spring [57]. It is intriguing that while the gene of interest on the group 3 substitution was a SOS1 homologue, it was the expression of the wheat homolog of AtHKT1;1, on a different linkage group, that was most significantly affected in the substitution lines above.

6. Vacuolar Na^+ compartmentation

The compartmentation of Na^+ ions into vacuoles provides an efficient mechanism to avert the toxic effects of Na^+ in the cytosol. The transport of Na^+ into the vacuoles is mediated by cation/H^+ antiporters that are driven by the electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes, the H^+-ATPase and the H^+-PPiase. Although the activity of these cation/H^+ antiporters was demonstrated more than 20 years ago [58], their molecular characterization was only possible after the Arabidopsis Genome-sequencing project. Following the cloning of AtNHX1, the first member of the NHX family of plant endosomal Na^+/H^+ antiporters (comprising six isoforms, AtNHX1–6) [59], AtNHX1 was shown to mediate both Na^+/H^+ and K^+/H^+ exchange in plant vacuoles [60–62], and in vacuoles from yeast expressing AtNHX1 [63]. Since then, the existence of NHX-like proteins has been demonstrated in all plants tested; whether gymnosperm or angiosperm, monocots or dicots [3]. Vacuolar NHX transporters have been shown to play significant roles in endosomal pH regulation [64], cellular K^+ homeostasis and cell expansion [65], vesicular trafficking and protein targeting [66–68].

Since under normal growth conditions, higher plants are not exposed to high Na^+ concentrations, the roles of vacuolar NHX-like Na^+/H^+ antiporters in Na^+ transport and Na^+ homeostasis become relevant only during the growth of the plants in the presence of soil solutions containing high NaCl concentrations, and the concomitant increase in cellular Na^+ concentrations. A correlation between the expression of genes encoding NHX antiporters in salt-tolerant cultivars and their salt tolerance was shown in cotton [69]. Similar results were observed in wheat [70] suggesting that the higher expression of endogenous vacuolar Na^+/H^+ antiporters in roots and shoots of the salt-resistant wheat genotypes facilitated Na^+ exclusion from the cytosol, improving salt tolerance [70]. The key role of *NHX* genes has been emphasized with the

generation of salt-tolerant transgenic plants through the overexpression of NHX genes in a wide variety of species [1,71]. Under these conditions, vacuolar Na^+/H^+ antiporters play a key role in mediating the transport of Na^+ into the vacuoles, lowering cytosolic Na^+ concentrations and ameliorating the toxic effects of Na^+ on metabolism [72].

The activity of vacuolar antiporters has been extensively demonstrated in vitro. $Na^+(K^+)$-dependent H^+ movements were shown in isolated tonoplast vesicles ([61,63,73–75], and others), reconstituted proteoliposomes [62], and intact vacuoles [64,76,77]. H^+-dependent Na^+ transport was also demonstrated in purified tonoplast vesicles with fluorescence quenching techniques [61], and by the accumulation of $^{22}[Na^+]$ in acidic vesicles [73]. A few non-invasive techniques have been developed for the measurement of Na^+ concentrations in planta and to assess Na^+/H^+ antiporter-mediated vacuolar Na^+ accumulation. These techniques include the use of microelectrodes [78], ^{23}Na NMR [79–81], and fluorescence dyes [82–84]. Although limited to single cell measurements, triplebarreled electrodes provide accurate and high temporal resolution for the measurement of Na^+ activity [78]. $^{23}Na^+$ NMR allows accurate ion measurements and with good spatial resolution. The only drawbacks of the method are the high cost of the equipment and that relatively long time needed for data acquisition. Fan et al. [79] used in vivo $^{23}Na^+$- and ^{31}P NMR to assess tonoplast Na^+/H^+ activity in excised roots of two barley cultivars differing in salt tolerance, and similar methodology was used to monitor cytosolic/vacuolar pH shifts and vacuolar Na^+ concentrations in isolated vacuoles [81] and cell suspensions [80]. Halperin and Lynch [82] used the tetraammonium salt of SBFI, sodium-binding benzofuran isophtalate, to measure changes cytosolic Na^+ in root hairs of *Arabidopsis*, and the acetoxymethyl (AM) ester of SBFI was used to characterize changes in cytosolic Na^+ in salt-sensitive (Jaya) and salt-tolerant (Pokkali) rice varieties [84]. In this work, the authors showed that Pokkali cells maintained low cytosolic Na^+ concentrations via an amiloride-sensitive sequestration of Na^+ into intracellular compartments, indicative of the action of vacuolar Na^+/H^+ antiporters. Fluorescent dye-mediated quantification of Na^+ concentrations provides a real-time, non-invasive, method for the measurements of intracellular Na^+ concentrations. The main drawbacks of this methodology are the difficulties associated with the partition of the dye into multiple compartments (i.e. cytosol and vacuole), and the relatively narrow window of the linear response of the dye (up to 40 mM Na^+) [84].

Indirect measurement of vacuolar accumulation is often attempted because of the technical difficulties described above. Higher Na^+ contents in aerial and root tissues of transgenic plants expressing NHX-like antiporters, grown in high salinity, have been demonstrated [63,61,85,86]. Whole plant and/or tissue Na^+ contents were reported with a view to assessing vacuolar Na^+ compartmentation brought about the action of the Na^+/H^+ antiporters. Although one would expect that plants will maintain a concentration gradient of Na^+ across the tonoplast, and relatively lower Na^+ concentrations in the cytosol, this situation will only hold when plants are facing a non-lethal salt-stress, since under lethal stress the H^+-electrochemical gradients driving the Na^+/H^+ antiporter will not be functional and as a consequence, the Na^+ concentration gradients will dissipate, resulting in Na^+ equilibrium in the tissue. Under these conditions, a comparison between the Na^+ content in salt-tolerant plants and non-tolerant plants would show very little difference in Na^+ content, although the salt-tolerant plants would display active growth in the presence of salt while the salt-sensitive plants would stop growing and eventually die. In addition, when measuring relative Na^+ contents in tissues with the aim of assigning a role(s) to the NHX-like antiporters in vacuolar Na^+ compartmentation, the possibility that cell specific roles are fulfilled by these antiporters needs to be considered.

7. Na^+ recirculation

The recirculation of Na^+ back to the roots by the phloem is still unresolved. Although evidence has been presented to support the notion of Na^+ recirculation to the roots could significantly alter the Na^+ concentrations in the leaves in several species and the extent of this recirculation could be related to the tolerance of plants to salinity ([1], and references therein), the transport systems associated with this phenomena have not yet been identified. It has been postulated that AtHKT1;1 could be involved in the recirculation of Na^+ by mediating the loading of Na^+ form the shoot into the phloem and then unloading it into the roots [19]. However, a recent publication demonstrated that although AtHKT1;1 controls the retrieval of Na^+ form the xylem, is not involved in Na^+ recirculation via the phloem [33].

8. Conclusions

There are many more HKT-type transporters in rice (and wheat) and their roles in Na^+ transport have not yet been assigned. The other families of putative Na^+ transporter proteins (CHX, CNGC, GLR, NHX) are also large in *Arabidopsis* and functional assignment remains to be determined for most of the proteins. The pathways for Na^+ transport that contribute to the fluxes in the root, stem and leaf tissues are just beginning to be determined at the cellular level. A closer connection between the whole plant and whole tissue Na^+ transport kinetics and the cellular (and apoplastic) routes of Na^+ has been most effectively made with the use of mutants and experimentation under the conditions of steady state Na^+ in the root medium. A continued effort in this direction is sure to yield more interesting data.

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References

[1] Tester, M. and Davenport, R. (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91, 503–527.
[2] Horie, T. and Schroeder, J.I. (2004) Sodium transporters in plants. Diverse genes and physiological functions. Plant Physiol. 136, 2457–2462.
[3] Pardo, J.M., Cubero, B., Leidi, E.O. and Quintero, F.J. (2006) Alkali cation exchangers: roles in cellular homeostasis and stress tolerance. J. Exp. Bot. 57, 1181–1199.
[4] Platten, J.D., Cotsaftis, O., Berthomieu, P., Bohnert, H., Davenport, R.J., Fairbairn, D.J., Horie, T., Leigh, R.A., Lin, H.-X., Luan, S., Maser, P., Pantoja, O., Rodrıguez-Navarro, A.,

Schachtman, A.-A., Schroeder, J.I., Sentenac, H., Uozumi, N., nor Very, A.-A., Zhu, J.-K., Dennis, E.S. and Tester, M. (2006) Nomenclature for HKT transporters, key determinants of plant salinity tolerance. Trends Plant Sci. 11, 372–374.
[5] Epstein, E. (1966) Dual pattern of ion absorption by plant cells and by plants. Nature 212, 1324–1327.
[6] Higinbotham, N. (1973) Electropotentials of plant cells. Annu. Rev. Plant Physiol. Plant Mol. Biol. 24, 25–46.
[7] Britto, D.T. and Kronzucker, H.J. (2006) Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci. 11, 529–534.
[8] Schachtman, D.P., Kumar, R., Schroeder, J.I. and Marsh, E.L. (1997) Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. Proc. Natl. Acad. Sci. USA 94, 11079–11084.
[9] Amtmann, A., Fischer, M., Marsh, E.L., Stefanovic, A., Sanders, D. and Schachtman, D.P. (2001) The wheat cDNA LCT1 generates hypersensitivity to sodium in a saltsensitive yeast strain. Plant Physiol. 126, 1061–1071.
[10] Antosiewicz, D.M. and Hennig, J. (2004) Overexpression of LCT1 in tobacco enhances the protective action of calcium against cadmium toxicity. Environ. Pollut. 129, 237–245.
[11] Leng, Q., Mercier, R.W., Hua, B.-G., Fromm, H. and Berkowitz, G.A. (2002) Electrophysiological analysis of cloned cyclic nucleotide-gated ion channels. Plant Physiol. 128, 400–410.
[12] Demidchik, B., Essah, P.A. and Tester, M. (2004) Glutamate activates cation currents in the plasma membrane of Arabidopsis root cells. Planta 219, 167–175.
[13] Maathuis, F.J.M. and Sanders, D. (2001) Sodium uptake in *Arabidopsis thaliana* is regulated by cyclic nucleotides. Plant Physiol. 127, 1617–1625.
[14] Talke, I.N., Blaudez, D., Maathuis, F.J.M. and Sanders, D. (2003) CNGCs: prime targets of plant cyclic nucleotide signaling? Trends Plant Sci. 8, 286–293.
[15] Balague, C., Lin, B.Q., Alcon, C., Flottes, G., Malmstrom, S., Kohler, C., Neuhaus, G., Pelletier, G., Gaymard, F. and Roby, D. (2003) HLM1, an essential signalling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel family. Plant Cell 15, 365–379.
[16] Li, X., Borsics, T., Harrington, H.M. and Christopher, D.A. (2005) *Arabidopsis* ArCNGC10 rescues potassium channel mutants of *E. coli*, yeast and *Arabidopsis* and is regulated by calcium/calmodulin and cyclic GMP in *E. coli*. Funct. Plant Biol. 32, 643–653.
[17] Gobert, A., Park, G., Amtmann, A., Sanders, D. and Maathuis, F.J.M. (2006) *Arabidopsis thaliana* cyclic nucleotide gated channel 3 forms a non-selective ion transporter involved in germination and cation transport. J. Exp. Bot. 57, 791–800.
[18] Garciadeblas, B., Senn, M.E., Banuelos, M.A. and Rodrıguez-Navarro, A. (2003) Sodium transport and HKT transporters: the rice model. Plant J. 34, 788–801.
[19] Berthomieu, P., Conéjéro, G., Nublat, A., Brackenbury, W.J., Lambert, C., Savio, C., Uozumi, N., Oiki, S., Yamada, K., Cellier, F., Gosti, F., Simonneau, T., Essah, P.A., Tester, M., Véry, A.-A., Sentenac, H. and Casse, F. (2003) Functional analysis of *AtHKT1* in Arabidopsis shows that Na^+ recirculation by the phloem is crucial for salt tolerance. EMBO J. 22, 2004–2014.
[20] Essah, P.A., Davenport, R.J. and Tester, M. (2003) Sodium influx and accumulation in Arabidopsis. Plant Physiol. 133, 307–318.
[21] Kader, M.A., Seidel, T., Golldack, D. and Lindberg, S. (2006) Expressions of OsHKT1, OsHKT2 and OsVHA are differently regulated under NaCl stress in saltsensitive and salt-tolerant rice (*Oryza sativa* L.) cultivars. J. Exp. Bot. 57, 4257–4268.
[22] Horie, T., Yoshida, K., Nakayama, H., Yamada, K., Oiki, S. and Shinmyo, A. (2001) Two types of HKT transporters with different properties of Na^+ and K^+ transport in *Oryza sativa*. Plant J. 27, 129–138.
[23] Rubio, F., Gassmann, W. and Schroeder, J.I. (1995) Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science 270, 1660–1663.
[24] Laurie, S., Feeney, K.A., Maathuis, F.J.M., Heard, P.J., Brown, S.J. and Leigh, R.A. (2002) A role for HKT1 in sodium uptake by wheat roots. Plant J. 32, 139–149.
[25] Shi, H., Ishitani, M., Kim, C. and Zhu, J.K. (2000) The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. PNAS USA 97, 6896–6901.
[26] Martinez-Atienza, J., Jiang, X., Garciablades, B., Mendoza, I., Zhu, J.K., Pardo, J.M. and Quintero, F.J. (2007) Conservation of the salt overly sensitive pathway in rice. Plant Physiol. 143, 1001–1012.
[27] Wu, S.J., Ding, L. and Zhu, J.K. (1996) *SOS1*, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 8, 617–627.
[28] Qiu, Q.S., Barkla, B.J., Vera-Estrella, R., Zhu, J.K. and Schumaker, K.S. (2003) Na^+/H^+ exchange activity in the plasma membrane of Arabidopsis. Plant Physiol. 132, 1041–1052.
[29] Sze, H., Padmanaban, S., Cellier, F., Honys, D., Cheng, N.H., Bock, K.W., Conejero, G., Li, X.Y., Twell, D., Ward, J.M. and Hirschi, K.D. (2004) Expression patterns of a novel CHX family highlight potential roles in osmotic adjustment and K^+ homeostasis in pollen development. Plant Physiol. 136, 2532–2547.
[30] Shabala, L., Cuin, T.A., Newman, I.A. and Shabala, S. (2005) Salinity-induced ion flux patterns from the excised roots of *Arabidopsis sos* mutants. Planta 222, 1041–1050.
[31] Cheng, N.H., Pittman, J.K., Zhu, J.K. and Hirschi, K.D. (2004) The protein kinase SOS2 activates the Arabidopsis H^+/Ca^{2+} antiporter CAX1 to integrate calcium transport and salt tolerance. J. Biol. Chem. 279, 2922–2926.
[32] Shi, H., Lee, B.-H., Wu, S.-J. and Zhu, J.K. (2003) Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat. Biotech. 21, 81–85.
[33] Davenport, R.J., Muñoz-Mayor, A., Jha, D., Essah, P.A., Rus, A. and Tester, M. (2007) The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in Arabidopsis. Plant Cell Env. 30, 497–507.
[34] Wang, B., Davenport, R.J., Volkov, V. and Amtmann, A. (2006) Low unidirectional sodium influx into root cells restricts net sodium accumulation in *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*. J. Exp. Bot. 57, 1161–1170.
[35] Volkov, V. and Amtmann, A. (2006) *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, has specific root ion-channel features supporting K^+/Na^+ homeostasis under salinity stress. Plant J. 48, 342–353.
[36] Kronzucker, H.J., Szczerba, M.W., Moazami-Goudarzi, M. and Britto, D.T. (2006) The cytosolic Na^+:K^+ ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using $^{42}K^+$ and $^{24}Na^+$. Plant Cell Environ. 29, 2228–2237.
[37] Steudle, E. (2000) Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531–1542.
[38] Chen, S., Li, J., Wang, S., Fritz, E., Huttermann, A. and Altman, A. (2003) Effects of NaCl on shoot growth, transpiration, ion compartmentation, and transport in regenerated plants of *Populus euphratica* and *Populus tomentosa*. Can. J. For. Res. 33, 967–975.
[39] Enstone, D.E., Peterson, C.A. and Ma, F. (2003) Root endodermis and exodermis: structure, function and responses to the environment. J. Plant Growth Regul. 21, 335–351.
[40] Hose, E., Clarkson, D.T., Steudle, E., Schreiber, L. and Hartung, W. (2001) The exodermis: a variable apoplastic barrier. J. Exp. Bot. 365, 2245–2264.
[41] Nublat, A., Desplans, J., Casse, F. and Berthomieu, P. (2001) *sas1*, an Arabidopsis mutant overaccumulating sodium in the shoot, shows deficiency in the control of the root radial transport of sodium. Plant Cell 13, 125–137.
[42] Hall, D., Evans, A.R., Newbury, J.J. and Pritchard, J. (2006) Functional analysis of CHX21: a putative sodium transporter in Arabidopsis. J. Exp. Bot. 57, 1201–1210.
[43] deBoer, A.H. and Volkov, V. (2003) Logistics of water and salt transport through the plant: structure and functioning of the xylem. Plant Cell Environ. 26, 87–101.
[44] Wegner, L.H. and de Boer, A.H. (1997) Properties of two-outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and longdistance signaling. Plant Physiol. 115, 1707–1719.
[45] Lacan, D. and Durand, M. (1996) Na^+–K^+ exchange at the xylem/symplast boundary. Its significance in the salt sensitivity of soybean. Plant Physiol. 110, 705–711.
[46] Mitchell, P. (1961) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biochem. J. 79, P23–P24.

[47] Shi, H., Quintero, F.J., Pardo, J.M. and Zhu, J.K. (2002) The putative plasma membrane Na^+/H^+ antiporter SOS1 controls long-distance Na^+ transport in plants. Plant Cell. 14, 465–477.
[48] Horie, T., Horie, R., Chan, W.-Y., Leung, H.-Y. and Schroeder, J.I. (2006) Calcium regulation of sodium hypersensitivities of *sos3* and *athkt1* mutants. Plant Cell Physiol. 47, 622–633.
[49] Rus, A., Yokoi, S., Atanbradralt, S., Reddy, M., Lee, B., Matsumoto, T.K., Koiwa, H., Zhu, J.K., Bressan, R.A. and Hasegawa, P.M. (2001) AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. PNAS USA 98, 14150–14155.
[50] Rus, A., Lee, B., Muñoz-Mayor, A., Sharkuu, A., Miura, K., Zhu, J.K., Bressan, R.A. and Hasegawa, P.M. (2004) AtHKT1 facilitates Na^+ homeostasis and K^+ nutrition in plants. Plant Physiol. 136, 2500–2511.
[51] Maser, P., Eckelman, B., Vaidyanathana, R., Horie, T., Fairbairn, D.J., Kubo, M., Yamagami, M., Yamaguchi, K., Nishimurae, M., Uozumi, N., Robertson, W., Sussman, M.R. and Schroeder, J.I. (2002) Altered shoot/root Na^+ distribution and bifurcating salt sensitivity in Arabidopsis by genetic disruption of the Na^+ transporter AtHKT1. FEBS Lett. 531, 157–161.
[52] Sunarpi, Horie, T., Motoda, J., Kubo, M., Yang, H., Yoda, K., Horie, R., Chan, W.Y., Leung, H.Y., Hattori, K., Konomi, M., Osumi, M., Yamagami, M., Schroeder, J.I. and Uozumi, N. (2005) Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na unloading from xylem vessels to xylem parenchyma cells. Plant J. 44, 928–938.
[53] Rus, A., Baxter, I., Muthukumar, B., Gustin, J., Lahner, B., Yakubova, E. and Salt, D.E. (2006) Natural variants of *AtHKT1* enhance Na^+ accumulation in two wild populations of *Arabidopsis*. PLoS Genet. 2, e210, doi:10.1371/journal.pgen.0020210.
[54] Gong, J.M., Waner, D.A., Horie, T., Li, S.L., Horie, R., Abid, K.B. and Schroeder, J.I. (2004) Microarray-based rapid cloning of an ion accumulation deletion mutant in *Arabidopsis thaliana*. PNAS USA 101, 15404–15409.
[55] Ren, Z.-H., Gao, J.-P., Li, L.-G., Cai, X.-L., Huahg, W., Chao, D.-Y., Zhu, M.-Z., Wang, Z.-Y., Luan, S. and Lin, H.-X. (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 37, 1141–1146.
[56] James, R.A., Davenport, R.J. and Munns, R. (2006) Physiological characterization of two genes for Na^+ exclusion in Durum wheat, Nax1 and Nax2. Plant Physiol. 142, 1537–1547.
[57] Mullan, D.J., Colmer, T.D. and Francki, M.G. (2007) Arabidopsis–rice–wheat gene orthologues for Na^+ transport and transcript analysis in wheat-*L. elongatum* aneuploids under salt stress. Mol. Genet. Genomics 277, 199–212.
[58] Blumwald, E. and Poole, R.J. (1985) Na^+/H^+ antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. Plant Physiol. 78, 163–167.
[59] Gaxiola, R.A., Rao, R., Sherman, A., Grisafi, P., Alper, S.L. and Fink, G.R. (1999) The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. PNAS USA 96, 1480–1485.
[60] Apse, M.P., Aharon, G.S., Snedden, W.A. and Blumwald, E. (1999) Overexpression of a vacuolar Na^+/H^+ antiport confers salt tolerance in Arabidopsis. Science 285, 1256–1258.
[61] Zhang, H.-X. and Blumwald, E. (2001) Transgenic salt tolerant tomato plants accumulate salt in the foliage but not in the fruits. Nat. Biotech. 19, 765–768.
[62] Venema, L., Quintero, F.J., Pardo, J.M. and Donaire, J.P. (2002) The *Arabidopsis* Na^+/H^+ exchanger catalyzes low affinity Na^+ and K^+ transport in reconstituted vesicles. J. Biol. Chem. 277, 2413–2418.
[63] Darley, C.P., van Wuytswinkel, O.C.M., van der Woude, K., Mager, W.H. and de Boer, A.H. (2000) *Arabidopsis thaliana* and *Saccharomyces cerevisiae* NHX1 genes encode amiloride sensitive electroneutral Na^+/H^+ exchangers. Biochem. J. 351, 241–249.
[64] Yamaguchi, T., Fukuda-Tanaka, S., Inagaki, Y., Saito, N., Yonekura-Sakakibara, K., Tanaka, Y., Kusumi, T. and Iida, S. (2001) Genes encoding the vacuolar Na^+/H^+ exchanger and flower coloration. Plant Cell Physiol. I42, 451–461.
[65] Apse, M.P., Sottosanto, J. and Blumwald, E. (2003) Ion homeostasis in *Arabiodpsis thaliana*: role of the vacuolar Na^+/H^+ antiporters. Plant J. 36, 229–239.
[66] Bowers, K., Levi, B.P., Patel, F.I. and Stevens, T.H. (2000) The sodium/proton exchanger Nhx1p is required for endosomal protein trafficking in the yeast *Saccharomyces cerevisiae*. Mol. Biol. Cell 11, 4277–4294.
[67] Sottosanto, J.B., Gelli, A. and Blumwald, E. (2004) DNA array analyses of *Arabidopsis thaliana* lacking a vacuolar Na^+/H^+ antiporter: impact of AtNHX1 on gene expression. Plant J. 40, 752–771.
[68] Brett, C.L., Tukaye, D.N., Mukherjee, S. and Rao, R. (2005) The yeast endosomal $Na^+(K^+)/H^+$ exchanger Nhx1 regulates cellular pH to control vesicle trafficking. Mol. Biol. Cell 16, 1396–1405.
[69] Wu, Y.Y., Chen, G.D., Meng, Q.W. and Zheng, C.C. (2004) The cotton *GhNHX1* gene encoding a novel putative tonoplast Na^+/H^+ antiporter plays an important role in salt stress. Plant Cell Physiol. 45, 600–607.
[70] Saqib, M., Zorb, C., Rengel, Z. and Schubert, S. (2005) The expression of the endogenous vacuolar Na^+/H^+ antiporters in roots and shoots correlates positively with the salt tolerance of wheat (*Triticum aestivum* L.). Plant Sci. 169, 959–965.
[71] Yamaguchi, T. and Blumwald, E. (2005) Developing salt tolerant crop plants: challenges and opportunities. Trends Plant Sci. 12, 615–620.
[72] Blumwald, E., Aharon, G.S. and Apse, M.P. (2000) Na^+ transport in plant cells. Biochim. Biophys. Acta 1465, 140–151.
[73] Garbarino, J. and DuPont, F.M. (1988) NaCl induces a Na^+/H^+ antiport in tonoplast vesicles from barley roots. Plant Physiol. 86, 231–236.
[74] Barkla, B.J., Charuk, J.H.M., Cragoe Jr., E.J. and Blumwald, E. (1990) Photolabeling of tonoplast from sugar beet cell suspensions by [^{3}H]-5-(*N*-methyl-*N*-isobutyl)-amiloride, an inhibitor of the vacuolar Na^+/H^+ antiport. Plant Physiol. 93, 924–930.
[75] Ballesteros, E., Blumwald, E., Donaire, J.P. and Belver, A. (1997) Na^+/H^+ antiport activity in tonoplast vesicles isolated from sunflower roots induced by NaCl stress. Physiol. Plant 99, 328–334.
[76] Niemitz, C. and Willenbrink, J. (1985) The function of tonoplast ATPase in intact vacuoles of red beet is governed by direct and indirect ion effects. Planta 166, 545–549.
[77] Blumwald, E., Cragoe Jr., E.J. and Poole, R.J. (1987) Inhibition of Na^+/H^+ antiport activity in sugar beet tonoplast by analogues of amiloride. Plant Physiol. 85, 30–33.
[78] Carden, D.E., Walker, D.J., Flowers, T.J. and Miller, A.J. (2003) Single-cell measurements of the contributions of cytosolic Na^+ and K^+ to salt tolerance. Plant Physiol. 131, 676–683.
[79] Fan, T.W.M., Higashi, R.M., Norlyn, J. and Epstein, E. (1989) In vivo ^{23}Na and ^{31}P NMR measurement of a tonoplast Na^+/H^+ exchange process and its characteristics in two barley cultivars. PNAS USA 86, 9856–9860.
[80] Gruwel, M.L.H., Rauw, V.L., Loewen, M. and Abrams, S.R. (2001) Effects of sodium chloride on pant cells; a ^{31}P and ^{23}Na NMR system to study salt tolerance. Plant Sci. 160, 785–794.
[81] Guern, J., Mathieu, Y., Kurkdjian, A., Manigault, P., Manigault, J., Gillet, B., Beloell, J.C. and Lallemand, J.Y. (1989) A ^{31}P NMR study of the modifications of vacuolar pH in isolated vacuoles induced by proton pumping and cation/H^+ exchanges. Plant Physiol. 89, 27–36.
[82] Halperin, S.J. and Lynch, J.P. (2003) Effects of salinity on cytosolic Na^+ and K^+ in root hairs of *Arabidopsis thaliana*: in vivo measurements using the fluorescent dyes SBFI and PBFI. J. Exp. Bot. 54, 2035–2043.
[83] Muhling, K.H. and Lauchli, A. (2002) Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. J. Plant Physiol. 159, 137–146.
[84] Anil, V.S., Krishnamurthy, H. and Mathew, M. (2007) Limiting cytosolic Na^+ confers salt tolerance to rice cells in culture: a two-photon microscopy study of SBFI-loaded cells. Physiol. Plant. 129, 607–621.
[85] Fukuda, A., Nakamura, A., Tagiri, A., Tanaka, H., Miyao, A., Hirchika, H. and Tanaka, Y. (2004) Function, intracellular localization and the importance of salt tolerance of a vacuolar Na^+/H^+ antiporter from rice. Plant Cell Physiol. 45, 146–159.
[86] Lu, S.Y., Jing, Y.X., Shen, S.H., Zhao, H.Y., Ma, L.Q., Zhou, X.J., Ren, Q. and Li, Y.F. (2005) Antiporter gene from *Hordeum brevisubulatum* (Trin.) link and its overexpression in transgenic tobaccos. J. Int. Plant Biol. 47, 343–349.