Published in final edited form as:

Trends Plant Sci. 2009 December ; 14(12): 660–668. doi:10.1016/j.tplants.2009.08.009.

HKT transporter-mediated salinity resistance mechanisms in *Arabidopsis* and monocot crop plants

Tomoaki Horie¹, Felix Hauser², and Julian I. Schroeder²

¹ Group of Molecular and Functional Plant Biology, Research Institute for Bioresources, Okayama University, Kurashiki, Okayama 710-0046, Japan

² Division of Biological Sciences, Cell and Developmental Biology Section, and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

Abstract

The salinization of irrigated lands is increasingly detrimental to plant biomass production and agricultural productivity as most plant species are sensitive to high concentrations of sodium (Na^+), which causes combined Na^+ toxicity and osmotic stress. Plants have multiple Na^+ transport systems to circumvent Na^+ toxicity. Essential physiological functions of major Na^+ transporters and their mechanisms mediating salinity resistance have been identified in *Arabidopsis*, including the SOS1, AtNHX and AtHKT1;1 transporters. As we discuss here, recent studies have demonstrated that a class of xylem–parenchyma-expressed Na^+ -permeable plant HKT transporters represent a primary mechanism mediating salt tolerance and Na^+ exclusion from leaves in *Arabidopsis*, and that major salt tolerance QTL in monocot crop plants are also based on this HKT-mediated mechanism. Sodium toxicity and salt tolerance in plants

Physiological studies have shown that salinity stress in plants is multifactorial, including osmotic stress [1] and cellular sodium (Na^+) toxicity, such as inhibition of vital enzymes and metabolic processes [2–14]. Photosynthetic processes are among the most sensitive to salinity and, therefore, salinity stress directly reduces carbon fixation and biomass production in plants [5,15–18]. Sodium transport processes have major roles in salinity tolerance, including organellar Na^+ sequestration [4,8,9,15,19,20]; Na^+ extrusion by plasma membrane Na^+ – H^+ exchange transporters, such as AtSOS1 [21,22] and exclusion of Na^+ from leaves and shoots [11,23–29]. In addition, reducing Na^+ uptake or increasing cytoplasmic potassium (K^+) levels relative to Na^+ increases Na^+ tolerance in plants [30–33]. However, given that multiple independent cationic nutrient uptake transporters mediate Na^+ uptake from the soil into roots (reviewed in Refs [6,34]), engineering of reduced Na^+ influx into plant roots is likely a more challenging endeavor. The identification and characterization of Na^+ -permeable transporters is therefore pivotal to understanding plant Na^+ toxicity and tolerance [13,35–38].

Recent research has demonstrated that members of the high-affinity K^+ transporter (HKT) transporter/channel family mediate important Na^+ tolerance mechanisms in plants. The *TaHKT2;1* gene from wheat (*Triticum aestivum*) (previously named *HKT1*), was the first HKT transporter gene found in plants [39]. It was shown to mediate high-affinity Na^+ – K^+ co-transport and also preferred Na^+ -selective low-affinity Na^+ transport in the presence of a

Corresponding author: Horie, T. (horie@rib.okayama-u.ac.jp).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

millimolar $[Na^+]$ in *Xenopus laevis* oocytes and yeast [40,41]. Members of the HKT transmembrane transporter family are among the most-studied Na^+ -permeable plant transporter proteins to date and have been identified and characterized in many other plant species [11,24–29,42–52]. The *Arabidopsis thaliana* genome includes only one *HKT* gene, *AtHKT1;1* (previously named *AtHKT1*) [42]. Multiple *HKT* genes are found in monocot genomes [28,29,49,53]. Studies of *Arabidopsis* plants carrying mutations in *AtHKT1;1* revealed that Na^+ -selective transport via *AtHKT1;1* has an essential role in Na^+ exclusion from leaves and K^+ homeostasis in leaves during salinity stress [11,24,26,27,54–56]. Here, we review findings of the functions of *AtHKT1;1* and its orthologs in rice (*Oryza sativa*) and durum wheat (*Triticum turgidum durum*), which mediate a major salinity-resistant mechanism both in dicots and monocots via Na^+ exclusion from leaves. We also review findings of *OsHKT2;1* in rice, which functions in mediating Na^+ influx into roots of K^+ -starved rice plants [51].

Sodium exclusion from leaves mediated by *AtHKT1;1* and *OsHKT1;5*

Biophysical transport and phylogenetic analyses showed that HKT transporters can be classified into two subgroups, class 1 and class 2 [57,58] (Figure 1). Class 1 HKT transporters show a preference for Na^+ transport over other cations, whereas class 2 HKT transporters show a larger K^+ permeability as well as Na^+ permeability in heterologous expression systems, with some exceptions [43,46,48,59].

AtHKT1;1 was identified as encoding a relatively Na^+ -selective class 1 transporter in *Arabidopsis*, with additional small K^+ uptake activity when expressed in an *Escherichia coli* K^+ -uptake mutant [42]. Mutations by T-DNA insertion, ethylmethane sulphonate (EMS) treatment or deletions in *AtHKT1;1* caused severe Na^+ overaccumulation in leaves, leading to leaf chlorosis under salinity stress [11,24,54]. Furthermore, *athkt1;1* T-DNA disruption mutations caused greatly reduced Na^+ concentrations in roots [11]. However, the total Na^+ content in whole *Arabidopsis* plants was similar in *athkt1;1* mutant and wild-type control plants, and, therefore, *AtHKT1;1* was concluded to regulate the Na^+ distribution between roots and shoots [11]. *AtHKT1;1* is expressed in the vasculature in roots and shoots of *Arabidopsis* [11,24]. *athkt1;1* EMS mutant plants accumulate significantly less Na^+ in the phloem sap compared with wild-type plants [24]. Together with findings of phloem tissue-specific expression of *AtHKT1;1* mRNA, a phloem Na^+ re-circulation model for *AtHKT1;1* was proposed in which Na^+ is loaded into phloem cells by *AtHKT1;1* and transferred from shoots to roots via a downward stream of phloem, preventing Na^+ overaccumulation in shoots [24].

The mechanism of action of *AtHKT1;1* has been characterized further. *AtHKT1;1* was found to localize at the plasma membrane of xylem parenchyma cells [26]. Analyses of xylem sap emerging from roots and in stems showed that *AtHKT1;1* mediates removal of Na^+ from root xylem vessels during salinity stress [26]. These findings led to an alternative model for the function of the *AtHKT1;1* transporter in protecting leaves from salinity stress by mediating removal of Na^+ from the xylem sap via Na^+ uptake into xylem parenchyma cells [26,27,55] (Figure 2a). In support of this model, enhancer trap-mediated *AtHKT1;1* overexpression in the root pericycle, which includes xylem parenchyma cells, causes enhanced inward Na^+ transport in the targeted cells, and results in improved salt tolerance in targeted *AtHKT1;1*-overexpressing lines compared with wild-type plants [60].

Independent analyses of the quantitative trait locus (QTL) in rice, named *SKCI* (shoot K^+ content), resulted in an identical model for the function of the rice gene *OsHKT1;5* [25]. The *SKCI* QTL was caused by point mutations in *OsHKT1;5* that replace several amino acid residues in the salt-tolerant cultivar Nona Bokra [25]. These point mutations enhance the

overall Na⁺ transport activity of OsHKT1;5, based on electrophysiological activity analyses of OsHKT1;5 expressed in *Xenopus* oocytes [25].

The OsHKT1;5 transporter was found to encode a Na⁺-selective transporter in *Xenopus* oocytes [25]. *OsHKT1;5* promoter-beta-glucuronidase (*GUS*) reporter analyses show expression in rice xylem parenchyma cells and OsHKT1;5 reduces Na⁺ levels in the xylem sap [25]. These findings are similar to the characterized functions of the close orthologs, *OsHKT1;5* and *AtHKT1;1*, which are members of the class 1 sub-family of HKT transporters [25,26,58] (Figure 1). Interestingly, the *Mesembryanthemum crystallinum* McHKT1;1 class 1 HKT transporter was also targeted to xylem parenchyma cells [48].

The findings that OsHKT1;5 and AtHKT1;1 mediate Na⁺ exclusion from leaves via Na⁺ removal from the xylem sap, do not exclude a role for AtHKT1;1 in Na⁺ loading into phloem vessels [24]. Research using the identical methods reported for phloem sap extraction [24] reproduced the significant reduction in Na⁺ accumulation in phloem extracts of *athkt1;1* null-mutant plants under salinity stress [26]. By contrast, physiological experiments using wild-type and *athkt1;1* mutant plants that were loaded with radioactive tracer ²²Na⁺ in the presence of 50 mM NaCl indicated that shoot Na⁺ contents of both wild-type and mutant plants were relatively unaffected over 2 days after Na⁺ loading, which led to the conclusion that the phloem sap Na⁺ recirculation model [24] is unlikely [55,61,62]. This controversy regarding the phloem Na⁺ recirculation model [24,55,62] highlights an important question, namely: where does the Na⁺ that is removed from the xylem sap end up?

An intracellular compartment that can accommodate substantial Na⁺ concentrations is the vacuole, where Na⁺ sequestration occurs via AtNHX Na⁺/cation-H⁺ antiporters [8]. Sodium efflux from roots mediated by the SOS1 Na⁺-H⁺ antiporter is another candidate mechanism for eliminating Na⁺ ions from the cytoplasm, as Na⁺ exclusion activity by SOS1 from the epidermal layer of the mature root zone [63] and the root tip region [23] have been shown. Further research is needed to address whether vacuolar Na⁺ sequestration is the only mechanism used by plants to deal with Na⁺ toxicity in their aerial tissues after HKT-mediated removal of Na⁺ from the xylem sap. Apart from these questions, present findings in *Arabidopsis* and rice agree with the model that a major role for AtHKT1;1 and OsHKT1;5 during salinity stress can be defined as 'Na⁺ exclusion from shoots' via Na⁺ removal from the xylem sap [25–27,64], which thus protects leaves from Na⁺ toxicity including vital photosynthetic reactions (Figure 2a).

A role for xylem parenchyma-HKT transporters in increasing K⁺ levels in leaves during salinity stress

Potassium protects plant cells from Na⁺ stress. Mutations in the rice and *Arabidopsis* Na⁺ transporters have been also found to reduce K⁺ accumulation in shoots during salt exposure, which is likely to further enhance salinity stress [25,26]. The reduced K⁺ accumulation in the xylem sap, shoots and roots of *hkt* mutants was inverted to enhanced Na⁺ accumulation in the same tissues. Specifically, enhanced Na⁺ levels in the xylem sap of *athkt1;1* mutants was accompanied by reduced xylem sap K⁺ levels [25,26]. As K⁺ counteracts Na⁺ toxicity, the reduced K⁺ accumulation in leaves of *hkt* mutants further compounds Na⁺ toxicity. However, the effect of *athkt1;1* disruption and point mutations found in OsHKT1;5 proteins on K⁺ accumulation is smaller than the inverse effect on Na⁺ accumulation [25,26]. These results suggest that AtHKT1;1 and OsHKT1;5 indirectly propel K⁺ release from xylem parenchyma cells into the xylem vessel.

A probable driving force for xylem parenchyma K⁺ release in combination with the Na⁺ removal function of AtHKT1;1 and OsHKT1;5 should be membrane depolarization. Na⁺

absorption into xylem parenchyma cells via HKT transporters would depolarize xylem parenchyma cells, which could activate ion channels that can mediate K^+ efflux [65–67]. Two independent depolarization-activated outward K^+ rectifying systems have been identified by patch clamping analyses using xylem parenchyma cells from barley (*Hordeum vulgare*) roots: (i) the K^+ outward-rectifying (KOR) channel; and (ii) the nonselective outward-rectifying (NOR) channel [66,67]. The KOR channel was found to be largely K^+ selective, whereas the NOR channel was shown to be predominantly permeable to cations including K^+ and Ca^{2+} [67]. These reports lead to the hypothesis that KOR and/or NOR channel activity are coupled to the AtHKT1;1-mediated depolarization at xylem parenchyma cells in *Arabidopsis* (Figure 2b). The cDNA encoding the shaker type outward-rectifying K^+ channel (SKOR) was identified in *Arabidopsis* [68]. Characterizations of the AtSKOR channel in *Xenopus* oocytes and loss-of-function *atskor* mutant plants have shown that this channel mediates K^+ release into the xylem vessels from xylem parenchyma cells [68]. Whether the NOR-type channel activity also contributes to the K^+ release from xylem parenchyma cells, will require identification of the NOR-encoding gene(s). Patch clamp analyses in combination with GFP fluorescence-labeled xylem parenchyma cells could enable further characterization of electrogenic K^+ efflux-mediating transporters in xylem parenchyma cells.

Findings on the inverted control of Na^+ and K^+ levels in the xylem sap of *hkt1* mutant plants, suggest that AtHKT1;1 and OsHKT1;5 provide two essential mechanisms towards mediating salt tolerance: (i) prevention of Na^+ overaccumulation in leaves; and (ii) promotion of K^+ accumulation in leaves [25,26] (Figure 2b).

Major salinity tolerance QTL in wheat are encoded by class 1 HKT genes

An important salinity tolerance locus *Kna1* in *T. aestivum* controls the selectivity of Na^+ and K^+ transport to shoots, resulting in a high K^+Na^+ ratio in leaves [69–72]. The underlying *Kna1* gene has been long sought [69–72] and has been mapped to the distal region of the long arm of chromosome 4D [72], but identifying the underlying gene was complicated by the polyploid nature of the wheat genome. *Nax1* and *Nax2* loci, which contribute to salt tolerance of wheat plants, were identified by QTL analyses using durum wheat [73,74]. Analogous to the findings in *Arabidopsis* and rice discussed earlier [11,25,26], the *Nax1* and *Nax2* loci were shown to reduce Na^+ transport from roots to shoots and maintain low Na^+ concentrations in the leaf blade by excluding Na^+ from the xylem [75]. The *Nax1* locus has been further shown to have a leaf-located function in exclusion of Na^+ from leaves, such that *Nax1* enhances removal of Na^+ from the xylem of the leaf sheath (the base of leaves), preventing Na^+ entry into the leaf blade [75]. Furthermore, the presence of *Nax1* and *Nax2* was shown to enhance K^+ accumulation in leaf blades and sheaths, suggesting that *Nax1* and *Nax2* mediate K^+ loading into the xylem [75]. The *Nax2* chromosomal region of a salt-tolerant durum wheat, Line149, was found to correspond to the *Kna1* region of *T. aestivum*, suggesting that *Kna1* and *Nax2* are orthologs [29]. Interestingly, sequencing analyses of known Na^+ transporter genes, including *HKTs* [39,40], *NHXs* [8,9,76] and *SOS1* [23], within the rough mapping regions of *Kna1*, *Nax1* and *Nax2* have suggested that all three of these major salinity tolerance QTL are attributable to polymorphisms in copies of wheat *HKT* genes, *TmHKT1;4-A1* and *TmHKT1;4-A2* (also named *TmHKT7-A1* and *-A2*) for *Nax1* and *TmHKT1;5* and *TaHKT1;5* for *Nax2* and *Kna1* [28,29]. Indeed, the wheat *TmHKT1;4* and *HKT1;5* genes encode class 1 HKT transporters that are orthologs of the transporters encoded by *AtHKT1;1* and *OsHKT1;5* [28,29] (Figure 1). These findings in wheat implicate that class 1 HKT Na^+ transporters mediate Na^+ exclusion from leaves and leaf Na^+ tolerance for all three of these major Na^+ tolerance QTL.

The *Kna1*, *Nax1* and *Nax2* QTL in wheat control Na^+ accumulation in leaf sheaths and in Na^+ removal from the xylem sap, suggesting that they share analogous or similar functions

with AtHKT1;1 in *Arabidopsis* and OsHKT1;5 and OsHKT1;4 (previously named OsHKT7) in rice (Figure 2) [25,26]. Together, the above studies suggest that class 1 HKT transporters (Figure 1) in *Arabidopsis*, rice and wheat have major functions in mediating Na⁺ tolerance and Na⁺ exclusion from leaves, by removing Na⁺ from the xylem sap [25–29,75]. This mechanism protects leaves and the photosynthetic machinery from Na⁺ overaccumulation in dicots and monocots (Figure 2a) and could provide a mechanism for engineering leaf Na⁺ exclusion and thus enhanced Na⁺ tolerance in plants [25,26]. Targeted overexpression of *AtHKT1;1* in the pericycle of *Arabidopsis* enhances salinity resistance, supporting this model [60]. Moreover, this recent *AtHKT1;1* overexpression study [60] and *athk1;1* T-DNA knockout analyses [27] both could not confirm a previous study reporting that *AtHKT1;1* enhances salt sensitivity [77]. Further research is needed to illuminate specialized functions and regulation mechanisms of these salinity tolerance-mediating HKT transporters.

Class 2 HKT transporters

The leaf Na⁺ exclusion mechanism in plants discussed above is mediated by class 1 HKT transporter sub-family members (Figure 1) [11,24–27,54,55,64]. A second clade of HKT transporters exists in plants, the HKT2 family [39,44] for which *in planta* functions are only now beginning to be revealed. TaHKT2;1 was the first HKT transporter identified in plants, which belongs to the class 2 subfamily [39] (Figure 1). The transcript level of several *HKT2*-type transporter genes has been shown to increase by K⁺ starvation in wheat, rice and barley [44,49,78]. As summarized below, several members of the HKT2 class of transporters are more permeable to K⁺ compared with the class 1 HKTs based on chimeric studies and biophysical transport analyses in *Xenopus* oocytes and yeast [39–41,44,57,59,79]. However, the *in vivo* physiological functions of this HKT2 transporter class remain unknown in plants, with the exception of the recently characterized function of OsHKT2;1 in rice [51], which has been demonstrated to be a unique member of class 2 HKT transporters in terms of its relative selectivity for Na⁺ over K⁺ [44,49,57].

Among the class 2 HKT transporters, the OsHKT2;1 transporter is distinct because it has a serine residue in the first putative selectivity pore-forming region ('loop') [80], which represents one of the important factors determining Na⁺ selectivity in HKT transporters [57], with some exceptions [43,46,48]. Other presently known members of the HKT2 class have a glycine residue at the corresponding position instead of a serine residue [39,40,44,49,79]. A different model has suggested that an unusual translation start at a non-ATG start site is responsible for the different ion selectivities of HKT transporters [50], but more recent work revised this hypothesis and concluded that differences in heterologous expression levels of *HKT* cDNA mutated in their 5' UTRs were responsible for the reported differential extracellular cation removal activities [81]. Consistent with the K⁺ permeability of class 2 HKT transporters, *in vivo* analyses of all four pore-forming loops in the bacterial HKT2 homolog, KtrB, showed that these glycine residues are required for K⁺ transport activity *in vivo* [82]. The transport selectivity of Na⁺ and K⁺ of HKT transporters depends on the ionic conditions and HKT2 transporters can mediate selective Na⁺ influx at high Na⁺ concentrations [40,41,44,47,57,83]. Furthermore, studies have shown that the relative cation selectivities of HKT transporters depend on the ionic conditions [39–41,44,83], which is typical for ion channels that show multiple occupancy by more than one cation at a time [79,84]. The importance of the glycine residue for K⁺ permeability at the filter position did not apply to some class 1 HKT transporters [43,48] and the ion selectivity of OsHKT2;1 reported differed among studies from relatively more Na⁺ selective [44,49] to showing a slightly larger relative K⁺ permeability [59] and no selectivity among mono-valent cations in heterologous expression systems [46]. Further research is needed in which HKT transporters are expressed in plant cells to determine the *in vivo* cation selectivities of HKT2-class transporters with four glycines in their pore loop

domains, as presently all cation selectivity studies on this class of plant transporters have been conducted in heterologous systems.

OsHKT2;1 transporter-mediated nutritional Na⁺ uptake in K⁺-starved rice roots

Salt toxicity arises mainly owing to Na⁺ influx into plant roots, which is probably mediated by more than one type of Na⁺ transporter [6,13,34,85–89]. No mutation that diminishes Na⁺ influx into plant roots had been isolated until recently. The OsHKT2;1 transporter was characterized as a Na⁺ transporter in heterologous expression systems [44,49,57], with evidence for additional K⁺ transport activity [46,59]. Analyses of three rice *oshkt2;1* disruption mutant alleles revealed a major role for OsHKT2;1 in mediating Na⁺ influx into K⁺-starved rice roots [51]. Plant physiological experiments showed that Na⁺ enhances growth of many different plant species [90,91]. Findings from *oshkt2;1* analyses provide genetic evidence that Na⁺ acts as a substitute nutrient for K⁺ in K⁺-starved rice plants under moderate Na⁺ concentrations [51], supporting this long-standing hypothesis [90,91]. Thus, *oshkt2;1* mutant alleles represent a first mutant gene that diminish Na⁺ influx into plant roots [51].

Given that many transporters are expected to contribute to Na⁺ influx into plant roots, it is not surprising that single gene mutants with greatly reduced Na⁺ influx have not been previously described [6,86,87]. Nevertheless, the OsHKT2;1-mediated short-term Na⁺ influx rates into K⁺-starved rice roots are as high as Na⁺ influx rates found for low-affinity Na⁺ influx transporters in plants [92,93], indicating that OsHKT2;1 is a primary Na⁺ transporter in rice roots when extracellular K⁺ is limiting. Given that OsHKT2;1 is conditionally induced by K⁺ starvation, the question arises whether this transporter mediates measurable K⁺ influx into intact rice roots. However, no clear differences in Rb⁺ (a K⁺ analog in transport experiments) influx rates were found among wild-type and *oshkt2;1* mutant alleles [51], consistent with data showing relative Na⁺ over K⁺ selectivity of OsHKT2;1 in heterologous expression systems [44,49].

OsHKT2;1-dependent Na⁺ influx is highly regulated and activated under K⁺ starvation conditions, but is rapidly downregulated by protein kinase inhibitors and also by salinity stress with a half-time of < 1.5 hr [51], suggesting that Na⁺ transport activity of OsHKT2;1 is post-translationally regulated by as yet unknown cellular signaling pathways. Analyses of OsHKT2;1 regulation mechanisms will provide insights into molecular mechanisms by which a plant root Na⁺ influx transporter can be controlled.

In spite of the lower K⁺ permeability of OsHKT2;1 compared with other HKT2 class transporters [44], many of the presently characterized HKT2 class genes are also induced by K⁺ starvation, including those in wheat, barley and rice [44,49,78]. Furthermore, anti-sense repression of the wheat gene *TaHKT2;1* (previously named *HKT1*) was shown to reduce Na⁺ influx into wheat roots [47]. Together with its root cortex expression pattern [39], induction by K⁺ starvation [78], and K⁺-starvation-induced Na⁺ influx currents in wheat cortex cells [88], *TaHKT2;1* might have a similar function in root Na⁺ influx to OsHKT2;1 in rice. Thus, it is interesting that class 2 HKT transporters can shift from a Na⁺–K⁺ co-transport mode to Na⁺ transport, depending on the ionic conditions [40,41,83]. Na⁺ influx into plant roots has been suggested to be dependent on multiple pathways and Na⁺ influx to toxic levels is independent of the status of K⁺ availability [87,89,94], consistent with findings that OsHKT2;1 is rapidly downregulated by salinity stress and does not contribute to salinity stress [51].

K⁺ starvation-induced Na⁺ influx is likely to be mediated by more than one transporter family in plants. The findings that the *TaHKT2;1* transporter mediates Na⁺–K⁺ uptake [40,41], indicated that other types of high-affinity K⁺ uptake transporter should exist in plants.

Arabidopsis genomic sequencing led to identification of the KUP–HAK–KT family, which is closely related to bacterial KUP K⁺ uptake transporters [95–99]. The findings that Na⁺–K⁺ uptake is a major high-affinity K⁺ uptake mechanism in aquatic plants [100,101], but not in land plants [102] further make clear that non-HKT transporters should mediate high-affinity K⁺ uptake. KUP/HAK/KT transporters function in high-affinity K⁺ uptake [103,104], and have also been reported to enable Na⁺ permeation [95,98]. Members of the KUP/HAK/KT transporter family are strongly induced by K⁺ starvation [96,98,104,105] and thus these transporters might also contribute to K⁺-starvation-induced Na⁺ influx into plant roots, depending on the plant species. Furthermore, some plants, such as *Arabidopsis*, do not have class 2 HKT Na⁺ transporters [13,42], and therefore it would be trivial to conclude that no other Na⁺ influx transporter classes exist in plant roots. Future research is likely to shed molecular insights into these classes of additional Na⁺ influx transporters [85–89,94,106].

Important future questions for HKT transporter-mediated salinity tolerance

As described above, research on *Arabidopsis* and rice has led to the uncovering of molecular genetic and mechanistic functions for a major salinity tolerance mechanism and class of HKT transporters that mediate leaf Na⁺ exclusion in dicot and monocot reference plants [11,24–27,54,55]. In spite of this important advance, there are many new questions. For example, no protein interactor and regulator of HKT transporters is currently known in plants. Recent research indicates roles for post-translational modification of HKT-dependent transport activity in rice [51]. Similarly, the abundance of a relatively Na⁺ selective transporter in the plasma membrane of xylem parenchyma cells (Figure 2) [26,48] calls for transport activity regulation mechanisms. Such HKT transporters would probably have damaging effects on xylem parenchyma membrane potential, if they were not downregulated when not needed at low extracellular Na⁺ levels. HKT-mediated membrane potential changes could cause hyperpolarized resting potentials of xylem parenchyma cells in the absence of Na⁺ stress [39,41], assuming baseline Na⁺ concentrations in the cytoplasm. Such HKT-mediated effects on membrane potential could be debilitating on physiological xylem transport of many other ions and nutrients.

Another question that remains is whether the physiological functions of these HKT1 transporters are fully understood. The answer is currently no, given the debate regarding whether AtHKT1;1 affects phloem Na⁺ levels (see above and Refs[24,55]). Other open questions include: where does Na⁺ go after being removed from the xylem? It is hard to imagine that all Na⁺ removed from xylem vessels remains in xylem parenchyma cells even though some Na⁺ will be sequestered in vacuoles [8,19]; independent of whether Na⁺ re-circulation via the phloem occurs, what is the identity of the Na⁺ transporters that mediate Na⁺ efflux from xylem parenchyma cells into the neighboring apoplastic space? Does the AtHKT1;1-type transporter also mediate outward Na⁺ transport *in vivo*, as proposed in the Na⁺ re-circulation model [24]? Alternatively, a SOS1-type Na⁺–H⁺ exchanger [23] in the plasma membrane of phloem companion cells would be suited for mediating Na⁺ efflux.

Several studies have suggested that HKT transporters function as ion channels [41,57,79,107], but to test this hypothesis directly, *in vivo* electrophysiological analyses are needed to determine whether reversal potentials of HKT transporters follow the electrochemical equilibrium (Nernst) potential for imposed ion gradients. Research on rice and the rice genome sequencing efforts showed that monocots have multiple HKT transporters [49]. In a *japonica* rice cultivar Nipponbare, five functional class 1 HKT transporters including OsHKT1;5 have been found to be expressed [49] (Figure 1). Recently, detailed ion selectivity analyses of OsHKT1;1 and OsHKT1;3 have been reported [59]. OsHKT1;1 and OsHKT1;3, which are two closely related class 1 transporters, have been demonstrated to show Na⁺ selective transport [59]. *In situ* hybridization experiments showed that the tissue specificity of

the expression of *OsHKT1;1*, *OsHKT1;3* and *OsHKT2;1* overlap in root periphery and vascular tissues of both roots and shoots, also showing some gene-specific expression patterns. These *OsHKT* mRNAs were also detected in osmocontractile leaf bulliform cells that are thought to function in leaf folding. These results suggest that Na⁺ transport via *OsHKT* transporters have roles in several different physiological aspects of rice [59]. Thus far, no gene knockout data on the monocot class 1 HKT transporters exist and only one HKT2 class transporter knockout has been reported [51]. In wheat, where *HKT1*-linked salinity resistance QTL have been characterized [28,29], this is complicated by the fact that wheat includes multiple copies of *HKT1;4* and *HKT1;5* genes with similar proposed functions as the *Arabidopsis AtHKT1;1* gene. No knockout mutants in the rice orthologs *HKT1;4* and *HKT1;5* [25] are available yet. Recent advances in creating transposon and T-DNA insertional mutagenesis lines in rice [108–111], rice transformation [112], and rice genome sequencing [113–115] render rice a suitable crop plant model system to characterize the functions of these HKT transporters. Further evidence from loss-of-function mutants of these genes will be needed to elucidate fully the roles of *HKT1;4* and *HKT1;5* in plant salinity tolerance. Another question that arises is whether these findings can be translated into engineering improved Na⁺ tolerance of crops, which is likely to require an in-depth understanding of the multiple HKT isoforms found in crop plants, their cellular targeting and their regulators and protein interactors. Nevertheless present studies suggest that manipulation of HKT-mediated transport provides an approach for engineering Na⁺ exclusion and protection of leaves from salinity stress.

Conclusions

Recent research points to a major function of specific class 1 HKT transporters in mediating salt tolerance and leaf Na⁺ exclusion in dicots and monocots, via reducing Na⁺ content in the xylem sap and thus protecting plant leaves from Na⁺ overaccumulation. This research provides an example that basic findings in *Arabidopsis* are relevant to crop plants. However, important questions remain. Elucidating a complete picture of how class 1 HKT transporters contribute to salinity tolerance in plants, together with uncovering the downregulation mechanisms of HKT2 transporter-mediated Na⁺ influx into roots under high Na⁺ concentrations, will be important steps in determining distinct and relevant salt tolerance mechanisms in plants. Further research will contribute to understanding mechanisms by which leaf metabolic processes and photosynthesis can be protected during salt stress, which will become increasingly important for engineering plants for food and renewable biomass production in light of globally increasing salinization of agricultural utilized lands.

Acknowledgments

This research was support by DOE grant DOE-DE-FG02-03ER15449 and NIEHS grant 1 P42 ESI0337 (to J.I.S.).

References

1. Tarczynski MC, et al. Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* 1993;259:508–510. [PubMed: 17734171]
2. Rains DW, Epstein E. Transport of sodium in plant tissue. *Science* 1965;148:1611. [PubMed: 17769920]
3. Cheeseman JM. Mechanisms of salinity tolerance in plants. *Plant Physiol* 1988;87:547–550. [PubMed: 16666181]
4. Barkla BJ, Blumwald E. Identification of a 170-kDa protein associated with the vacuolar Na⁺/H⁺ antiport of *Beta vulgaris*. *Proc Natl Acad Sci U S A* 1991;88:11177–11181. [PubMed: 1662387]
5. Munns R. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. *Plant Cell Environ* 1993;16:15–24.

6. Schroeder JI, et al. Perspectives on the physiology and structure of inward-rectifying K⁺ channels in higher plants: Biophysical implications for K⁺ uptake. *Annu Rev Biophys Biomol Struct* 1994;23:441–471. [PubMed: 7919789]
7. Murguía JR, et al. A salt-sensitive 3' (2'), 5'-bisphosphate nucleotidase involved in sulfate activation. *Science* 1995;267:232–234. [PubMed: 7809627]
8. Apse MP, et al. Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiport in *Arabidopsis*. *Science* 1999;285:1256–1258. [PubMed: 10455050]
9. Gaxiola RA, et al. The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification. *Proc Natl Acad Sci U S A* 1999;96:1480–1485. [PubMed: 9990049]
10. Nelson DE, et al. Myo-inositol-dependent sodium uptake in ice plant. *Plant Physiol* 1999;119:165–172. [PubMed: 9880357]
11. Mäser P, et al. Altered shoot/root Na⁺ distribution and bifurcating salt sensitivity in *Arabidopsis* by genetic disruption of the Na⁺ transporter AtHKT1. *FEBS Lett* 2002a;531:157–161.
12. Ward JM, et al. Plants pass the salt. *Trends Plant Sci* 2003;8:200–201. [PubMed: 12758034]
13. Horie T, Schroeder JI. Sodium transporters in plants. Diverse genes and physiological functions. *Plant Physiol* 2004;136:2457–2462. [PubMed: 15375202]
14. Song CP, et al. A probable Na⁺(K⁺)/H⁺ exchanger on the chloroplast envelope functions in pH homeostasis and chloroplast development in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 2004;101:10211–10216. [PubMed: 15220473]
15. Greenway H, Munns RA. Mechanisms of salt tolerance in non-halophytes. *Annu Rev Plant Physiol* 1980;31:149–190.
16. Flowers TJ. Salinisation and horticultural production. *Sci Hort* 1999;78:1–4.
17. Tsugane K, et al. A recessive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. *Plant Cell* 1999;11:1195–1206. [PubMed: 10402422]
18. Munns R. Prophylactically parking sodium in the plant. *New Phytol* 2007;176:501–504. [PubMed: 17953536]
19. Blumwald E, Poole R. Na⁺/H⁺-antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. *Plant Physiol* 1985;78:163–167. [PubMed: 16664191]
20. Blumwald E, Poole RJ. Salt tolerance in suspension cultures of sugar beet : induction of Na⁺/H⁺ antiport activity at the tonoplast by growth in salt. *Plant Physiol* 1987;83:884–887. [PubMed: 16665356]
21. Shi H, et al. The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na⁺/K⁺ antiporter. *Proc Natl Acad Sci U S A* 2000;97:6896–6901. [PubMed: 10823923]
22. Shi H, et al. Overexpression of a plasma membrane Na⁺/H⁺ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. *Nat Biotechnol* 2003;21:81–85. [PubMed: 12469134]
23. Shi H, et al. The putative plasma membrane Na⁺-H⁺ antiporter SOS1 controls long-distance Na⁺ transport in plants. *Plant Cell* 2002;14:465–477. [PubMed: 11884687]
24. Berthomieu P, et al. Functional analysis of AtHKT1 in *Arabidopsis* shows that Na⁺ recirculation by the phloem is crucial for salt tolerance. *EMBO J* 2003;22:2004–2014. [PubMed: 12727868]
25. Ren ZH, et al. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat Genet* 2005;37:1141–1146. [PubMed: 16155566]
26. Sunarpi. Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na⁺ unloading from xylem vessels to xylem parenchyma cells. *Plant J* 2005;44:928–938. [PubMed: 16359386]
27. Horie T, et al. Calcium regulation of sodium hypersensitivities of *sos3* and *athkt1* mutants. *Plant Cell Physiol* 2006;47:622–633. [PubMed: 16540484]
28. Huang S, et al. A sodium transporter (HKT7) is a candidate for *Nax1*, a gene for salt tolerance in durum wheat. *Plant Physiol* 2006;142:1718–1727. [PubMed: 17071645]
29. Byrt CS, et al. HKT1;5-like cation transporters linked to Na⁺ exclusion loci in wheat, *Nax2* and *Kna1*. *Plant Physiol* 2007;143:1918–1928. [PubMed: 17322337]
30. Shubert S, Läuchli A. Sodium exclusion mechanisms at the root surfaces of two maize cultivars. *Plant Soil* 1990;123:205–209.
31. Gaxiola R, et al. A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. *EMBO J* 1992;11:3155–3164.

32. Wu SJ, et al. *Sos1*, a genetic locus essential for salt tolerance and potassium acquisition. *Plant Cell* 1996;8:617–627. [PubMed: 12239394]
33. Zhu JK, et al. Genetic analysis of salt tolerance in *Arabidopsis*: Evidence for a critical role of potassium nutrition. *Plant Cell* 1998;10:1181–1191. [PubMed: 9668136]
34. Demidchik V, et al. Nonselective cation channels in plants. *Annu Rev Plant Biol* 2002;53:67–107. [PubMed: 12221989]
35. Blumwald E. Sodium transport and salt tolerance in plants. *Curr Opin Cell Biol* 2000;12:431–434. [PubMed: 10873827]
36. Zhu JK. Plant salt tolerance. *Trends Plant Sci* 2001;6:66–71. [PubMed: 11173290]
37. Zhu JK. Salt and drought stress signal transduction in plants. *Annu Rev Plant Physiol Plant Mol Biol* 2002;53:247–273.
38. Apse MP, Blumwald E. Na^+ transport in plants. *FEBS Lett* 2007;581:2247–2254. [PubMed: 17459382]
39. Schachtman DP, Schroeder JI. Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. *Nature* 1994;370:655–658. [PubMed: 8065452]
40. Rubio F, et al. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* 1995;270:1660–1663. [PubMed: 7502075]
41. Gassmann W, et al. Alkali cation selectivity of the wheat root high-affinity potassium transporter *HKT1*. *Plant J* 1996;10:869–882. [PubMed: 8953248]
42. Uozumi N, et al. The *Arabidopsis HKT1* gene homologue mediates inward Na^+ currents in *Xenopus* oocytes and Na^+ uptake in *Saccharomyces cerevisiae*. *Plant Physiol* 2000;121:1249–1259. [PubMed: 10759522]
43. Fairbairn DJ, et al. Characterization of two distinct HKT1-like potassium transporters from *Eucalyptus camaldulensis*. *Plant Mol Biol* 2000;43:515–525. [PubMed: 11052203]
44. Horie T, et al. Two types of HKT transporters with different properties of Na^+ and K^+ transport in *Oryza sativa*. *Plant J* 2001;27:129–138. [PubMed: 11489190]
45. Rus A, et al. AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. *Proc Natl Acad Sci U S A* 2001;98:14150–14155. [PubMed: 11698666]
46. Golldack D, et al. Characterization of a HKT-type transporter in rice as a general alkali cation transporter. *Plant J* 2002;34:1–14.
47. Laurie S, et al. A role for HKT1 in sodium uptake by wheat roots. *Plant J* 2002;32:139–149. [PubMed: 12383080]
48. Su H, et al. Expression of the cation transporter McHKT1 in a halophyte. *Plant Mol Biol* 2003;52:967–980. [PubMed: 14558658]
49. Garciadeblás B, et al. Sodium transport and HKT transporters: the rice model. *Plant J* 2003;34:788–801. [PubMed: 12795699]
50. Haro R, et al. HKT1 mediates sodium uniport in roots. Pitfalls in the expression of HKT1 in yeast. *Plant Physiol* 2005;139:1495–1506. [PubMed: 16258014]
51. Horie T, et al. Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+ -starved roots for growth. *EMBO J* 2007;26:3003–3014. [PubMed: 17541409]
52. Takahashi R, et al. Cloning and functional comparison of a high-affinity K^+ transporter gene *PhaHKT1* of salt-tolerant and salt-sensitive reed plants. *J Exp Bot* 2007;58:4387–4395. [PubMed: 18182440]
53. Huang S, et al. Comparative mapping of HKT genes in wheat, barley, and rice, key determinants of Na^+ transport, and salt tolerance. *J Exp Bot* 2008;59:927–937. [PubMed: 18325922]
54. Gong JJ, et al. Microarray-based rapid cloning of an ion accumulation deletion mutant in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 2004;101.
55. Davenport RJ, et al. The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in *Arabidopsis*. *Plant Cell Environ* 2007;30:497–507. [PubMed: 17324235]
56. Horie T, et al. Functions of HKT transporters in sodium transport in roots and in protecting leaves from salinity stress. *Plant Biotech* 2008;25:233–239.

57. Mäser P, et al. Glycine residues in potassium channel-like selectivity filters determine potassium selectivity in four-loop-per-subunit HKT transporters from plants. *Proc Natl Acad Sci U S A* 2002b; 99:6428–6433.
58. Platten JD, et al. Nomenclature for HKT transporters, key determinants of plant salinity tolerance. *Trends Plant Sci* 2006;11:372–374. [PubMed: 16809061]
59. Jabnoune M, et al. Diversity in expression patterns and functional properties in the rice HKT transporter family. *Plant Physiol.* (in press)
60. Møller IS, et al. Shoot Na⁺ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na⁺ transport in *Arabidopsis*. *Plant Cell.* (in press)
61. Møller IS, Tester M. Salinity tolerance of *Arabidopsis*: a good model for cereals? *Trends Plant Sci* 2007;12:534–540. [PubMed: 18023242]
62. Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 2008;59:651–681. [PubMed: 18444910]
63. Shabala L, et al. Salinity-induced ion flux patterns from the excised roots of *Arabidopsis sos* mutants. *Planta* 2005;222:1041–1050. [PubMed: 16079998]
64. Rus A, et al. Natural variants of AtHKT1 enhance Na⁺ accumulation in two wild populations of *Arabidopsis*. *PLoS Genet* 2006;2:e210. [PubMed: 17140289]
65. Schroeder JI, et al. Voltage dependence of K channels in guard-cell protoplasts. *Proc Natl Acad Sci U S A* 1987;84:4108–4112. [PubMed: 16593851]
66. Wegner LH, Raschke K. Ion channels in the xylem parenchyma of barley roots. *Plant Physiol* 1994;105:799–813. [PubMed: 12232243]
67. Wegner LH, De Boer AH. Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K⁺ homeostasis and long-distance signaling. *Plant Physiol* 1997;115:1707–1719. [PubMed: 12223889]
68. Gaymard F, et al. Identification and disruption of a plant shaker-like outward channel involved in K⁺ release into the xylem sap. *Cell* 1998;94:647–655. [PubMed: 9741629]
69. Gorham J, et al. Chromosomal location of a K/Na discriminating character in the D genome of wheat. *Theor Appl Genet* 1987;74:584–588.
70. Gorham J, et al. Partial characterization of the trait for enhanced K⁺-Na⁺ discrimination in the D genome of wheat. *Planta* 1990;180:590–597.
71. Luo MC, et al. Engineering of interstitial foreign chromosome segments containing the K⁺/Na⁺ selectivity gene *Kna1* by sequential homoeologous recombination in durum wheat. *Theor Appl Genet* 1996;93:1180–1184.
72. Dubcovsky J, et al. Mapping of the K⁺/Na⁺ discrimination locus *Kna1* in wheat. *Theor Appl Genet* 1996;92:448–454.
73. Munns R, et al. Genetic control of sodium exclusion in durum wheat. *Aust J Agri Res* 2003;54:627–635.
74. Lindsay MP, et al. A locus for sodium exclusion (*Nax1*), a trait for salt tolerance, mapped in durum wheat. *Funct Plant Biol* 2004;31:1105–1114.
75. James RA, et al. Physiological characterization of two genes for Na⁺ exclusion in durum wheat, *Nax1* and *Nax2*. *Plant Physiol* 2006;142:1537–1547. [PubMed: 17028150]
76. Yokoi S, et al. Differential expression and function of *Arabidopsis thaliana* NHX Na⁺/H⁺ antiporters in the salt stress response. *Plant J* 2002;30:529–539. [PubMed: 12047628]
77. Rus A, et al. AtHKT1 facilitates Na⁺ homeostasis and K⁺ nutrition in planta. *Plant Physiol* 2004;136:2500–2511. [PubMed: 15347798]
78. Wang TB, et al. Rapid up-regulation of *HKT1*, a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. *Plant Physiol* 1998;118:651–659. [PubMed: 9765551]
79. Corratge C, et al. Molecular and functional characterization of a Na⁺-K⁺ transporter from the Trk family in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *J Biol Chem* 2007;282:26057–26066. [PubMed: 17626012]

80. Kato Y, et al. Evidence in support of a four transmembrane-pore-transmembrane topology model for the *Arabidopsis thaliana* Na⁺/K⁺ translocating AtHKT1 protein, a member of the superfamily of K⁺ transporters. *Proc Natl Acad Sci U S A* 2001;98:6488–6493. [PubMed: 11344270]
81. Bañuelos MA, et al. Effects of polylinker uATGs on the function of grass HKT1 transporters expressed in yeast cells. *Plant Cell Physiol* 2008;49:1128–1132. [PubMed: 18539606]
82. Tholema N, et al. All four putative selectivity filter glycine residues in KtrB are essential for high affinity and selective K⁺ uptake by the KtrAB system from *Vibrio alginolyticus*. *J Biol Chem* 2005;280:41146–41154. [PubMed: 16210320]
83. Rubio F, et al. Genetic selection of mutations in the high affinity K⁺ transporter HKT1 that define functions of a loop site for reduced Na⁺ permeability and increased Na⁺ tolerance. *J Biol Chem* 1999;274:6839–6847. [PubMed: 10066736]
84. LeMasurier M, et al. KcsA: it's a potassium channel. *J Gen Physiol* 2001;118:303–314. [PubMed: 11524460]
85. Amtmann A, et al. Multiple inward channels provide flexibility in K⁺/Na⁺ discrimination at the plasma membrane of barley suspension culture cells. *J Exp Bot* 1997;48:431–440.
86. Roberts S, Tester M. A patch clamp study of Na⁺ transport in maize roots. *J Exp Bot* 1997;48:431–440.
87. Tyerman S, et al. Pathways for the permeation of Na⁺ and Cl[−] into protoplasts derived from the cortex of wheat roots. *J Exp Bot* 1997;48:459–480.
88. Buschmann PH, et al. Enhancement of Na⁺ uptake currents, time-dependent inward-rectifying K⁺ channel currents, and K⁺ channel transcripts by K⁺ starvation in wheat root cells. *Plant Physiol* 2000;122:1387–1397. [PubMed: 10759535]
89. Maathuis FJ, Sanders D. Sodium uptake in *Arabidopsis* roots is regulated by cyclic nucleotides. *Plant Physiol* 2001;127:1617–1625. [PubMed: 11743106]
90. Mengel, K.; Kirkby, EA. Principles of Plant Nutrition. Vol. 3. International Potash Institute; 1982.
91. Flowers TJ, Läuchli A. Sodium versus potassium: Substitution and compartmentation. *Inorg Plant Nutr* 1983;15b:651–681.
92. Rains DW, Epstein E. Sodium absorption by barley roots: role of the dual mechanisms of alkali cation transport. *Plant Physiol* 1967;42:314–318. [PubMed: 16656509]
93. Essah PA, et al. Sodium influx and accumulation in *Arabidopsis*. *Plant Physiol* 2003;133:307–318. [PubMed: 12970496]
94. Davenport RJ, Tester M. A weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. *Plant Physiol* 2000;122:823–834. [PubMed: 10712546]
95. Fu HH, Luan S. AtKuP1: a dual-affinity K⁺ transporter from *Arabidopsis*. *Plant Cell* 1998;10:63–73. [PubMed: 9477572]
96. Kim EJ, et al. AtKUP1: an *Arabidopsis* gene encoding high-affinity potassium transport activity. *Plant Cell* 1998;10:51–62. [PubMed: 9477571]
97. Quintero FJ, Blatt MR. A new family of K⁺ transporters from *Arabidopsis* that are conserved across phyla. *FEBS Lett* 1997;415:206–211. [PubMed: 9350997]
98. Santa-Maria GE, et al. The *HAK1* gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. *Plant Cell* 1997;9:2281–2289. [PubMed: 9437867]
99. Gierth M, Mäser P. Potassium transporters in plants-involvement in K⁺ acquisition, redistribution and homeostasis. *FEBS Lett* 2007;581:2348–2356. [PubMed: 17397836]
100. Smith FA, Walker NA. Transport of potassium in *Chara australis*. I A symport with sodium. *J Membr Biol* 1989;108:125–137.
101. Walker NA, Sanders D. Sodium-coupled solute transport I charophyte algae: a general mechanism for transport energization in plant cells? *Planta* 1991;185:443–445.
102. Maathuis F, et al. The physiological relevance of Na⁺-coupled K⁺-transport. *Plant Physiol* 1996;112:1609–1616. [PubMed: 12226467]
103. Rigas S, et al. TRH1 encodes a potassium transporter required for tip growth in *Arabidopsis* root hairs. *Plant Cell* 2001;13:139–151. [PubMed: 11158535]

104. Gierth M, et al. The potassium transporter AtHAK5 functions in K⁺ deprivation-induced high-affinity K⁺ uptake and AKT1 K⁺ channel contribution to K⁺ uptake kinetics in *Arabidopsis* roots. *Plant Physiol* 2005;137:1105–1114. [PubMed: 15734909]
105. Nieves-Cordones M, et al. A putative role for the plasma membrane potential in the control of the expression of the gene encoding the tomato high-affinity potassium transporter HAK5. *Plant Mol Biol* 2008;68:521–532. [PubMed: 18726559]
106. Volkov V, Amtmann A. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, has specific root ion-channel features supporting K⁺/Na⁺ homeostasis under salinity stress. *Plant J* 2006;48:342–353. [PubMed: 17052322]
107. Accardi A, Miller C. Secondary active transport mediated by a prokaryotic homologue of ClC Cl[−] channels. *Nature* 2004;427:803–807. [PubMed: 14985752]
108. Hirochika H, et al. Retrotransposons of rice involved in mutations induced by tissue culture. *Proc Natl Acad Sci U S A* 1996;93:7783–7788. [PubMed: 8755553]
109. Hirochika H. Retrotransposons of rice: Their regulation and use for genome analysis. *Plant Mol Biol* 1997;35:231–240. [PubMed: 9291976]
110. Jeon JS, et al. T-DNA insertional mutagenesis for functional genomics in rice. *Plant J* 2000;22:561–570. [PubMed: 10886776]
111. Miyao A, et al. Target site specificity of the *Tos17* retrotransposon shows a preference for insertion within genes and against insertion in retrotransposon-rich regions of the genome. *Plant Cell* 2003;15:1771–1780. [PubMed: 12897251]
112. Hiei Y, et al. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the DNA. *Plant J* 1994;6:271–282. [PubMed: 7920717]
113. Goff SA, et al. A draft sequence of the rice genome (*Oryza sativa* L. ssp Japonica) *Science* 2002;296:92–100.
114. Sakata K, et al. An automated annotation system and database for rice genome sequence. *Nucleic Acids Res* 2002;30:98–102. [PubMed: 11752265]
115. Yu J, et al. A draft sequence of the rice genome (*Oryza sativa* L. ssp Indica). *Science* 2002;296:79–92. [PubMed: 11935017]
116. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;17:540–552. [PubMed: 10742046]
117. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797. [PubMed: 15034147]
118. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 2003;52:696–704. [PubMed: 14530136]

Figure 1.

Phylogenetic tree of angiosperm HKT transporter proteins illustrating the two major HKT transporter classes. The class 1 HKT transporters that have been shown or suggested to mediate Na^+ removal from xylem vessels are shaded in red. The proteins shaded in grey represent class 2 HKT transporters and the remaining HKT transporters correspond to class 1 HKT transporters. The tree was constructed using phym1 on a gblocks curated muscle alignment [116–118]. The asterisk marks the *OsHKT1;2* pseudogene of rice cv. Nipponbare [49]. The three internal stop codons were overridden to create a putative full-length *OsHKT1;2* protein solely for comparison here. A third class of HKT transporters found in *Physcomitrella patens* and related organisms is not shown here. Abbreviations: At, *Arabidopsis thaliana*; Ec,

Eucalyptus camaldulensis; Hv, *Hordeum vulgare*; Mc, *Mesembryanthemum crystallinum*; Os, *Oryza sativa*; Pa, *Phragmites australis*; Pt, *Populus trichocarpa*; Ss, *Suaeda salsa*; Ta, *Triticum aestivum*; Th, *Thellungiella halophila*; Tm, *Triticum monoccocum*; Tt, *Triticum turgidum*.

Figure 2.

A model for AtHKT1;1 and OsHKT1;5 functions in mediating Na⁺ exclusion from leaves by removing Na⁺ from the xylem sap during salinity stress. **(a)** AtHKT1;1 and OsHKT1;5 mediate Na⁺ unloading from xylem vessels at the plasma membrane of xylem parenchyma cells [25, 26]. The Na⁺ uptake function of AtHKT1;1 and OsHKT1;5 prevents Na⁺ overaccumulation in shoots and leaves, thus protecting vital salt-sensitive photosynthetic processes from salinity stress [11,25–27]. **(b)** Working hypothesis for the coupling of Na⁺ unloading from the xylem by class 1 HKT transporters with K⁺ loading into xylem vessels via depolarization-activated K⁺ channels. HKT-mediated Na⁺ uptake from xylem vessels would induce membrane depolarization of xylem parenchyma cells, which in turn could activate outward-conducting

K⁺ channels (i.e. depolarization-activated outward-rectifying K⁺ channels). This mechanism would result in promoting the concomitantly observed K⁺ accumulation in the xylem and leaves, which contributes to protection of leaves from salinity stress [25,26].