HKT transporters mediate salt stress resistance in plants: from structure and function to the field.

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Abstract

Plant cells are sensitive to salinity stress and do not require sodium as an essential element for their growth and development. Saline soils reduce crop yields and limit available land. Research shows that HKT transporters provide a potent mechanism for mediating salt tolerance in plants. Knowledge of the molecular ion transport and regulation mechanisms and the control of *HKT* gene expression are crucial for understanding the mechanisms by which HKT transporters enhance crop performance under salinity stress. This review focuses on HKT transporters in monocot plants and in *Arabidopsis* as a dicot plant, as a guide to efforts towards improving salt tolerance of plants thus increasing the production of crops and bioenergy feedstocks.

Introduction

Potassium (K^+) and sodium (Na^+) are alkali metals and have similar chemical properties and content ratio in non-saline soils. Nevertheless, physiological impacts of these elements on the metabolism and growth of plants are quite different. K^+ is known to be an essential macro-nutrient in plants [1,2] and maintaining high K^+/Na^+ ratios in shoots (or leaves) has been suggested to be a major strategy for glycophyte plants to cope with salinity stress [3]. The osmotic effect of salt stress can lead to various physiological and morphological changes, such as inhibition of cell expansion, cell division and nutrient balance. While high salt concentrations inhibit plant growth, plants are endowed to some degree with a tolerance against salt accumulation. Plants alleviate the toxic effect of Na^+ by excluding Na^+ from leaf tissue [4] and by sequestration of Na^+ into vacuoles. The precise control of Na^+/K^+ selective accumulation in shoot and root tissues is an essential task to maintain cellular cation homeostasis in the presence of imposed high salt concentration and high external osmolarity [5–7].

Initial studies in the reference plant *Arabidopsis thaliana* identified the AtHKT1;1 transporter as a largely Na^+ selective transporter [8] and found that the *AtHKT1;1* gene provides a key mechanism for protecting leaves from Na^+ over-accumulation and salt stress (Table 1) [4,9,10]. Diverse approaches for the isolation of strong salt tolerance genes using a combination of conventional and novel molecular genetic approaches have been performed with the aim to improve salt tolerance of crop species and to make saline soils accessible for agriculture [11,12].

The initial “class II” HKT transporter family member was first isolated as a K^+ uptake transporter in wheat, which exhibited Na^+/K^+ co-transport activity [13,14]. Later studies showed that indeed two classes of HKT transporters are found in plants, several of the class II HKT transporters (“HKT2s”) mediate Na^+/K^+ transport activity, whereas the class I HKT transporters (“HKT1s”) usually mediate relatively Na^+ selective transport [8,15]. Further evidence on HKTs in various plants has proven the physiological significance of HKTs in saline environments [16–18]. The family of HKTs belongs to the HKT/Trk/Ktr-type K^+ transporter superfamily found in microorganisms and plants [19], which indicates that

HKT/Trk/Ktr-type K^+ transporters may be crucial for their growth and development.

Fundamental structure properties of HKTs affecting their function

Knowledge of the structure and function relationship and regulation of HKTs is a key to modifying HKT-linked traits. HKTs belong to a class of K^+ transporters [20]. AtHKT1;1 consists of four repeated transmembrane domain-pore loop-transmembrane domain motifs, similar to ion conducting pore-forming units of K^+ channels (Fig. 1) [21]. Homologous bacterial KtrB form a dimer recruiting KtrA octamer subunits at the cytosolic side (Fig. 2A) [22]. Most pore loops of HKT/Trk/Ktr-type K^+ transporters contain a hallmark Gly residue, which corresponds to the first Gly of a “GYG” signature selectivity sequence of K^+ channels [23]. The replacement of glycine by serine in loop 1 of AtHKT1;1 and *Vibrio alginolyticus* KtrB results in Na^+ selectivity of the transporters rather than in a K^+ uptake system (Fig. 1) [15,24]. These findings support the proposed evolutionary correlation between the three classes of K^+ transport systems, HKT/Trk/Ktr-type K^+ transporters, K^+ channels and Kdp-type K^+ pumps which are present in prokaryotic cells [25]. The X-ray crystal structures of TrkH from *V. parahaemolyticus* and KtrB from *Bacillus subtilis* support the related ion permeation mechanism of these transporters (Fig. 2) [26,27]. Considering the analogy of HKT/Trk/Ktr-type K^+ transporters with K^+ channels and Kdp-type K^+ pumps, the Na^+ transport property of HKT found in AtHKT1;1 may be evolutionarily acquired from a K^+ selective prototype. Note that a distinct class of K^+ transporters found in plants, the “Kup/HAK/KT” K^+ transporters differ from the above transporters [28].

TaHKT2;1 isolated from wheat possesses Na^+ -coupled K^+ transport activity [13,28,29]. Consistently, the related cyanobacterial Ktr system shows Na^+ activation of K^+ uptake [30]. This suggested that even K^+ -selective HKT/Trk/Ktr-type transporters likely respond to external increased Na^+ concentrations. However not every HKT/Trk/Ktr-type K^+ transporter possesses Na^+ activation including an ortholog from *T. trypanosoma*, which is insensitive to Na^+ for its K^+ transport activity [31].

Several regions have been identified to be involved in the regulation and

gating of HKT/Trk/Ktr-type transporters. The conserved single positive residue in the middle of the eighth and last transmembrane domain of HKT/Trk/Ktr-type transporters, which is not present in K⁺ channels, is crucial for classifying it as a “transporter” (Fig. 1) [32]. The reconstitution of VpTrkH in liposome membranes without the cytosolic regulatory subunit TrkA confirmed permeation of Rb⁺, a K⁺ analog, through the transporter (Fig. 2) [26]. An intramembrane loop in the sixth transmembrane domain functions as gate that controls KtrB activity in *V. alginolyticus* [33,34]. An intramembrane domain loop and the conserved positive residue in the eighth and last transmembrane domain was positioned beneath of the ion selectivity filter to prevent ion permeation in the closed state of TrkH/KtrB [26,27].

The bacterial TrkA/KtrA subunit termed KTN/RCK or KtrA/TrkA, contains the nucleotide binding sites for ADP/ATP in the cytosol, which alters the gating of TrkH/KtrB-mediated K⁺ permeation (Fig. 1 and 2) [24,30,35]. The tertiary structure of TrkA/KtrA supported the notion that the nucleotide binding altered the conformation of TrkA/KtrA itself as well as of TrkH/KtrB [27,36,37]. A counterpart to the regulatory subunit TrkA/KtrA has not been identified in plant genomes.

Regulatory mechanisms of *AtHKT1;1* gene expression

Arabidopsis HKT1;1 null mutant plants showed an accumulation of Na⁺ in shoots when cultivated on medium supplemented with NaCl, which indicated that *AtHKT1;1* is a part of the salt tolerance mechanism [4,9,38]. Tissue specific expression analyses of *AtHKT1;1* showed expression in the root stele and leaf vasculature [4]. Further biochemical immuno-localization analyses found that AtHKT1;1 protein is targeted to the plasma membrane of xylem parenchyma cells and suggested that AtHKT1;1 functions in the removal of Na⁺ from the xylem sap [39]. An alternate, non-strictly exclusive model reported expression of *AtHKT1;1* in the phloem and that AtHKT1;1 activity in the phloem might contribute to circulation of Na⁺ in the whole plant (Fig. 3, Table 1) [9]. A soil microbe, *Bacillus subtilis* GB03, enhanced Na⁺-tolerance of *Arabidopsis* seedlings via the induction of the expression of *AtHKT1;1* in shoots and the repression of the expression in roots [40]. In contrast to the aforementioned phenomenon, reduced expression of

AtHKT1;1 in roots of certain *Arabidopsis* accessions conferred Na⁺ tolerance [41,42]. Time-dependent and tissue-specific expression of *AtHKT1;1* likely optimizes Na⁺ flux across the plasma membrane of xylem parenchyma cells and thus contributes to the Na⁺ distribution in whole plants.

Cytokinin (CK) signaling, which is mediated by a two-component system, plays a significant role as a negative regulator in the resistance against salt stress. CK application conferred repression of *AtHKT1;1* in shoots and roots through both type-B response regulator, ARR1 and ARR2, resulting in an increase in sodium contents in the shoot but a decrease in roots of wild-type *Arabidopsis* plants, analogous to *athkt1;1* mutant lines (Fig. 3) [43]. Independently it was reported that the expression level of *AtHKT1;1* in whole plants increased in a mutant defective in the CK synthetic enzymes, *ipt1,3,5,7* [44]. These studies together imply that CK can enhance the sodium accumulation in shoots by down-regulating expression levels of *AtHKT1;1*.

Two transcription factors, ABI4 and AtZIP24 have been reported as negative regulators of *AtHKT1;1* expression. The loss of function mutant of *ABI4*, an ABA responsive transcription factor, acquired salt tolerance with decreased Na⁺ accumulation and increased *AtHKT1;1* gene expression levels (Fig. 3). *ABI4*-overexpressor plants exhibited suppressed *AtHKT1;1* expression leading to decreased salt tolerance [45]. These findings are interesting as other responses to ABA can enhance salt tolerance [46], whereas surprisingly ABA could enhance salt sensitivity via *AtHKT1;1* regulation [45]. Further research into these opposing ABA responses will be of interest. AtZIP24 belongs to a group of bZIP-type transcription factors that are induced by salt stress [47]. In *AtZIP24* RNAi lines transcript levels of *AtHKT1;1* were increased, suggesting that AtZIP24 represses *AtHKT1;1* expression under salt stress.

Positively-regulating transcription factors that induce *AtHKT1;1* expression remain unknown. The *AtHKT1;1* promoter sequence contains a small RNA binding region -2.6 kb upstream of the *AtHKT1;1* start codon, which was found to be more strongly methylated in leaves compared to roots (Fig. 3A). Differences in methylation may contribute to higher level of *AtHKT1;1* expression in roots [48]. As another transcriptional *cis* regulatory element in the *AtHKT1;1* promoter, a direct

repeat sequence located -5.3 kb to -3.9 kb upstream of the transcriptional start codon of *AtHKT1;1* was found through the comparative approach on salt sensitivity in several *Arabidopsis* cultivars (Fig. 3) [41,48]. The repeat sequence is relevant for the root specific expression of *AtHKT1;1*, thereby preventing an increase of Na⁺ content in shoots. A grafting approach demonstrated that the *AtHKT1;1* activity in roots contributed to regulate Na⁺ concentrations in the shoot. Furthermore the Na⁺ distribution found in enhancer trap lines, which were engineered to overexpress *AtHKT1;1* only in stelar root cells, supports this role of *AtHKT1;1* in roots [42]. Patch-clamp analyses revealed a Nernstian behavior of *AtHKT1;1*-mediated channel-like currents with a strong selectivity for Na⁺ over K⁺ *in vivo* [49].

Physiological significance of HKT transporters in salinity resistance in monocots

Seven functional *OsHKT* genes were found to exist in a japonica rice (*Oryza sativa*) cultivar (Table 1) and similar composition of the *HKT* gene family was observed in wheat and barley [50,51]. *OsHKT2;1/2* and *OsHKT2;2*, are similar class II transporters from salt tolerant indica rice varieties Nona Bokra and Pokkali, respectively [52]. These were suggested to contribute to the salt tolerant phenotype due to maintenance of K⁺ acquisition through their K⁺-Na⁺ co-transport under salinity stress [53]. The *OsHKT2;2* gene in a japonica cultivar is found to be a pseudo-gene [50]. The *OsHKT2;1* transporter functions as a unique class II Na⁺ transporter [52,54]. A primary role of *OsHKT2;1* was found to be mediation of nutritional Na⁺ absorption and Na⁺ uptake into K⁺-starved rice plants to compensate K⁺ deficiency [54].

QTL analyses of salt tolerant rice and wheat varieties have led to the identification of class I HKT transporters that are salt tolerance determinants mediating Na⁺ exclusion from shoots or leaves [55–57]. The *HKT1;5* locus from Nona Bokra plants was narrowed down as a QTL that maintains higher K⁺ contents in shoots under salinity stress, but was found to encode a Na⁺ selective transporter preferentially expressed in xylem parenchyma cells in roots [55]. The *HKT1;5* loci from the wheat relative (*Triticum monococcum*) and bread wheat (*Triticum aestivum*), which were named *Nax2* and *Kna1* locus, respectively [57–60], have been demonstrated to be the salt tolerance determinant loci in wheat [61,62]. In

particular, the introgression of the *Nax2* locus into a commercial durum wheat cultivar significantly enhanced salt tolerance, resulting in a 25% increase in grain yield compared to control lines in salt-impacted soil in the field [61]. Products of *TmHKT1;5-A* (*Nax2*) and *TaHKT1;5-D* (*Kna1*) were found to be Na⁺ selective transporters and expressed in root stelar cells [61,62]. These findings indicate that AtHKT1;1 in *Arabidopsis* and HKT1;5 in rice and wheat share a similar physiological role in protecting leaves from salinity stress by mediating xylem Na⁺ unloading in roots [16,18]. In wheat, another important QTL named *Nax1* contributes to the retrieval of Na⁺ from root xylem and Na⁺ sequestration in leaf sheaths during salinity stress [60,63]. The *TmHKT1;4-A2* gene from *T. monococcum* was found to be a likely candidate for the *Nax1* locus [56]. The rice *OsHKT1;4* gene has also been proposed to play an important role in Na⁺ sequestration in leaf sheaths [64] but the detailed physiological function remains to be elucidated. In contrast to the case of rice and wheat, relatively low dependence of restricted Na⁺ loading into xylem upon overall salinity tolerance of barley plants has been reported [65,66]. Salt tolerant barley varieties exhibited higher K⁺/Na⁺ ratios in the xylem sap by maintaining efficient K⁺ loading into xylem in roots [65].

In conclusion, soil salinity is becoming an increasingly serious threat and therefore development of salt-tolerant crops is an urgent goal [16,17]. Studies have provided a large body of evidence that understanding the molecular bases of HKT transporters provides an opportunity to improve the salt tolerance of plants. Fundamental research into the underlying mechanisms will assist and promote further efforts to bring findings of basic research to applications in the field.

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The introgression of the *Nax2* locus that encodes a Na⁺ selective TmHKT1;5 transporter from the wheat relative *Triticum monococcum* into a commercial durum wheat cultivar has been demonstrated to improve salt tolerance of the cultivar conferring the ability to produce 25% more grain yields than control cultivar in salt-impacted agricultural lands.

These results provided strong evidence that increases in the activity of HKT1;5-mediated xylem Na⁺ unloading is a promising strategy to increase salt tolerance of crop plants.

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Analyses of barley varieties that show different salt sensitivity indicated that xylem Na⁺ unloading is not a predominant salt tolerance mechanism in barley in contrast to rice and wheat plants. Interestingly, salt tolerant barley varieties show a higher K⁺/Na⁺ ratio in xylem sap under salt stress maintaining higher K⁺ loading activity into xylem vessels through voltage-gated K⁺ channels, suggesting the importance of K⁺ acquisition and K⁺ distribution to shoots upon salt stress.

Figure legends

Figure 1. Schematic image of HKT/Trk/Ktr transport system consisting of ion translocating membranous proteins and cytosolic subunits based on combined data on these transporters in bacteria and plants. Cytosolic octameric ATP-binding regulatory subunits are found in bacteria, but missing in plant HKTs. Yellow circles represents ATP/ADP. Left panel, Red circles indicate the positions involving the $\text{Na}^+\text{-K}^+$ selectivity filter, the gating and the regulation of the transport activity (see text). Y shows an N-linked glycosylation site found in AtHKT1;1 in *in vitro* expression analysis. The C-terminal end of the transmembrane domain KtrB mediates the dimeric stability of KtrB. Right panel, The counter-clockwise arrangements of the 4-fold MPM motif and octameric cytosolic subunits.

Figure 2. Sequence similarity of plant HKT and the potassium transporters from *B. subtilis* (KtrAB) and *V. parahaemolyticus* (TrkHA). A. Crystal structure of the membrane spanning KtrB transporter subunit in side view (left) and top view (right). The box shows the complete multimeric KtrB transport protein structure (green) with the cytoplasmic regulatory subunit KtrA (blue). B. Approximated structural model of AtHKT1 based on KtrB in side view (left) or top view (right). The model was obtained from ModBase (uniprot:Q84TI7; Template PDB Code: 4j7cI). C. Side view (left) and top view (right) of the membrane-spanning TrkH transporter subunit. The box shows the complete multimeric TrkH structure (green) with the cytoplasmic regulatory subunit TrkA (blue). In all panels the purple sphere depicts a potassium ion. The structures are colored according to the sequence conservation with blue being the highest and red being the least conserved sequence. The MAFFT alignment to determine the degree of conservation comprises the sequences from the structure and the sequences used in Hauser and Horie [3].

Figure 3. A. Diagram of upstream sequence of ATHKT1;1 gene. The tandem repeat sequence, methylation site and ABI4 binding element (ABE) is found as *cis* elements [41,45,48]. B. Schematic model illustrating AtHKT1;1 and the DNA binding proteins, ABI4, bZIP24, ARR1 and ARR12, which all have been reported as negative regulators of *AtHKT1;1* expression [43,45,47].

Figure 2

A

B

AtHKT1;1

C

100
50
3

Figure 3

Table 1.

Expression profiles of *HKT* genes of *Arabidopsis thaliana* and *Oryza sativa*

Information of *OsHKT* genes is extracted from the Rice Expression Profile Database (RiceXPro; <http://ricexpro.dna.affrc.go.jp/>).

Gene name	Locus ID	Primary tissues expressed	Timing of expression
AtHKT1;1	At4g10310	Root Leaf petiole Leaf veins	Germinating period to maturity
OsHKT1;1	LOC_Os04g51820 Os04g0607500	Leaf blade Leaf sheath Anther Lemma Palea	Vegetative Reproductive
OsHKT1;3	LOC_Os02g07830 Os02g0175000	Leaf blade	Vegetative
OsHKT1;4	LOC_Os04g51830 Os04g0607600	stem	Reproductive Ripening
OsHKT1;5	LOC_Os01g20160 Os01g0307500	Leaf sheath Root stem	Reproductive Ripening
OsHKT2;1	LOC_Os06g48810 Os06g0701700	Leaf sheath Root Lemma Palea	Vegetative Reproductive
OsHKT2;3	LOC_Os01g34850 Os01g0532600	Leaf blade Leaf sheath Root	Vegetative Reproductive
OsHKT2;4	LOC_Os06g48800 Os06g0701600	Leaf blade Leaf sheath	Vegetative Reproductive Ripening