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Model of Cation Transportation Mediated by High-Affinity Potassium Transporters (HKTs) in Higher Plants

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Abstract

Trk/Ktr/HKT transporters probably were evolved from simple K^+ channels KcsA. HKT transporters, which mediate Na^+-uniport or Na^+/K^+-symport, maintain K^+/Na^+ homeostasis and increase salinity tolerance, can be classified into three subfamilies in higher plants. In this review, we systematically analyzed the characteristics of amino acids sequences and physiological functions of HKT transporters in higher plant. Furthermore, we depicted the hypothetical models of cations selection and transportation mediated by HKT transporters according to the highly conserved structure for the goal of better understanding the cations transportation processes.

Keywords: HKT transporters, Cation transport, K^+/Na^+ homeostasis, Na^+-uniport, Na^+/K^+-symport

Introduction

Sodium (Na), unlike potassium (K), is not an essential nutrient element for the most of higher plants but may be a beneficial element for some species [1-3]. In higher plants, Na^+ could act as an osmoticum and temporarily substitute for K^+ in deficiency or insufficiency of K^+ [4-6]. Na^+ is able to stimulate growth of fungi and plants as long as the accumulation and compartmentalization are efficiently controlled below a limited concentration at the cell and tissue levels [6-9]. Excessive Na^+ in the external environment could lead to the detrimental effects on plant growth, and even cause plant death. The toxic levels did not defined in detail and were supposed to depend on cell types [9], but it is viewed that the cytosolic concentration of Na^+ should not be higher than 10–30 mM [10]. Additionally, tissue K^+/Na^+ ratio is a widely used parameter in discriminating genotypes for salinity tolerance of higher plants [11-20]. Plants can maintain high cytosolic K^+/Na^+ ratio through excluding Na^+ from shoots and accumulating K^+ in shoots [21-28].

For resisting Na^+ toxicity, plants developed three mechanisms for salinity tolerance to maintain potassium/sodium homeostasis (Figure 1): 1) Na^+ exclusion from the shoot, 2) Na^+ tissue tolerance and 3) osmotic tolerance [29,30]. Till now, a series of transporter systems have been reported which help plants to improve salinity tolerance by inhibiting Na^+ influx, enhancing Na^+ efflux, or mediating the sequestration of Na^+ into the cell vacuoles (Figure 1). Simplified model for mechanisms of K^+/Na^+ absorption, recirculation and extrusion by different classes of Na^+ channels/transporters are shown in Figure 1, such as nonselective cation channels (NSCC) [31-33], cation-Cl^- co-transporter (CCC) [34], low-affinity cation transporter (LCT) [35,36], salt overly sensitive 1 (SOS1) [37-41], Na^+/H^+ antiporter NHX1 [42-46] and high affinity potassium transporter (HKT/HAK) [27,28,47-50]. Plant root cells generally take up Na^+/K^+ from soil through some channels (NSCCs, AKT1, LCT1 and CCC), transporters (KUP/HAK/KT and HKT) and apoplastic. Channel permeations and apoplastic are the main pathways of Na^+ influx under salt tress. The SOS pathway mediates efflux of Na^+ cross the plasma membrane to the soil solution or apoplast. NHX1 partitions Na^+ within vacuole and jointly regulates the cytosol Na^+ concentrations. AtHKT1;1, OsHKT1;5, TaHKT1;5 and TmHKT1;4/5 retrieve Na^+ from the xylem into the xylem parenchyma cell and prevent the shoot from Na^+ over-accumulation damage. It is hypothesized that AtHKT1;1 mediates

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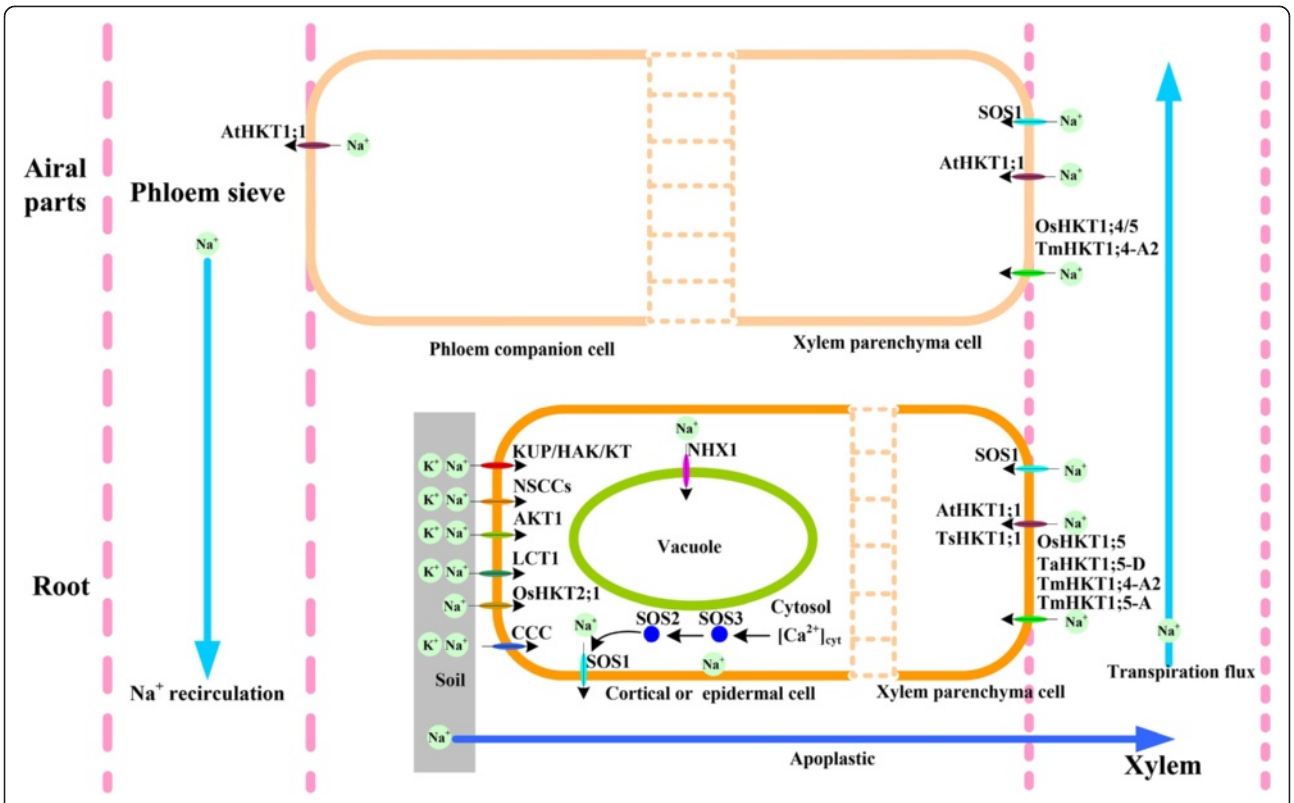

Figure 1 K^+/Na^+ homeostasis in higher plants. Plant root cells generally absorb Na^+/K^+ from soil through different channels (NSCCs, AKT1, LCT1, CCC), transporters (KUP/HAK/KT and HKT) and apoplastic. Channel permeations and apoplastic are the main pathways of Na^+ influx under salt stress. In the SOS pathway Na^+ crosses the plasma membrane to the apoplast or soil solution and the NHX1 partitions Na^+ within vacuole and jointly regulate the cytosol Na^+ concentrations and play a vital role in response to salt stress. AtHKT1;1, OsHKT1;5, TaHKT1;5 and TmHKT1;4/5 retrieve Na^+ from the xylem into xylem parenchyma cell and prevent the shoot from damage caused by Na^+ over-accumulation. It is hypothesized that AtHKT1;1 mediates recycling Na^+ from the shoot to root through removal of Na^+ from the xylem and loading Na^+ into the phloem sieves. These processes assure a normal K^+/Na^+ homeostasis and maintain a high K^+/Na^+ ratio to rescue plants when suffering from salt stress. NSCC, nonselective cation channels; CCC, cation-Cl – co-transporter; LCT, low-affinity cation transporter; SOS1, salt overly sensitive 1; NHX1, Na^+/H^+ antiporter 1.

recycling of Na^+ from the shoot to root through removal of Na^+ from the xylem and loading Na^+ into the phloem sieves. These processes assure a normal K^+/Na^+ homeostasis and also maintain a high K^+/Na^+ ratio to rescue plants when suffering salt stress.

HKT transporters belong to a superfamily of Trk/Ktr/HKT and play a vital physiological roles in plants. Plant HKT transporter is a multiple cation uptake system, which can mediate Na^+ uniport, Na^+/K^+-symport and even Mg^{2+}/Ca^{2+} permeation. Function of plant HKTs depends on its structure. Therefore, for better understanding how HKT transporters work in higher plants, it is necessary to construct a model of cations uptake mediated by HKT transporters through systematically analyzing the conserved structures of HKTs. In this article we hypothesized a model of cations selection and transportation mediated by HKT transporters.

Three subfamilies of HKT transporters

HKT genes encode high affinity potassium transporters in plants and available evidences support that HKTs can be classified into three subfamilies (i.e. subfamily I, subfamily II and subfamily III) according to the phylogenetic analysis based on amino acids of HKTs (Figure 2). Till now, we can retrieve more than one hundred members of HKT transporters from published papers and gene (or protein) databases. The number of HKT transporters in higher plants shows a striking difference among different species. Researchers already identified several *HKT*-like genes in wheat, at least nine in rice, but unique in *Arabidopsis* and *Physcomitrella patens*, since *TaHKT2;1* (originally named *HKT1*) was firstly isolated from wheat roots. It is certain that the monocotyledon contain more HKT transporters than dicotyledon. In addition, HKT transporters of subfamily I were isolated both in the dicotyledon and monocotyledon, but HKTs of subfamily II were isolated only in the monocotyledon. Some HKT transporters have been found in the more primitive higher plants, such as *Selaginella moellendorffii* and *Physcomitrella patens*. Phylogenetic analysis showed that this kind of HKTs should be classed to subfamily III (Figure 2).

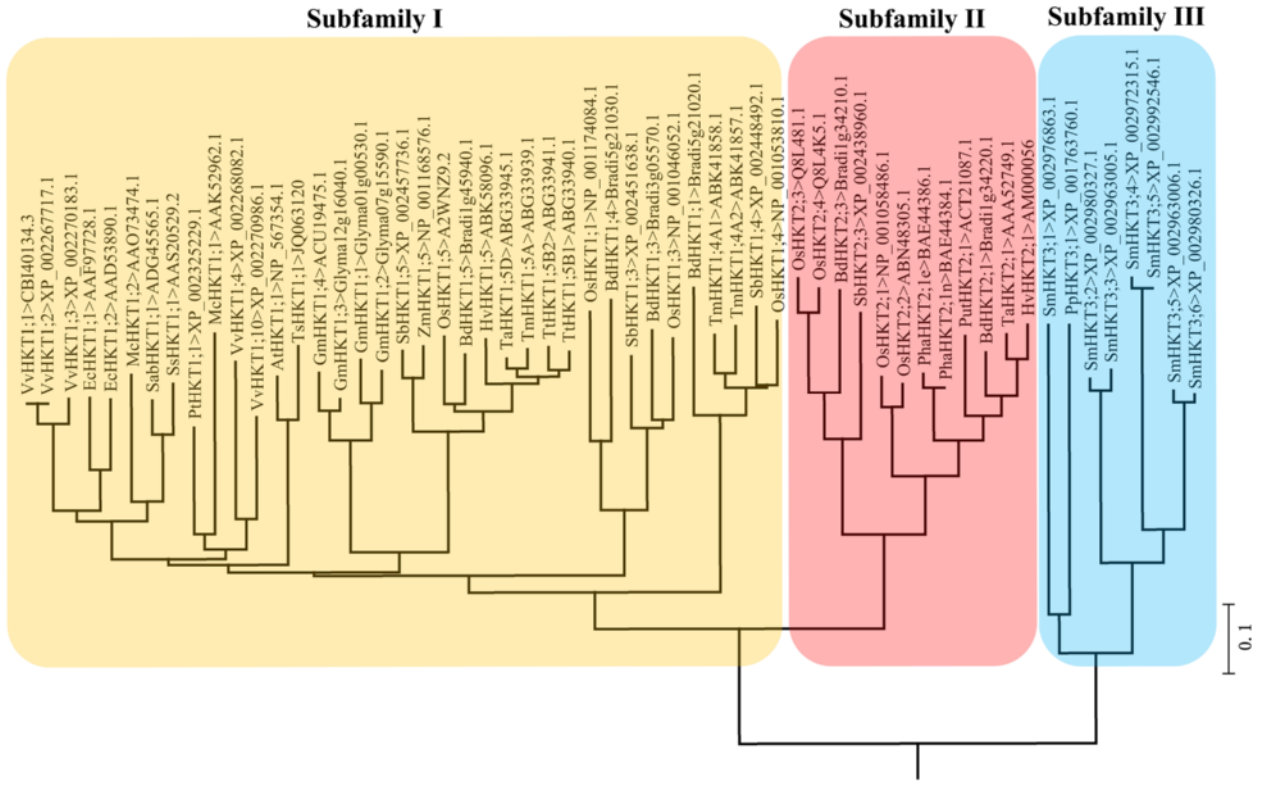

Figure 2 Phylogenetic analysis of HKT transporters in higher plants. Subfamily I of HKT transporters are all characterized by "Ser" in the first loop (P_A). Subfamily II and III have the GlyGlyGlyGly-type characteristic in the amino acid sequences exception of OsHKT2;1. At, *Arabidopsis thaliana*; Ts, *Thellungiella salsuginea*; Pt, *Populus trichocarpa*; Mc, *Mesembryanthemum crystallinum*; Vv, *Vitis vinifera*; Ec, *Eucalyptus camaldulensis*; Sb, *Sorghum bicolor*; Ss, *Suaeda salsa*; Zm, *Zea mays*; Sab, *Salicornia bigelovii*; Os, *Oryza sativa*; Hv, *Hordeum vulgare*; Bd, *Brahypodium distachyom*; Tm, *Triticum monococcum*; Ta, *Triticum aestivum*; Tt, *Triticum timopheevii*; Gm, *Glycine max*; Put, *Puccinellia tenuiflora*; Pha, *Phragmites australis*; Sm, *Selaginella moellendorffii*; Pp, *Physcomitrella patens*.

Subfamily I members of HKT transporters contain a highly conserved serine (Ser) residue in the first motif MP_AM, whereas subfamily II members primarily have glycine (Gly) residue with the exception of OsHKT2;1. Mutation from Ser to Gly change the affinity to cations [6,48,51,52]. Subfamily III members are similar to subfamily II with typical GlyGlyGlyGly-type feature. It is hypothesized that subfamily III transporters have the characteristics of K^+-Na^+ co-transport but there are few reports [52].

Structure of HKT transporters in higher plants

HKTs in Plant and Ktr/Trks in bacteria/fungi contain four MPM motifs which might be evolved from simple K^+ channels KcsA [47,53-62]. Two transmembrane helices (M_1 and M_2) and a reentrant loop (P segment) compose the basic motif (MPM motif). Hydropathy plot analysis of Trk/Ktr/HKT systems initially supported a structural model comprising of 8–12 transmembrane segments [53,63-65]. Although every MPM evolved from bacteria KcsA, the four MPM motifs are not simple repeats and they have their own features which determine the selectivity of cations. In fact, the similarity between every two MPMs is less than 30%. Alignment analysis suggested that the fourth MPM motif is the most conserved subunit which is almost similar to KcsA. Bacterial Trk and Ktr are associates with an ion-conducting transmembrane subunit and at least one peripheral regulatory subunit derived from the cleavage of the cytoplasmic C-terminal domain of Trk/Ktr channel [54,55]. Whereas, no regulatory subunit is found in the single amino acid chain systems of fungal Trk and plant HKT transporters till now.

In higher plants, HKT transporters contain some highly conserved amino acid residues which may play a vital function. A Gly or Ser residue in MP_AM motif (first motif) determines the permeability of K^+ or Na^+ [53]. Plant HKTs act as a Na^+-K^+ symporter when Gly exists in MP_AM motif. However, HKT transporters merely show Na^+ selective-permeability when Gly is substituted by Ser. Therefore, plant HKTs can be classified to SerGlyGlyGly-type and GlyGlyGlyGly-type.

According to the classical structural model, HKT transporters contain four MPM motifs, which might be

evolved from the simple K^+ KcsA. But, multiple alignments show that the fourth motif MP_DM is divided into two segments (Figure 3). The fourth signature Gly is located in the first segment and the second segment contains three highly conserved amino acid residues, which are cysteine (Cys), lysine (Lys) and arginine (Arg). Two highly conserved positive amino acid i.e. Arg (R) and Lys (K) residues in the MP_DM (Figure 3) are not replaceable. These positive residues, which are conserved in many K^+ channels, contribute to cation transport activity [66].

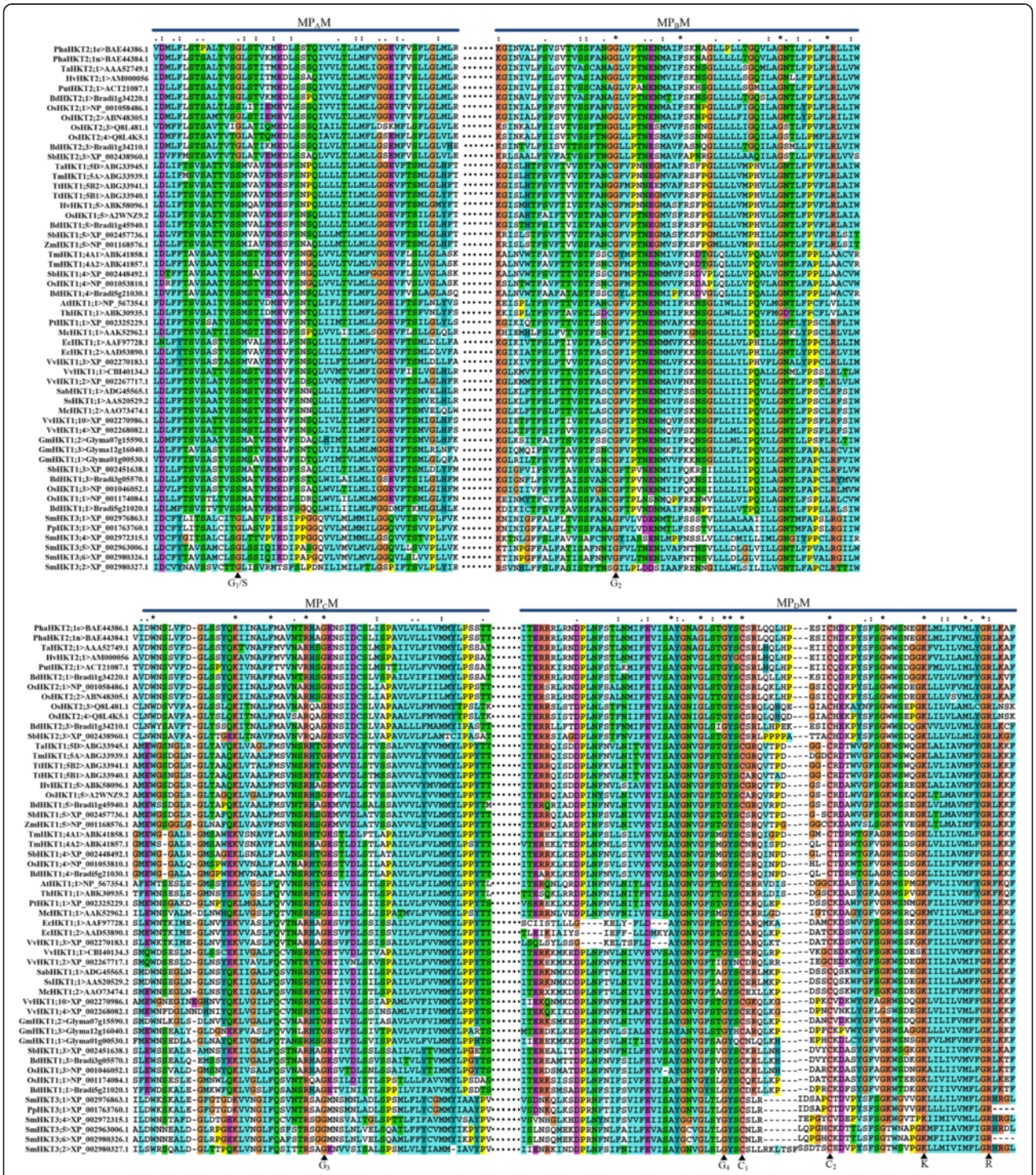

Figure 3 Multiple alignment of plant HKTs. The highly conserved signature residues were marked with bold triangle. G: glycine (Gly); S: serine (Ser); C: cysteine (Cys); K: lysine (Lys); R: arginine (Arg).

Kato *et al.* thought that both Lys and Arg residues face towards the ion conducting pore side, and a salt bridge(s) exists between positive residues in MP_DM motif and conserved negative residues in the pore region to reduce electrostatic repulsion against cation permeation caused by the positive residue(s) [66]. This salt bridge may help stabilize HKTs configuration [66]. Therefore, the MP_DM motif may be regarded as an independent functional motif because of the separate location and having the quite different role comparing with the other MPM motifs. In addition, it deserves paying close attention to another highly conserved amino acid, cysteine (C) in the fourth motif (C1 and C2 marked with bold triangle in Figure 3).

For obtaining more information about the structure of HKT, the transmembrane structure and hydrophobic features were analyzed through the HMMTOP method (http://www.enzim.hu/hmmtop/index.php). Results of hydrophobicity prediction showed that C-terminal is faced toward intracellular and N-terminal is faced toward extracellular in most of the plant HKT transporters (Table 1). And this result suggests that N-terminal of

Table 1 The location of N/C terminal and signature residues, and potential transmembrane helix number (THN)

Name	N-Ter	G_1/S	G_2	G_3	G_4	C_1	C_2	K	R	THN	C-Ter
AtHKT1;1	inside	H	H	o	H	i	i	H	i	12	inside
ThHKT1;1	inside	H	H	o	H	O	o	H	i	10	inside
OsHKT1;1	outside	I	H	o	i	i	i	i	H	10	outside
EcHKT1;1	inside	H	H	o	H	O	o	H	i	12	inside
PtHKT1;1	outside	H	H	i	H	o	o	H	i	11	inside
McHKt1;1	inside	H	H	i	H	o	o	H	i	12	inside
McHKT1;2	inside	I	H	o	H	O	o	H	i	10	inside
OsHKT1;3	outside	i	H	o	H	i	i	H	o	10	outside
TmHKT1;4A1	inside	o	H	i	H	o	o	H	i	10	inside
TmHKT1;4A2	inside	o	H	i	H	o	o	H	i	10	inside
SbHKT1;4	outside	o	H	i	H	o	o	H	i	11	inside
OsHKT1;4	outside	H	O	i	H	o	o	H	i	11	inside
TmHKT1;5	outside	H	H	i	H	o	o	H	i	13	inside
TaHKT1;5	outside	H	O	o	H	O	o	H	i	9	inside
TtHKT1;5B2	outside	H	O	o	H	O	o	H	i	9	inside
HvHKT1;5	inside	o	H	i	o	o	o	o	H	10	inside
ZmHKT1;5	inside	H	H	i	o	o	o	o	H	10	inside
OsHKT1;5	outside	H	O	i	O	o	O	O	O	10	outside
OsHKT2;1	outside	H	H	o	o	i	i	H	o	12	outside
PhaHKT2;1e	outside	H	H	o	o	O	O	o	H	11	inside
TaHKT2;1	inside	H	O	i	o	o	o	o	H	12	inside
HvHKT2;1	outside	H	H	i	o	o	o	o	H	11	inside
PutHKT2;1	inside	H	H	i	o	o	o	o	H	12	inside
PhaHKT2;1n	outside	H	H	o	o	O	O	o	H	11	inside
OsHKT2;2	outside	H	O	o	O	O	O	O	O	8	outside
SbHKT2;3	outside	H	O	o	H	O	o	H	i	9	inside
OsHKT2;3	outside	H	O	i	H	o	o	H	i	11	inside
OsHKT2;4	inside	H	H	i	H	o	o	H	i	12	inside
PpHKT3;1	outside	H	H	H	O	O	O	O	O	8	outside
SmHKT3;1	inside	o	O	o	H	H	i	H	o	9	outside
SmHKT3;2	inside	o	H	i	H	o	o	H	i	10	inside
SmHKT3;4	inside	H	H	o	H	o	o	H	i	11	outside
SmHKT3;5	outside	H	H	i	H	i	i	H	o	11	inside

Abbreviations represent: H, membrane helix; I, inside loop; i, inside helix tail; O, outside loop; o, outside helix tail. G: glycine (Gly); S: serine (Ser); C: cysteine (Cys); K: lysine (Lys); R: arginine (Arg).

HKT may be in charge of catching ions but C-terminal is responsible for regulating the permeability. This can explain why HKT transporters mediate a cation from external environment into cytoplasm. The number of transmembrane helixes in HKT transporter ranges from eight to thirteen. There are some loops (the longer part of a sequence outside of the membrane, which can form a domain or a simpler structure) and (or) tails (the elongation of the membrane helix, it can be followed by a loop or another tail, forming a short loop interacting with the outside or inside part of the membrane) between two transmembrane helixes. According to the classical model, the signature Gly (G) and Ser (S) residues were thought to be probably seated in loops between two transmembrane helixes. In fact, the situation may be more complicated because the signature Ser/Gly can be situated in the every structure — membrane helix, inside loop, inside tail, outside loop and outside tail. However, the widespread pattern are: 1) G_1/S, G_2 and G_4 (especially G_1/S) are mainly located in membrane helix; 2) Nearly all the third glycine residues lie in the helix tail; 3) Two conserved cysteine residues (C_1 and C_2) do not lie in the transmembrane helixes except for SmHKT3;1 transporter; 4) For lysine and arginine, if one lies in helix another lies in helix tail with few exceptions (Table 1). In addition, we depicted the typical structure of AtHT1;1 transporter (shown in Figure 4). Except for the twelve transmembrane helixes, AtHT1;1 transporter contains sixteen helix tails but only five loops.

There are two interesting exceptions OsHKT2;2 and PpHKT3;1 which functions did not follow the universal principles. OsHKT2;2 share 91% identity with OsHKT2;1 in amino acid sequences. OsHKT2;1 mediates Na^+ uptake both in plant and heterologous systems. In contrast, OsHKT2;2, previously found to be a pseudogene in Nipponbare rice (*japonica* rice) but not in *indica* rice [60]. Furthermore, at millimolar Na^+ concentrations, OsHKT2;2 mediated Na^+ influx into plant cells without adding extra cellular K^+ [67]. PpHKT3;1 (originally named PpHKT1) transporter is the unique one in *Physcomitrella patens* and characterizes with Gly in the first motif. However, PpHKT1 transporter mediated K^+ and Na^+ influx but not high-affinity Na^+ uptake because *Pphkt1* mutant plants maintain normal K^+ and Na^+ influx [52]. Screening of the transmembrane and topology structure, we found that OsHKT2;2 and PpHKT1 transporter only contain eight transmembrane helixes, and signature conserved residues are mainly located outside of the cytomembrane (Table 1). These different structure characteristics may be the reason for the different phenomena on cations uptake.

Various cations transport characteristics based on amino acid sequences in higher plants

SerGlyGlyGly-type characteristic determines the HKT as a Na^+-uniporter. All HKT members in subfamily I are characterized by SerGlyGlyGly. Either in dicotyledons or monocotyledons, most of the HKT members of subfamily I have been looked upon as Na^+-specific transporters [60]. In *Arabidopsis* genome, *AtHKT1;1* is the unique member, which is mainly expressed in xylem parenchyma cells [27,48,68,69]. AtHKT1;1 mediates Na^+ but small degree K^+ influx into cells when heterologously expressed in *Xenopus laevis oocytes* and *Saccharomyces cerevisiae* [47]. In addition all the identified mutants of *AtHKT1;1* have been found to be salt sensitive and Na^+ over-accumulation in aerial organs but Na^+ under-accumulation in roots [27,48,68,70,71]. Thus AtHKT1 decreases Na^+ concentration

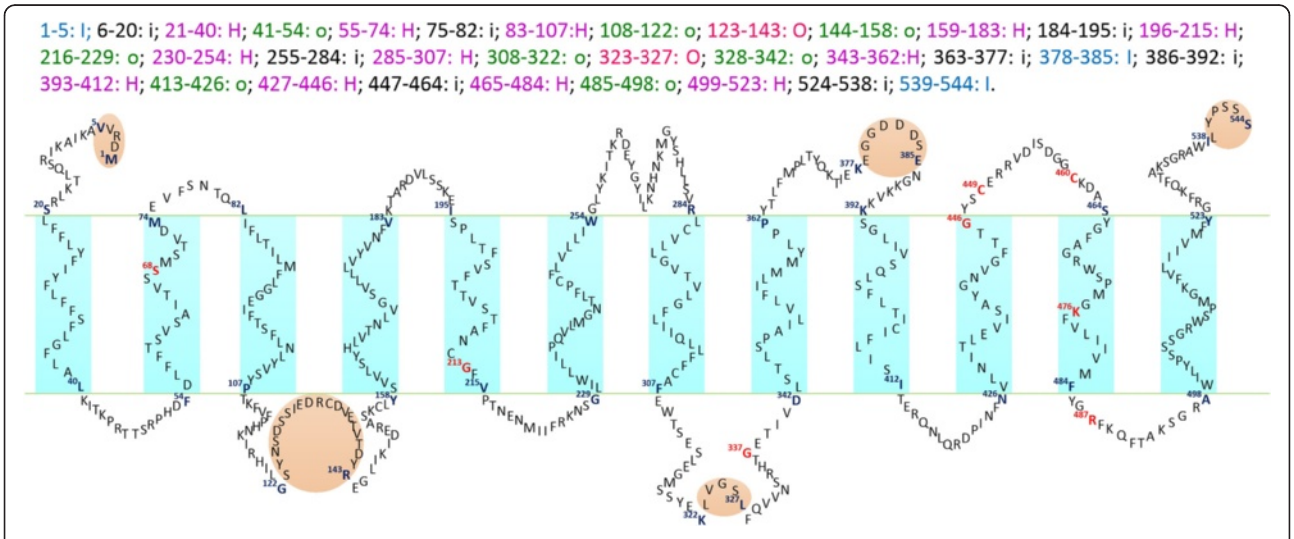

Figure 4 Structure of AtHKT1;1 transporter. The letters with red font represent the highly conserved amino acid resides which may play crucial functions on cation selection and transport. H, membrane helix; I, inside loop; i, inside helix tail; O, outside loop; o, outside helix tail.

in the transpiration stream and increase salinity tolerance following two patterns: 1) Na^+ retrieval in the root through unloading sodium directly from the xylem sap to xylem parenchyma cells, and 2) Na^+ recirculation in shoot through removal of Na^+ from the xylem sap and then transporting Na^+ from phloem companion cells into the phloem sieves. Both pathways can effectively minimize the over-accumulation of Na^+ in shoot and thus protect the leaves from salt damage when suffering from salt stress (Figure 5). Additionally, earlier investigations indicated that over-expression of *AtHKT1;1* in specific cell types could modify Na^+ transport process with the reduction of shoot Na^+ accumulation and thus improve salinity tolerance. Møller *et al.* [72] revealed that Na^+ accumulation was decreased from 37 to 64% in shoot because of increased influx of Na^+ into root stellar cells when overexpressed *AtHKT1;1* in the mature root stele [72]. Rice obtained higher Na^+ exclusion and salinity tolerance when *AtHKT1;1* was expressed in the root cortical and epidermal cells [73]. These results have implied that the alteration of a specific Na^+ transport process in specific cell types leads to a decrease of shoot Na^+ accumulation, which is a mechanism of salt stress in higher plants [73,74]. In the various mechanisms of salt tolerance (mentioned above), osmotic tolerance or tissue tolerance, mediated by other channels and transporters, might be more important in enabling *Arabidopsis* plants to grow in saline conditions than Na^+ exclusion [75-77].

EcHKT1;1/2 from *Eucalyptus camaldulensis* can mediate both Na^+ and K^+ influx when expressed in *Xenopus*

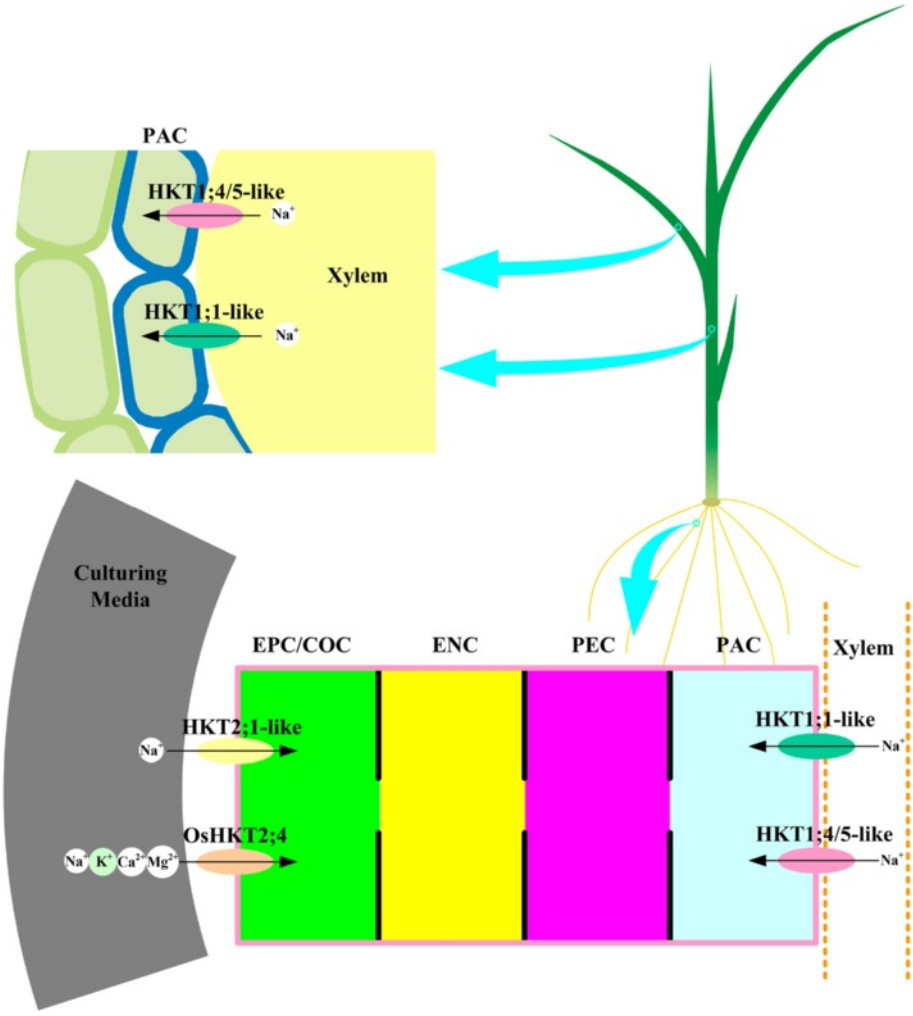

Figure 5 Functions of HKT transporters in higher plants. The transporters of HKT2;1-like, including OsHKT2;1, HvHKT2;1 and TaHKT2;1, mediate Na^+ uptake from culturing media merely in K^+-starved environments. HKT transporters, such as AtHKT1;1, OsHKT1;5, TaHKT1;5-D, TmHKT1;4-A2 and TmHKT1;5-A are involved in Na^+ exclusion from xylem to xylem parenchyma cell in order to minimize the accumulation of Na^+ in the shoot through the transpiration stream, and this is the key process for salinity tolerance of plants. OsHKT2;4 is a very special member in HKT family, and it is not only conducts as a transporter of Na^+-K^+ symport but also mediate Ca^{2+} (maybe other divalent cations) uptake like a cation channel. Cell types depicted include: epidermal cell (EPC), cortical cell (COC), endodermis cell (ENC), pericycle cell (PEC), parenchyma cell (PAC).

oocytes [56,78]. McHKT1;1/2, characterized from *Mesembryanthemum crystallinum*, can conduct K^+-Na^+ co-transport or K^+ uptake in heterologous expression systems [59]. McHKT1;1 and TsHKT1;2 were up-regulated after a sudden increase of external NaCl [59,79,80]. TaHKT1;5-D, TmHKT1;4-A2(Nax1) and TmHKT1;5-A (Nax2), AtHKT1;1 homologs, mediate Na^+ uptake in xylem parenchyma cells and Na^+ loading into the phloem sap, thereby improve the salt tolerance [81-87]. OsHKT1;5 (OsSKC1), located in parenchyma cells surrounding the xylem vessels, is likely to function in loading Na^+ from the xylem into the xylem parenchyma cells [26]. TmHKT1;4-A2 expressed in roots and leaf sheaths of a salt-tolerant durum wheat line 149, and mediated Na^+ influx from xylem sap to the xylem parenchyma cells [82,88]. TaHKT1;5-D and TmHKT1;5-A mediated Na^+ transportation from roots xylem then and maintained a high K^+-to-Na^+ ratio in the leaves [81,84,86]. In addition, functional analysis in *Xenopus laevis oocytes* revealed that OsHKT1;1 and OsHKT1;3 are permeable to Na^+ only, but are strongly different in terms of affinity and direction of transport (inward only or reversible) [60,89].

OsHKT2;1 is the unique member characterized by SerGlyGlyGly in subfamily II. OsHKT2;1 is mainly expressed in cortical and endodermal cells of roots and vascular bundle regions of leaves [6]. OsHKT2;1 displays three models of ion selectivity according to external K^+ and/or Na^+ in heterologous expression systems i.e. OsHKT2;1 acts as 1) Na^+-K^+ co-transporter at submillimolar level of external Na^+ and K^+, 2) Na^+ uniport when the external Na^+ content is within or above the millimolar range or when the external K^+ is in the submillimolar range 3) and nonconductive states within the millimolar to 10 mM range of external K^+ [89]. The *in vivo* functional analysis demonstrated that Na^+ enhanced growth of rice under K^+ starvation conditions, and OsHKT2;1 is the central transporter for nutritional Na^+ uptake in case of K^+-starved rice roots [6,67].

GlyGlyGlyGly-type feature decides the Na^+/K^+-symport. The first motif MP_AM contains a Gly residue in all the HKT members of subfamily II with the exception of OsHKT2;1 (previously named OsHKT1) [45,65,74,90,91]. In wheat and barley roots, TaHKT2;1(TaHKT1) and HvHKT2;1 (HvHKT1) mediate Na^+ uptake at K^+-starved situation [61,92]. OsHKT2;2 is one of the typical HKT transporters of subfamily II with GGGG-type amino acids sequence in rice, which has been found to be permeable to both K^+ and Na^+ [57,67,91]. Kader *et al.* [93] reported that expression of the *OsHKT2;2* gene is detected in the phloem of leaves when treated with 150 mM NaCl [93]. TaHKT2;1 in wheat, PhaHKT2;1 in *Phragmites australis* and HvHKT2;1/2 in Tibetan wild barely have been shown at least two transport modes in heterologous expression systems, K^+-Na^+ co-uptake and Na^+ influx at high Na^+ concentrations [66,69,94-97]. However, OsHKT2;4 showed different cation selectivity. OsHKT2;4 transporter, unlike with the other subfamily II HKT transporters, mediates robust inward K^+ currents even without the addition of extracellular Na^+ in heterologous expression systems, and also functions as a Mg^{2+} and Ca^{2+} permeable channel in the absence of competing K^+ ions [98-100]. This implies that OsHKT2;4 is likely to be more important in K^+ homeostasis as a K^+ transporter/channel than a Na^+-K^+ co-transporter [99,100].

HKT transporters in subfamily III are similar to subfamily II members with the characteristics of GlyGlyGlyGly, but their functions are uncertain. The phylogenetic analysis reveals that all the HKTs of subfamily I and II are belong to flowering plants, but the remainders are collected from some primitive higher plants such as PpHKT in *Physcomitrella patens* and SmHKTs in *Selaginella moellendorffii* (Figure 2). Thereby, these HKTs may be categorized into subfamily III because they are more identical with the ancestral transporters Trk in yeast. *PpHKT3;1* (originally named PpHKT1) which was identified as a unique *HKT* gene in *Physcomitrella patens* [5,101,102]. Regretfully, *Pphkt1* mutant plants maintained normal K^+ and Na^+ influx and thus PpHKT1 transporter did not mediate high-affinity Na^+ uptake [52]. Consequently, the functions of subfamily III HKTs still remain unknown, and further studies are imperative.

Cation selection model mediated by plant HKT transorters

In bacteria, archaea, fungi and plants the Trk/Ktr/HKT transporters are the key factors of osmotic regulation, pH homeostasis and resistance to drought and high salinity [16,72-74,103]. These cation transporters are functionally diverse i.e. Na^+ uniporter, Na^+/K^+ symporter and even divalent cation transporter [9,15-20,50,104,105]. However, some key informations are still unclear: 1) How do HKTs specifically catch the cations? 2) How do the energy transfers and exchanges since the K^+/Na^+ transport mediated by HKTs is an active pathway? 3) What is the mechanism(s) to monitor K^+/Na^+ concentration to regulate gene expression and transport activities? The crystal structure of a Ktr K^+ transporter from *Bacillus subtilis* and TrkH from *Vibrio parahaemolyticus* showed that Ktr and TrkH were resembled K^+ channel [106,107]. KtrB and TrkH assemble with KtrA and TrkA respectively. The activities of Trk and Ktr are upregulated by ATP respectively via TrkA and KtrA [106,107]. This suggests a mechanism for how ATP activates the activity of TrkH and Ktr by inducing conformational changes.

Additionally, two highly conserved positively charged arginine (R) and lysine (K) residues are present in the MP_DM helix of plant HKT transporters (Figure 3). Lacking of arginine (R) or lysine (K) could cause the functional

loss of HKT transporters (Figure 6). Cation transporters require a barrier to prevent free diffusion of ions along their electrochemical gradient, and it is possible that the positive residues within the transporter' pore could help to regulate its activities. Individual replacement of positively charged residues in the MP_DM helices with glutamine (Gln) did not abolish the cation uptake activity of plant HKTs, indicating that exchange of one of the positively charged residues in the MP_DM helix of plant HKTs with a hydrophilic residue can be tolerated [66]. Replacing of two or more positively charged residues with glutamine caused a considerable loss of activity in TaHKT2;1 [66]. It is hypothesized that lysine and arginine residues form a salt bridge(s) in the MP_DM to help to stabilize HKTs configuration [66]. Here we are suggesting another model for explain how the positive arginine and lysine work (Figure 6b and c). Generally, the arginine and lysine are positively charged and the electrostatic repulsion will refuse cation permeation from pore folded by HKT into the cell. The MP_DM helix with positive residues can be looked as a cation barrier or switch. A certain activator would arouse the conformational change of barrier and then the switch will turn on (Figure 6b and c). ATP is a general energy driving ion transportation on membrane and this process is companied with the transporter phosphorylation which will trigger structural change and ion permeation [106-108]. Plant HKTs may be activated by this manner in a view of the universal mechanism about molecular switch mediated by phosphorylation. Probably, the highly conserved hydroxyl amino acid resides, such as serine (S), threonine (T) and tyrosine (Y) in the MP_DM (S, T and Y rich region in Figure 3), contribute to this phosphorylation process. However, no evidence indicates that HKTs are related with activity of ATPase up to date. Researchers still need to keep searching for which ATPase mediates the phosphorylation process.

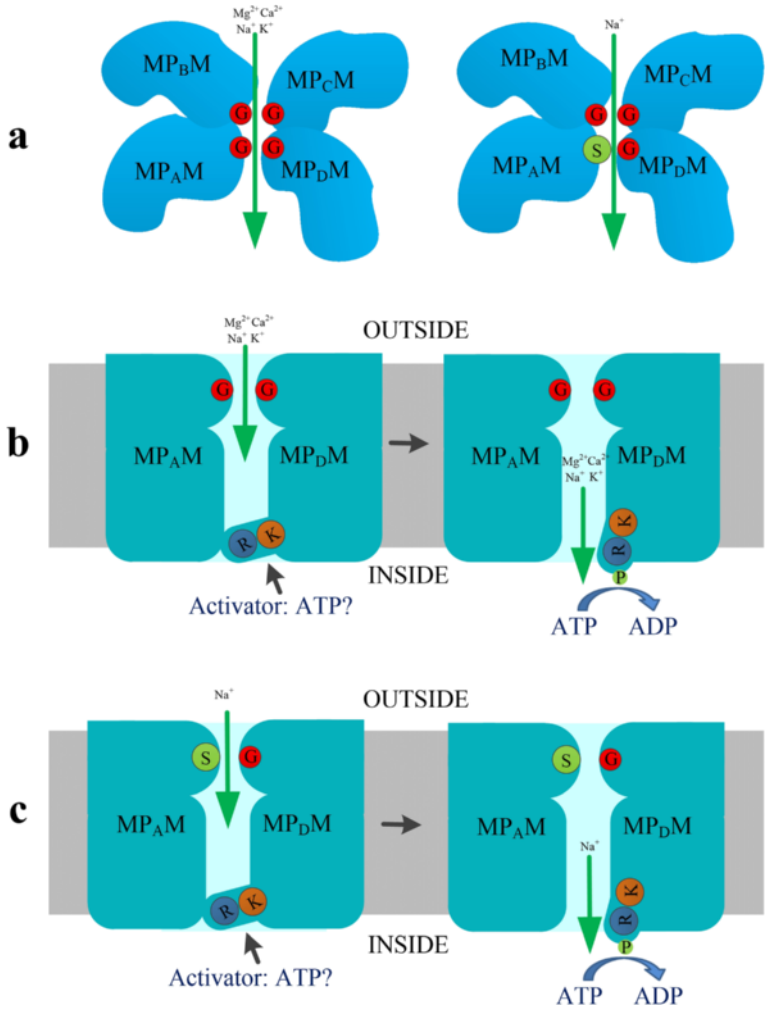

Figure 6 Model of cation trapping and selection of plant HKTs. a) Four glycine (G) residues form a trap space and allow Na^+, K^+, Mg^{2+} and Ca^{2+} across. Serine (S) and three glycine (G) residues form a trap space and allow Na^+ across. **b)** and **c)** Positive arginine (R) and lysine (K) residues form a cation barrier to stop cation across.

Considering all the former evidences, we think that plant HKT transporter may be able to perform ion transportation following this hypothesis cation selection model (Figure 6). Plant HKTs include SerGlyGlyGly-type and GlyGlyGlyGly-type transport. SerGlyGlyGly-type HKTs mainly mediate Na^+ uniport but GlyGlyGlyGly-type HKTs are diversified characteristics for cations selectivity. This class of HKTs can mediate Na^+-K^+symport and even divalent cations transport [57,67,100]. According to the helical wheel model structure [54], the four signature residues form a space which works as a cation trapping site (Figure 6). Gly is the smallest amino acid and Ser has polarity. Therefore, the space assembled by GlyGlyGlyGly is more flexible than SerGlyGlyGly. The more flexible space lets plant HKTs to catch more type of cations, such as divalent Mg^{2+}/Ca^{2+} and bigger K^+. As an activator, ATP can drive structure conversion of plant HKTs and the switch on (Figure 6). That process possibly accompanies with phosphorylation of serine, threonine or (and) tyrosine in the MP_DM motif. This cation selection model could be used to explain why GGGG-type HKTs show more complicated features on cation selectivity than SGGG-type.

Hypothesis on HKT polymer

TrkH transporter may play a role of K^+ transport through assembling to tetramer [54,106]. Interestingly, there are two highly conserved cysteine residues (C1 and C2 marked with bold triangle in Figure 3) in MP_DM motif according to the multiple alignments. These conserved cysteine residues (C_1 and C_2) mainly lie in helix tail but not transmembrane helixes (Table 1 and Figure 4). Functional complementation experiments in yeast trk1trk2 mutant and Na^+ hypersensitive mutant suggests that these two cysteine residues are indispensable (Figure 6). In tissues of organisms, a crucial function of cysteine residues is to cross link of proteins or protein subunits through disulfide bonds. This indicates that chains of HKT may be able to assemble a dimer or a tetramer through the two cysteine residues (Figure 7). In this model, cysteine residues can stabilize the structure configuration of HKTs. Positive resides of two or four group of arginine (Arg) and lysine (Lys) can make a cation barrier or switch which usually turn off, but the switch will be turned on when an activator binds to the MP_DM motif (Figure 6). Additionally, more Gly or Ser residues will be involved in the forming of cation trap/space according to the HKT polymer

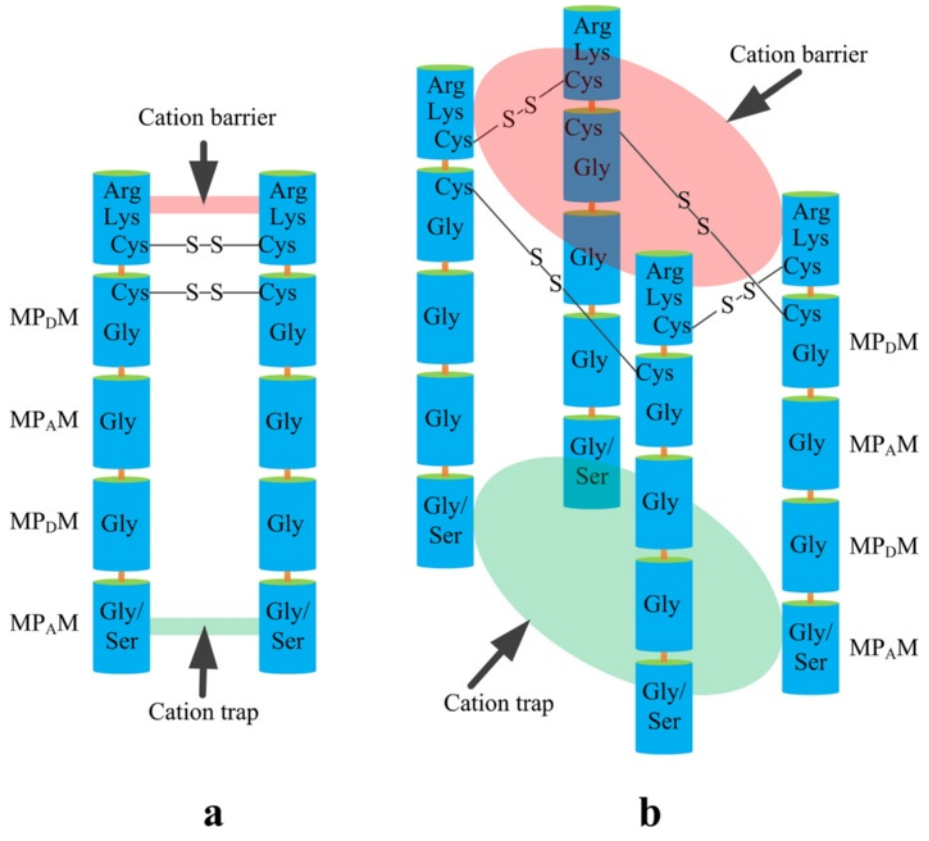

Figure 7 Hypothesis of HKT polymer model. Two highly conserved cysteine (Cys) residues form disulfide bonds to help HKTs to assemble a dimer or tetramer and stabilize the structures. Positive arginine (Arg) and lysine (Lys) residues form a cation barrier to stop cation across. Two/four serine (Ser) or glycine (Gly) residues form a cation trap for plant HKTs specifically selecting cation. **a)** dimer model and **b)** tetramer model.

model, and this situation provides plant HKTs with more flexibility on cations selection.

Conclusions and future directions

Trk/Ktr/HKT were generally thought to be evolved from the bacterial KcsA K^+ channel [109] and contain four conserved MPM motifs [55,58,65,110]. Results of phylogenetic analysis showed that plant HKTs can be classified to three subfamilies. Subfamily I was characterized by SerGlyGlyGly but subfamily II and III were GlyGlyGlyGly-type HKTs exception of OsHKT2;1. Till now, the physiological functions of HKTs in higher plant have been well understood. GGGG-type HKTs are Na^+-K^+ co-transporters, and SGGG-type HKTs present Na^+ specific-selectivity.

Former model about cation selectivity of plant HKTs emphasizes on the vital function of first motif MP_AM based on the diversity of signature residues. In fact, there are some key questions still unsolved. Firstly, it is needed to be further clarified in the details of molecular mechanism that how plant HKTs specifically trap a certain cation. Secondly, what energy materials take part in the active transport mediated by plant HKTs, and how the energy transfers and exchanges. We supposed that the fourth motif MP_DM also have same importance for cation permeation conducted by plant HKTs since this motif is more conserved than other MPMs. Highly conserved positive residues arginine and lysine in MP_DM may be a cation barrier/switch which prevents cation permeation along the pore folded by HKT into intracellular. A certain activator, most probably ATP, binds to MP_DM motif (or another motif) and drives the conformational change of HKTs, and then the cation switch turned on (Figure 7). Moreover, protein chains can be cross-linked through disulfide bonds condensed by cysteine resides. Interestingly, there are exactly two highly conserved cysteine residues in motif MP_DM. Therefore, we hypothesize that plant HKTs possibly assemble to a dimer or tetramer through the two conserved Cys residues based on tetrameric model for the Trk family of symporters [54,106]. However, the model of cations transport through HKT transporters still need be supported by more experimental evidences i.e. 1) functional identification about specific amino acid mutations, 2) high-resolution distribution of HKT in membrane, 3) determination of chemicals related to energy transformation 4) and especially crystal structure interpretation of plant' HKTs.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

S contributed overall project coordination, data analysis and interpretation. L and L searched the database, obtained the full amino acid sequences of HKTs and analyzed the structures of HKTs. Ma developed all figures. All authors participated in writing and revising. S and L contributed equally to this work. All authors read and approved the final manuscript.

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