

Discerning the Dynamics of Sodium Transport in Plants Crucial for Developing Crops Resilient to Salt Stress

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Abstract

This review focuses on sodium (Na^+) transport in plants which is a complex process. Understanding about the genes that are implicated in Na^+ transport is of prime importance since their overexpression can lead to transgenic crop plants that tolerate high levels of salt stress conditions. Movement of Na^+ from the soil into the roots is accomplished by non-selective cation channels (NSCCs) besides high affinity potassium transporters (HKTs). Its egress takes place at the membrane level by salt overly sensitive pathway (SOS), which is well characterized genetically. Information about the genes associated with tissue specific expressions for Na^+ sequestration into the vacuoles is largely obscure, but is being unravelled slowly. Such a comprehensive understanding about the Na^+ movement from the soil to the root, its loading into xylem, long-distance transport to the leaf blade, and compartmentalization into the vacuole in a tissue-specific manner appears crucial for developing climate-resilient crop plants in future.

Key words: Na^+ transport, Vacuolar sequestration, Non-selective cation channels, High affinity potassium transporter, Salt overly sensitive pathway

Transport of Na^+ in Plants

It is of prime importance for us to understand the physiological, biochemical and molecular mechanisms underlying the salt stress tolerance so that we can address the challenges associated in improving the final yields including grain quality in crops growing under saline conditions. It is vital therefore, to concentrate our research endeavour to combat soil salinity, water stress and nutritional quality of grains growing under severe stress conditions. Such circumstances demand involvement of biotechnological interventions for improving the crop productivity (1).

Absorption of Na^+ at the soil-plant interface by non-selective cation channels (NSCCs), high affinity potassium transporters (HKTs) and low affinity cation transporters (LCTs)

Na^+ may not be required by all plants, but Na^+ promotes plant growth and may be essential to certain plants (2). Na^+ is also known to compensate for some of the functions carried out by K^+ since it is chemically similar, when the availability of K^+ is limited. But, how do plants sense the concentrations of Na^+ ions in their environment? Salt overly sensitive 1 (SOS1 or Na^+/H^+ antiporters), histidine kinases, *Arabidopsis thaliana* histidine kinase 1 (AHK1/

ATHK1) have been proposed as potential salt/osmosensors (3,4,5). $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCX), NSCC/NADPH oxidase tandem, cyclic nucleotide receptors, purino receptors, annexins, H^+ ATPase/GORK (gated outward rectifying channel) tandem have also been suggested (6). But, our current knowledge about plant early Na^+ sensors and subsequent signaling events are totally limited. In this review, absorption of Na^+ from the soil, its transport across the plant body, its exclusion at the membrane level and the plant responses to excess Na^+ are briefly described.

Absorption of Na^+ through non-selective cation channels (NSCC)

Ingress of Na^+ from the soil occurs across the plasma membrane of the root hair epidermal cells by passive means. Depending upon the salinity levels, the chemical driving force for Na^+ transport will also vary. Generally, negative electrical membrane potential (-140 mV) differences are maintained across the plasma membrane. Blumwald et al. (7) pointed out that even low extracellular Na^+ levels will establish a large electrochemical potential gradient which favours passive transport of Na^+ from the environment into the cytosol. Also, Cheesman (8) showed that Na^+ import is passive at low K^+ levels. Na^+ is imported into majority of the plants by passive means, mostly through Non-Selective Cation Channels (NSCC). Besides NSCCs, High Affinity Potassium Transporters (HKTs), Low Affinity Cation Transporter1 (LCT1), Glutamate-like Receptor Homologues (GLRs) and Cyclic Nucleotide Gated Channels (CNGC) (Fig. 1) also help in Na^+ transport (9,10). NSCCs are not used solely for Na^+ transport. They help in influx of many other cations also. Both CNGCs and GLRs, the two important families of NSCCs are associated with Na^+ uptake. Of the many CNGCs, CNGC1, CNGC3, CNGC4 and to some extent CNGC10 have been reported to assist in Na^+ absorption (11,12,13,14) reported that GLR1.1 and GLR1.4 conduct Na^+ , K^+ , and Ca^{2+} and GLR3.7 helps in the uptake of Na^+ , Ca^{2+} and Ba^{2+} . Thus, multiple channels help the Na^+ to enter the root cells. But, which one pre-

dominantly works under low or high concentrations of Na^+ ions is totally not clear.

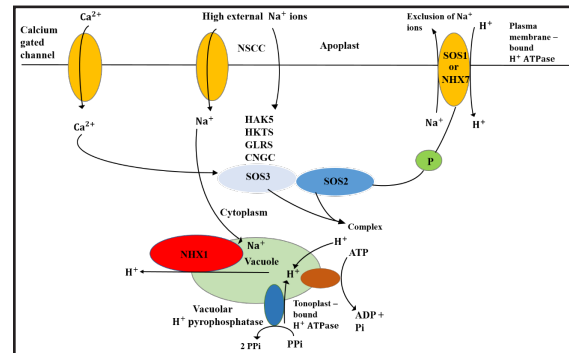


Fig. 1: Transport of Na^+ from soil to the root cells through NSCC, HAK5, HKTs, GLR and CNGC. Na^+ is excluded out through SOS pathway at the plasma membrane with the exchange of H^+ by plasma membrane – bound ATPase. Na^+ is also exported to vacuoles through NHX. When Na^+ goes into vacuole, it comes into cytoplasm. Two enzymes namely vacuolar membrane – bound ATPase and vacuolar pyrophosphatase help in the process.

Absorption of Na^+ through high affinity potassium transporters (HKTs)

Corratge-Faillie et al. (15), Hauser and Horie (16) reported that class II HKTs act for the uptake of Na^+ and some of them like TaHKT2;1 (from *Triticum aestivum*) function as a high-affinity Na^+/K^+ symporter, but under NaCl stress, it acts as a low affinity Na^+ uniporter. While HKTs with serine (SGGG) residue (SGGG-type HKTs) are permeable to only Na^+ , HKTs with glycine residue (GGGG-type HKTs) help to permeate both Na^+ and K^+ in plants (17). Out of the nine HKT members that have been detected in rice, OsHKT2;1 and OsHKT2;2 (formerly known as HKT1, HKT2 and belong to SGGG-type) have been reported to mediate Na^+ uptake from the soil (18,19). Over all, it appears that class I HKTs mediate the movement of Na^+ and K^+ within the plant and are associated with salt stress tolerance (20,21,22), class II HKTs promote uptake of both Na^+ and K^+ under conditions of K^+ starvation (23,24,25,26). Symport of Na^+ and K^+ is also ideal for the plants under the condi-

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tions where shortage of K^+ prevails in the soil. This may ensure the survival of the plants under conditions where K^+ concentrations are meagre (27). Rice *OsHKT2;1* is induced in response to K^+ deficiency, but its mutants *oshkt2;1* accumulate less Na^+ , but not less K^+ . These findings indicate that HKT2;1 contributes to the uptake of Na^+ . Functional diversity thus exists among the two subclasses of HKTs. Besides their uptake, HKT channels play vital roles in the homeostasis of Na^+ - K^+ ions in the plants (28,29). Horie et al. (18,23) suggested that Na^+ can compensate for the deficiency of K^+ to some extent in plants. Further, Horie et al. (23) and Yao et al. (19) found that *OsHKT2;2* transports Na^+ under salt stress in a salt tolerant rice variety Pokkali. Overexpression of sodium, potassium permeable *HvHKT2;1* in the barley, enhances Na^+ levels in the shoots and mitigates salt stress. Experiments suggest that barley can tolerate Na^+ better when compared to wheat (24). It is interesting to note that class II HKT homologues have not been detected in *Arabidopsis*, while they are common in monocots (15). But the relative importance of the presence/absence of these two classes of HKTs in monocots and dicots is not immediately known.

Absorption of Na^+ through LCTs

Schachtman et al. (30), Amtmann et al. (31), and Uruguchi et al. (32) found out a candidate transporter LCT1 from wheat. This transporter transports multiple cations like Na^+ , Rb^+ , Ca^{2+} , Li^+ , and Cs^+ . But this Na^+ uptake function of LCT1 may be unique to wheat only as has been pointed out by Schachtman et al. (30). However, we still do not know the functions of majority of the porters from these three family members. This infers that there are multiple porters for Na^+ uptake that act in parallel and the uptake system appears as a complex regulatory mechanism.

Long distance transport of Na^+

In general, Na^+ gets transported radially through both apoplastic and symplastic pathways (33). Na^+ reaches the endodermis beyond which its transport is needed across the plasma membrane (10). In rice, significant amounts of

Na^+ are transported to the shoot by bypass flow, unlike *Arabidopsis* (34). Application of silicon decreases the apoplastic bypass flow of Na^+ and Cl^- (35). Likewise, phi cells with a thick cell wall generally hinder the movement of Na^+ in the xylem (36). Apoplastic entry of Na^+ is generally hindered by apoplastic barriers such as root endodermis and exodermis that can otherwise avert bypass flow (37,38). Both endodermis and exodermis contain Casparian strips (a paracellular deposition of lignin in the cell walls that helps transport into symplastic system from apoplast) and the suberin lamella, which are made up of long-chain fatty acids (38,39,40,41). The long-chain fatty acids surrounding exodermis and endodermis cell walls form a hydrophobic barrier that modulate not just water but also ion uptake (42). Therefore, suberization can intercept entry of Na^+ ions into vascular tissues both in dicots and monocots (38,43). Evidence now exists that deposition of suberin enhances in response to salinity stress in higher order roots. Interestingly, suberin in higher quantities in the exodermis coupled with sequestration of Na^+ into vacuoles has been observed with lower Na^+ content in leaves (44,45). These results point out that development of Casparian strips and suberization surrounding exodermis and endodermis is an important strategy to prevent the movement of Na^+ ions into the vascular stream. For long-distance ion transport, SOS1 is also necessary and helps in xylem loading/unloading in *Arabidopsis* (46) (Fig. 2), Na^+ partitioning in tomato (47) and responsible for ion fluxes in roots (48).

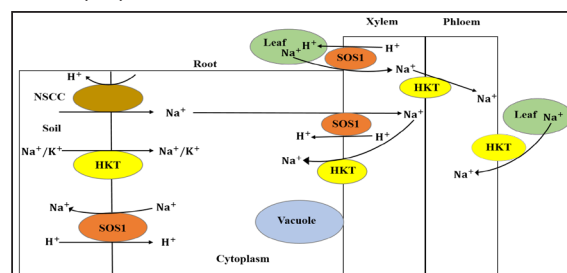


Fig. 2: Transporters that mediate ions. Through NSCC (channel), enters into cytoplasm in exchange of protons (H^+). HKT mediates both and from soil into the root cells. goes out of the cells through SOS1 pathway back into the soil or

through the SOS1 into xylem cells. that enters into xylem can go back into root cells through HKT. gets into xylem from leaf also via SOS1, but enters into phloem via HKT system. Leaf also contributes to via HKT.

Exclusion of Na⁺ from the xylem

Na⁺ ions that enter the cytoplasm of the roots now move into the xylem of the root stele via the symplastic pathway (Fig. 2). Most likely, Cation/H⁺ Exchanger (CHX21) is a candidate in the radial transport of Na⁺ ions. Na⁺ that reaches the stele, enters into the xylem and transpiration stream, for transport from the root cells to the shoot cells. In conditions of severe salt stress, xylem loading of Na⁺ is perhaps a passive process. But, under moderate stress, salt overly sensitive 1 (SOS1) is likely a candidate that can mediate the loading of Na⁺ into xylem (49) (Fig. 2). In many plants, retrieval of Na⁺ ions from the xylem occurs in the basal regions of roots and shoots (50). It appears now that HKTs, especially AtHKT1 play crucial roles in xylem unloading (51). But, in the case of rice, Na⁺ is retrieved from xylem by OsHKT1;5 (52).

Na⁺ efflux or exclusion from the root

Na⁺ efflux from the root cells into the external medium is poorly understood till date in glycophytes. To generate salt tolerant plants, it is indispensable to unravel the mechanisms associated with Na⁺ exclusion to the outside environment and thus restrain the cytosolic levels of Na⁺. In some of the halophytes, Na⁺ ions are excluded through salt glands located on the leaf margins. But, in glycophytes, such an egress mechanism is generally not noticed. Exclusion of Na⁺ at the plasma membrane level largely imparts higher degrees of tolerance in crop plants (53,54). Several bodies of evidence indicate that the mechanism of SOS1 is the vital and the major transporter that has been genetically characterized for Na⁺ efflux at the plasma membrane level in the root tips. Besides SOS1, Rapid Transmembrane Sodium Cycling (RTSC) has been proposed for the efflux, though not totally accepted. Further, alternative mechanisms

have been put forward where Na⁺ proceeds through apoplast, but not through the plasma membrane. Corroborating evidences indicate that exclusion of Na⁺ across the plasma membrane is certainly modest, but not high as usually reported using tracer experiments (55). In plants, efflux systems can certainly act as “overflow valves”. For example, where external Na⁺ concentrations are low, K⁺-stimulated Na⁺ outflow from roots has been noticed (56). In other words, K⁺ ions may be preferred by plants over that of Na⁺ ions under stress conditions (54,57,58). It has been observed that few ions like K⁺ are effluxed despite its low availability in the soil (55,59). Over all, the experiments point out that besides SOS1 pathway; other pathways exist for Na⁺ ion exclusion from the plants. However, genes associated with such pathways need to be validated.

Vacuolar compartmentation/sequestration of Na⁺ ions or movement of Na⁺ ions into vacuoles

Among the diverse strategies that the plants adapt to cope with salt stress tolerance, sequestration of Na⁺ ions into the vacuole are one of the key components and of primary importance. But what is not known is the tissue-specific (root apex, main root, stem, leaf, petiole, pedicel, flower etc.) expression of genes related to Na⁺ sequestration into the vacuoles. Few proteins such as Na⁺/H⁺ exchangers (NHX) that are selective for transporting Na⁺ ions are localized on the vacuolar membrane (tonoplast). NHX proteins are accountable for the compartmentalization or sequestration of Na⁺ ions into the vacuole. Movement of Na⁺ against a concentration gradient across the plasma membrane and tonoplast is generally coupled to the H⁺-translocating pumps (49,60). In plants growing under salt stress conditions, H⁺ translocation is noticed because of the enhanced transcription of the genes such as plasma membrane and vacuolar-type ATPases (*P-ATPase* and *V-ATPases*) (7,61,62). Both plasma membrane and tonoplast transporters are entangled in the efflux of Na⁺ ions into the soil and vacuole respec-

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tively by coupling the transport of H^+ (7,62) (Fig. 2). Further, enhanced salt and drought stresses have been recorded in transgenic plants overexpressed with *vacuolar Na⁺/H⁺ antiporter* and *H⁺-pyrophosphatase* genes (*VPPase*) (63,64,65). These experiments infer that gene associated with Na^+ transport is crucial for determining the salt stress tolerance.

Sequestration of toxic ions through Na^+ / H^+ porters into the vacuoles reduces the load of Na^+ in the cytoplasm and prevents denaturation of proteins (66,67,68). When *AtNHX1* from *Arabidopsis thaliana* was overexpressed, transgenic lines exhibited salt stress tolerance (7,63). Tissue specific expressions of several NHX porters like *OsNHX1*, *OsNHX2*, *OsNHX3* and *OsNHX5* have been found in rice and *Sorghum bicolor* (66,67,69,70). Such a tissue specific expression helps the plants to effectively maintain low Na^+ levels in the cytoplasm under saline conditions. To understand the vacuolar sequestration of Na^+ and its link with salt stress tolerance, Wu et al. (71) have conducted experiments in wheat. Salt stress has clearly been positively correlated with tissue-specific Na^+ sequestration and vacuolar sequestration ability in wheat roots. Cytosolic Na^+ concentrations were higher in the root meristem of wheat tolerant to salt stress in comparison with sensitive lines. On the other hand, vacuolar Na^+ levels were higher in salt susceptible lines than the tolerant genotypes. It must also be noted that root elongation zone contained high Na^+ levels. Such an accumulation in the root elongation zone infers that Na^+ acts as an osmoticum and maintains the turgor that might help bonafide growth of roots.

Genetic engineering and salt stress tolerance utilizing Na^+ porters

Reducing the concentrations of Na^+ conductance in the plant is one way to achieve salt stress tolerance. It is not just the amount of Na^+ but the low Na^+/K^+ ratio, especially in the root cells which is vital for accomplishing salinity tolerance (72,73,74). Similarly, enhancing Na^+

efflux by epidermal cells of the roots using the SOS pathway and NHXLP proteins is another way for generating crop plants that can endure soil salinity (7,63,73). *Sodium proton exchanger* genes (*NHX*) such as *AtNHX1* have enhanced the salt stress tolerance in Arabidopsis upon overexpression (63,75,76). Another important way to mitigate salt stress is by incorporating genes like vacuolar H^+ -ATPase and H^+ -pyrophosphatase that help in sequestering the Na^+ ions into vacuoles (63,64,65,77,78). HKT1;5 also appears as an important gene for achieving salt stress tolerance. This gene has been introgressed from *Triticum monococcum* into *Triticum turgidum* for salt tolerance with enhanced grain yields in the field (79). Remarkably, overexpression of Na^+ - and K^+ -permeable HKT transporter HvHKT2;1 in barley enhanced the tolerance (24). It is also crucial to genetically engineer genes linked to Na^+ and K^+ loading and unloading into vascular tissues. Besides deploying genetic engineering technologies, it is pivotal for us to exploit gene editing technologies such as CRISPR-Cas9.

Conclusions

Gravity of salinity is increasing day after day globally. Therefore, crops must be evolved that can cope with high salt stress conditions. While SOS pathway is genetically characterized, other genes associated with Na^+ exclusion at the plasma membrane level need to be unveiled. Taking cognizance of the above facts, it appears that two different classes of transporters such as sodium proton antiporters (NHX) and antiporter-like proteins (NHXLP) and HKTs (associated with Na^+ uptake and long-distance transport of Na^+) help to exclude Na^+ at the membrane level or sequester into vacuoles, and maintain K^+ homeostasis respectively. One of the facets that needs our consideration and also demanding is upregulation of Na^+ exclusion to the outside environment or inclusion into the vacuole and simultaneously silencing of NSCC and HKTs for obtaining plants with synergistic effect. This is certainly challenging, but needs a strategy. Entry of Na^+ ions into the apoplast

are inhibited by exodermis and endodermis which can avert bypass flow. But the genes that modulate suberization of the cells need to be selectively incorporated for better resilience of the crops to salt stress. Further, tissue-specific sequestration of Na⁺ ions into the vacuoles must be further explored for understanding and incorporation of such genes into crop plants.

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Conflict of interest

Authors declare no conflict of interest. All authors have read and approved the manuscript.

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