Coordination of AtHKT1;1 and AtSOS1 Facilitates Na\(^+\) and K\(^+\) Homeostasis in *Arabidopsis thaliana* under Salt Stress

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**Abstract**  Reducing Na\(^-\) accumulation and maintaining K\(^+\) stability in plant is one of the key strategies for improving salt tolerance. AtHKT1;1 and AtSOS1 are not only the salt tolerance determinants themselves, but also mediate K\(^+\) uptake and transport indirectly. To assess the contribution of AtHKT1;1 and AtSOS1 to Na\(^-\)' homeostasis and K\(^+\) nutrition in plant, net Na\(^-\) and K\(^+\) uptake rate, Na\(^-\) and K\(^+\) distributions in *Arabidopsis thaliana* wild type (WT), *hkt1;1* mutant (*athkt1;1*) and *sos1* mutant (*atsos1*) were investigated. Results showed that under 2.5 mM K\(^+\) plus 25 or 100 mM NaCl, *athkt1;1* shoot concurrently accumulated more Na\(^-\) and less K\(^+\) than did WT shoot, suggesting that AtHKT1;1 was critical for controlling Na\(^-\) and K\(^+\) distribution in plant; while *atsos1* root accumulated more Na\(^-\) and absorbed lower K\(^+\) than did WT root, implying that AtSOS1 was determiner of Na\(^-\) excretion and K\(^+\) acquisition. Under 0.01 mM K\(^+\), *athkt1;1* absorbed lower Na\(^-\) than did WT with 100 mM NaCl, suggesting that AtHKT1;1 is involved in Na\(^-\) uptake in roots; while *atsos1* shoot accumulated less Na\(^-\) than did WT shoot no matter with 25 or 100 mM NaCl, implying that AtSOS1 played a key role in controlling long-distance Na\(^-\) transport from root to shoot. We present a model in which coordination of AtHKT1;1 and AtSOS1 facilitates Na\(^-\) and K\(^+\) homeostasis in *A. thaliana* under salt stress: under the normal K\(^+\) and Na\(^-\) distributions in *Arabidopsis thaliana* wild type (WT), *hkt1;1* mutant (*athkt1;1*) and *sos1* mutant (*atsos1*) were investigated. Results showed that under 2.5 mM K\(^+\) plus 25 or 100 mM NaCl, *athkt1;1* shoot concurrently accumulated more Na\(^-\) and less K\(^+\) than did WT shoot, suggesting that AtHKT1;1 was critical for controlling Na\(^-\) and K\(^+\) distribution in plant; while *atsos1* root accumulated more Na\(^-\) and absorbed lower K\(^+\) than did WT root, implying that AtSOS1 was determiner of Na\(^-\) excretion and K\(^+\) acquisition. Under 0.01 mM K\(^+\), *athkt1;1* absorbed lower Na\(^-\) than did WT with 100 mM NaCl, suggesting that AtHKT1;1 is involved in Na\(^-\) uptake in roots; while *atsos1* shoot accumulated less Na\(^-\) than did WT shoot no matter with 25 or 100 mM NaCl, implying that AtSOS1 played a key role in controlling long-distance Na\(^-\) transport from root to shoot. We present a model in which coordination of AtHKT1;1 and AtSOS1 facilitates Na\(^-\) and K\(^+\) homeostasis in the original and straightforward strategy that decreases the accumulation of Na\(^-\) in plants is reducing Na\(^-\) influx in roots. AtHKT1;1 (High-affinity K\(^+\) Transporter) isolated from *Arabidopsis thaliana* appears to function as a Na\(^-\) selective uniporter that controls Na\(^-\) uptake when studied in heterologous expression systems (Uozumi et al. 2000). In planta, earlier experiments had revealed that *AtHKT1;1* promoter in *A. thaliana* was active in the vascular tissues of the root and leaf (Berthomieu et al. 2003; Mäser et al. 2002), and the further investigation using transgenic *ProAtHKT1;1:GUS* plants showed that it specifically expressed in xylem parenchyma cells (Sunarpi et al. 2005). Transporter of Na\(^-\) as it is, AtHKT1;1 has attracted much attentions so far. Rus et al. (2001) and Zhang et al. (2008) pointed out that a potential role of AtHKT1;1 in Na\(^-\) uptake by *A. thaliana* roots.

**Keywords:** *Arabidopsis thaliana*, AtHKT1;1, AtSOS1, Na\(^-\), K\(^+\), Salt stress

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Introduction

Soil salinity is a major abiotic stress in plant agriculture worldwide (Zhu 2001; Flowers 2004). Most crops are very sensitive to salt: Na\(^-\) disequilibrium is a primary consequence of ionic stress and leads to adverse effects on water uptake, cytosolic enzyme activities, photosynthesis and metabolism, which causes growth inhibition and leads ultimately to plant death (Niu et al. 1995; Zhu et al. 2003). K\(^+\) is the most abundant cation in plant cells and can comprise as much as 10% of plant dry weight versus Na\(^-\) (Leigh and Wyn Jones 1984; Véry and Sentenac 2003). K\(^+\) has important functions as a major osmolyte in vacuoles, in turgor-driven movements; as a cofactor for enzymes, for maintaining the plasma membrane potential; and especially, as an important factor to improve salt-tolerance of plants under salt stress (Zimmermann and Sentenac 1992; Mäser et al. 2001).

The key of salinity resistance is reducing accumulation of Na\(^-\) and maintaining stabilization of K\(^+\) in plants. There is now more comprehensive understanding about the transport systems and regulatory mechanisms that mediate K\(^+\) and Na\(^-\) homeostasis in plants (Epstein 1998; Zhu 2002, 2003). First, the original and straightforward strategy that decreases the accumulation of Na\(^-\) in plants is reducing Na\(^-\) influx in roots. AtHKT1;1 (High-affinity K\(^+\) Transporter) isolated from *Arabidopsis thaliana* appears to function as a Na\(^-\) selective uniporter that controls Na\(^-\) uptake when studied in heterologous expression systems (Uozumi et al. 2000). In planta, earlier experiments had revealed that *AtHKT1;1* promoter in *A. thaliana* was active in the vascular tissues of the root and leaf (Berthomieu et al. 2003; Mäser et al. 2002), and the further investigation using transgenic *ProAtHKT1;1:GUS* plants showed that it specifically expressed in xylem parenchyma cells (Sunarpi et al. 2005). Transporter of Na\(^-\) as it is, AtHKT1;1 has attracted much attentions so far. Rus et al. (2001) and Zhang et al. (2008) pointed out that a potential role of AtHKT1;1 in Na\(^-\) uptake by *A. thaliana* roots.
However, the acknowledged function of AtHKT1;1 is Na⁺ unloading from the xylem to surrounding parenchyma cells (Sunarpi et al. 2005; Davenport et al. 2007; Horie et al. 2009) and Na⁺ recirculation from shoots to roots (Berthomieu et al. 2003). Na⁺ withdrawal from the xylem and recirculation in the phloem are effective strategies to reduce Na⁺ accumulation in shoots. Intriguingly, this course of Na⁺ unloading from the xylem to surrounding parenchyma cells effects K⁺ transfer, which leads to depolarisation of plasma membrane of xylem parenchyma cells, and then stimulates K⁺ efflux into the xylem via membrane depolarization activated K⁺ channels such as SKOR (Stelar K⁺ Outward Rectifier) (Wegner and Raschke 1994; Wegner and De Boer 1997; Horie et al. 2009; Hauser and Horie 2010). Finally, the process of Na⁺ unloading indirectly mediates K⁺ transport by control K⁺ release from the xylem parenchyma cells into the xylem sap toward the shoot (Gaymard et al. 1998; Liu et al. 2006).

Another important Na⁺ transporter as it is, AtSOS1 (Salt Overly Sensitive 1) is also located in the plasma membrane of xylem parenchyma cells (Shi et al. 2002). Although AtSOS1 has the same expression location with AtHKT1;1, it possesses contrary function to AtHKT1;1. Analysis by using a AtSOS1 promoter:GUS fusion revealed preferential expression of AtSOS1 at the plasma membrane of xylem parenchyma cells, loading Na⁺ directly into the xylem or mediating cytoplasmic Na⁺ efflux to the neighbouring apoplastic spaces, and then Na⁺ diffusing into xylem (Shi et al. 2002; Guo et al. 2012). In addition, Shi et al. (2002) also found that the activity of AtSOS1 was sharply induced by salt stress, and AtSOS1 could improve Na⁺ efflux to the apoplastic spaces with reducing Na⁺ accumulation in A. thaliana. Furthermore, it was obtained that the extrusion of Na⁺ from the cytoplasm by AtSOS1 protected the K⁺ permeability of the membrane, and the AKT1 (Arabidopsis K⁺ Transporter 1) K⁺ channel in particular, from inhibition by Na⁺ (Qi and Spalding 2004). Meanwhile, AtSOS1 might involve in long-distance Na⁺ transport from root to shoot, maintaining pH homeostasis and Ca²⁺ transfer in plants (Shi et al. 2002; Oh et al. 2010).

The above researches indicate that AtHKT1;1 and AtSOS1 are not only the salt tolerance determinants themselves, but also medicate the Na⁺ homeostasis and K⁺ nutrition in A. thaliana by regulating the other Na⁺ or K⁺ transporter indirectly. However, it is still unclear that how they work together. In the present work, net Na⁺ and K⁺ uptake rate, Na⁺ and K⁺ accumulation, and Na⁺ and K⁺ distribution were investigated in WT, athkt1;1 and atsos1. Finally, we hypothesized a function model of AtHKT1;1 and AtSOS1 in facilitating Na⁺ homeostasis and K⁺ nutrition in A. thaliana: AtHKT1;1 and AtSOS1 were mainly involved in Na⁺ unloading and Na⁺ exclusion, respectively, under the normal K⁺ plus mild or severe salt stress, which kept the low Na⁺ level in plants; Under the low K⁺ plus mild or severe salt stress, AtHKT1;1 and AtSOS1 preferentially worked on Na⁺ uptake into the root and Na⁺ loading into the xylem, respectively, which could insure Na⁺ instead of K⁺ to keep the osmotic equilibrium and cell turgor under low K⁺ condition.

Results

Ion Uptake and Distribution in athkt1;1 in Response to Mild Salt Stress

Under 25 mM NaCl, compared with WT, net Na⁺ uptake rate and shoot Na⁺ concentration were increased by 79.9% and 165.3%, respectively, in athkt1;1 (Fig. 1A, C), while root Na⁺ concentration was decreased by 32.8% in athkt1;1 (Fig. 1B). On the contrary, net K⁺ uptake rate and shoot K⁺ concentration in athkt1;1 were lower than those in WT under 25 mM NaCl (Fig. 1D, F), while root K⁺ concentration in athkt1;1 was higher than it in WT with or without 25 mM NaCl (Fig. 1E). The results showed that loss-of-function of AtHKT1;1 not only changed net Na⁺ and K⁺ uptake rate, but also changed Na⁺ and K⁺ distribution in plant under mild stress.

Ion Uptake and Distribution in atsos1 in Response to Mild Salt Stress

Compared with WT, net Na⁺ uptake rate and root Na⁺ concentration were increased by 177.2% and 102%, respectively, in atsos1 under 25 mM NaCl (Fig. 1A, B), while shoot Na⁺ concentration had no difference (Fig. 1C). Compared with 0 mM NaCl, 25 mM NaCl decreased root and shoot K⁺ concentrations significantly in both WT and atsos1; however, there were no difference between WT and atsos1 with or without 25 mM NaCl (Fig. 1E, F). Net K⁺ uptake rate in atsos1 was lower than it in WT with or without 25 mM NaCl, but it was not affected by the external Na⁺ concentration in both WT and atsos1 (Fig. 1D). The results indicated that loss-of-function of AtSOS1 only affected net Na⁺, K⁺ uptake rate and root Na⁺ accumulation under mild stress.

Ion Uptake and Distribution in athkt1;1 in Response to Severe Salt Stress

Under 100 mM NaCl, compared with WT, net Na⁺ uptake rate had no change in athkt1;1 (Fig. 2A); root Na⁺ concentration was decreased by 57.2%, shoot Na⁺ concentration was increased by 15.4% in athkt1;1 (Fig. 2B, C). Net K⁺ uptake rate and shoot K⁺ concentration in athkt1;1 were lower than those in WT (Fig. 2D, F), while root K⁺ concentration had no
significantly difference between WT and athkt1;1 under 100 mM NaCl (Fig. 2E). The results showed that loss-of-function of AtHKT1;1 not only changed net K$^+$ uptake rate, but also changed Na$^+$ and K$^+$ distribution in plant under severe stress.

Ion Uptake and Distribution in atsos1 in Response to Severe Salt Stress

Compared with WT, net Na$^+$ uptake rate, root and shoot Na$^+$ concentration were increased by 105.5%, 34.3% and 40.3%, respectively, in atsos1 under 100 mM NaCl (Fig. 2A, B, C). Net K$^+$ uptake rate in atsos1 was lower than it in WT with or without 100 mM NaCl (Fig. 2D). Compared with WT, root K$^+$ concentration was decreased significantly, while shoot K$^+$ concentration had no difference in atsos1 under 100 mM NaCl (Fig. 2E, F). The results indicated that loss-of-function of AtSOS1 not only changed net Na$^+$ and K$^+$ uptake rate, but also changed Na$^+$ and K$^+$ distribution in plant under severe stress.

Ion Uptake and Distribution in athkt1;1 in Response to low K$^+$ (0.01 mM) plus Mild Salt Stress

Under 0.01 mM K$^+$ with 25 mM NaCl, compared with WT, net Na$^+$ uptake rate, root and shoot Na$^+$ concentrations had no change in athkt1;1 (Fig. 3A, B, C). Overall it appeared that the K$^+$ uptake and accumulation had the similar variation tendency to Na$^+$ in athkt1;1 (Fig. 3D, E, F). The results showed that loss-of-function of AtHKT1;1 had no influence on Na$^+$, K$^+$ uptake and accumulations under low K$^+$ plus
mild salt stress. In other words, the activity of AtHKT1;1 was restricted by low K⁺ condition.

Ion Uptake and Distribution in *atsos1* in Response to Low K⁺ (0.01 mM) plus Mild Salt Stress

Compared with WT, net Na⁺ uptake rate had no difference in *atsos1* (Fig. 3A); root Na⁺ concentration was increased by 99.6%, while shoot Na⁺ concentration was decreased by 27.4%, respectively, in *atsos1* under 0.01 mM K⁺ with 25 mM NaCl (Fig. 3B, C). In contrast, compared with WT, root K⁺ concentration was decreased by 42.5%, shoot K⁺ concentration was increased by 112.7% in *atsos1* under 0.01 mM K⁺ with 25 mM NaCl (Fig. 3E, F). The results indicated that loss-of-function of AtSOS1 only changed Na⁺ and K⁺ distribution in plant under low K⁺ plus mild salt stress.

Ion Uptake and Distribution in *athkt1;1* in Response to Low K⁺ (0.01 mM) plus Severe Salt Stress

Under 0.01 mM K⁺ with 100 mM NaCl, compared with WT, net Na⁺ uptake rate decreased in *athkt1;1* (Fig. 4A); root Na⁺ concentration was decreased by 25%, shoot Na⁺ concentration was increased by 27.4%, respectively, in *athkt1;1* (Fig. 4B, C). Net K⁺ uptake rate was decreased significantly in *athkt1;1* with 100 mM NaCl (Fig. 4D), while root K⁺ concentration in *athkt1;1* was higher than it in WT under 100 mM NaCl (Fig. 4E). The results showed that loss-of-function of AtHKT1;1 could change net Na⁺, K⁺ uptake rate and distribution in plant under low K⁺ plus severe salt stress.

Fig. 2. Effects of severe salt stress (100 mM NaCl) on net Na⁺ (A), K⁺ (D) uptake rate and Na⁺ (B, C), K⁺ (E, F) concentration of WT, *athkt1;1* and *atsos1* roots (B, E) and shoots (C, F) under 2.5 mM K⁺ condition. 7-week-old seedlings were exposed to modified Hoagland nutrient solutions supplemented with additional 100 mM NaCl for 3 d (the treatment without salt stress as Control). Values are means ± SE (n = 6) and bars indicate SE. Columns with different letters indicate significant difference at *P* < 0.05 (Duncan’s test).
Ion Uptake and Distribution in *atsos1* in Response to low K⁺ (0.01 mM) plus Severe Salt Stress

Compared with WT, net Na⁺ uptake rate and root Na⁺ concentration had no change in *atsos1* (Fig. 4A, B); shoot Na⁺ concentration was decreased by 12% in *atsos1* under 0.01 mM K⁺ with 100 mM NaCl (Fig. 4C). K⁺ uptake rate and root K⁺ concentration were decreased significantly in *atsos1* with 100 mM NaCl (Fig. 4D, E). Under 100 mM NaCl, compared with WT, shoot K⁺ concentration had no change in *atsos1* (Fig. 4F). The results showed that loss-of-function of AtSOS1 could change net K⁺ uptake rate and Na⁺, K⁺ distribution in plant under low K⁺ plus severe salt stress.

Discussion

Coordination of AtHKT1;1 and AtSOS1 Facilitates Na⁺ and K⁺ Homeostasis in *A. thaliana* under the Normal K⁺ Plus Mild or Severe Salt Stress

Under the normal K⁺ plus mild or severe salt stress, the reduction of net Na⁺ uptake rate was not found in *athkt1;1* (Fig. 1A, Fig. 2A). Berthomieu et al. (2003) used excised roots to examine Na⁺ uptake in *sas2-1* (loss-of-function of *AtHKT1;1*), which was 20% higher than WT, indicating that AtHKT1;1 was not involved in Na⁺ uptake by roots in *A. thaliana*. However, a contribution of AtHKT1;1 to Na⁺...
uptake could not be ruled out, because other transporters may take the place of AtHKT1;1 to mediate Na⁺ uptake in sas2-1. Therefore, here we only reached the conclusion that AtHKT1;1 was not the only way that controlled Na⁺ entry into A. thaliana roots under this condition. In the present work, compared with WT, Na⁺ distribution had changed in athkt1;1, which accumulated the more shoot Na⁺ concentration and the less root Na⁺ concentration (Fig. 1B, C, Fig. 2B, C), indicating that AtHKT1;1 mutation led Na⁺ excessive accumulation in shoots (Berthomieu et al. 2003; Davenport et al. 2007). It could be interpreted that AtHKT1;1 was a determinant that controlled Na⁺ unloading in xylem (Mäser et al. 2002; Sunarpi et al. 2005; Devenport et al. 2007). The Na⁺ unloading from xylem to xylem parenchyma cells could regulate Na⁺ distribution between shoots and roots (Sunarpi et al. 2005; Devenport et al. 2007). The course of Na⁺ unloading by AtHKT1;1 was broken, which would cause membrane hyperpolarisation of xylem parenchyma cells and the activity of K⁺ channel SKOR was obviously restrained, and then the process of K⁺ loading from xylem to shoot by SKOR was ended (Gaymard et al. 1998; Horie et al. 2009; Hauser and Horie 2010; Shabala et al. 2010). Based on this, the mutation of AtHKT1;1 gene could disrupt K⁺ homeostasis in A. thaliana, resulting in the less K⁺ concentration in shoots and the more K⁺ concentration in roots (Fig. 1E, F, Fig. 2E, F). The similar results were
found in many other studies (Davenport et al. 2007; Mäser et al. 2002; Sunarpi et al. 2005). In addition, because of the course of $K^+$ translocation toward to shoot was blocked, the process of $K^+$ uptake might be inhibited in feedback. In this work, the net $K^+$ uptake rate was decreased in $athkt1;1$ no matter under 25 or 100 mM NaCl (Fig. 1D, Fig. 2D). Therefore, this study implied that the mainly role of AtHKT1;1 was protection leaf from Na$^+$ stress by retrieval of Na$^+$ from the xylem, and mediated K$^+$ uptake and distribution indirectly under the normal K$^+$ plus mild salt stress. And this function was more significant under the normal K$^+$ plus severe salt stress, because it exhibited K$^+$ exclusion in $athkt1;1$ (Fig. 2D) and growing condition of $athkt1;1$ was restrain observably under 100 mM NaCl (not shown).

Contrary to AtHKT1;1, AtSOS1 mediates long-distance Na$^+$ transport from root to shoot and Na$^+$ exclusion from root to soil. Expression of AtSOS1 gene improved salt tolerance of yeast cells that devoided of endogenous Na$^+$ transporters, meanwhile, a role of AtSOS1 in Na$^+$ efflux in plant cells also was supported by ion content analysis of callus cultures generated from $atsos1$ mutant plants (Shi et al., 2002). In the present work, the results further supported this point: compared with WT, the increase of net Na$^+$ uptake rate and root Na$^+$ concentration were observed obviously in $atsos1$ under the normal K$^+$ plus mild or severe salt stress (Fig. 1A, B, Fig. 2A, B). In addition, Shi et al. (2002) also found that under 25 mM NaCl, $atsos1$ shoot accumulated the less Na$^+$ than did WT shoot, while under 100 mM NaCl, $atsos1$ accumulated the more Na$^+$ than did WT, presenting a model in which SOS1 functions in loading Na$^+$ into the xylem under mild salt stress, whereas under severe salt stress, it may function in retrieving Na$^+$ from the xylem stream. However, compared with WT, there was no reduction of root Na$^+$ concentration was observed in $atsos1$ under the normal K$^+$ plus mild or severe salt stress (Fig. 1C, Fig. 2C) in this study. This finding indicated that under the normal K$^+$ plus mild or severe salt stress, AtSOS1 appeared to limit loading Na$^+$ into the xylem sap because no reduction of root Na$^+$ concentration was found in $atsos1$, which could be attributable to defective Na$^+$ extrusion at the root epidermal cells in the $atsos1$ mutant, because the $atsos1$ root had more Na$^+$ than did WT root. Furthermore, under severe salt stress, the increase of shoot Na$^+$ concentration (Fig. 2C) was also ascribed to deficiency of Na$^+$ extrusion in $atsos1$roots, rather than the function of AtSOS1 in retrieving Na$^+$ from the xylem stream was restricted. Guo et al. (2012) proposed that SOS1 controlled Na$^+$ loading, and was not involved in Na$^+$ retrieving in xylem, and the direction of Na$^+$ flux was depended on the transport activities of SOS1 and HKT under different Na$^+$ concentration. In this work, compared with WT, K$^+$ distribution had no change generally in $atsos1$ under mild or severe salt stress (Fig. 1E, F, Fig. 2F), whereas net K$^+$ uptake rate decreased sharply, especially under severe salt stress, it even showed K$^+$ excretion in $atsos1$ (Fig. 1D, Fig. 2D). Wu et al. (1996) pointed out that $atsos1$ was unable to grow on substrate with low levels (below 1 mM) of K$^+$, and the uptake experiment using $^{86}$Rb$^+$ showed that $atsos1$ was defective in high-affinity K$^+$ uptake. The expression of K$^+$ transporter gene $TPK1$ showed a larger decrease in $atsos1$ compared to that in WT in $A. thaliana$ under salt stress, as well as the activity of its protein (Oh et al., 2010). According to above physiological and molecular results, we could reasonably explain the conclusion proposed by Qi and Spalding (2004) that extrusion of Na$^+$ from cytoplasm by AtSOS1 was necessary for protecting K$^+$ permeability of the membrane from inhibition by Na$^+$ in $A. thaliana$. As a consequence, this study implied that the mainly role of AtSOS1 in protecting root from Na$^+$ stress by Na$^+$ excretion, and regulating K$^+$ uptake by facilitating the activity of K$^+$ transporters under the normal K$^+$ plus mild or severe salt stress.

Coordination of AtHKT1;1 and AtSOS1 Facilitates Na$^+$ and K$^+$ Homeostasis in $A. thaliana$ under the Low K$^+$ Plus Mild or Severe Salt Stress

Many researches had pointed out an interesting fail-safe mechanism of using Na$^+$ as a nutritional substitute for K$^+$ under K$^+$ deficiency (Flowers and Läuchli 1983; Rodríguez-Navarro and Rubio 2006). In this work, compared with the normal K$^+$ plus mild or severe salt stress, net Na$^+$ uptake rate were increased sharply in WT, $athkt1;1$ and $atsos1$ under low K$^+$ plus mild or severe salt stress (e.g. compared Fig.1A with Fig.3A, net Na$^+$ uptake rate was about 60 nmol/g. RFW/min in WT under 2.5 mM K$^+$ plus 25 mM NaCl, while it was increased to about 250 nmol/g. RFW/min in WT under 0.01 mM K$^+$ plus 25 mM NaCl), suggesting that Na$^+$ as the osmotic adjustment substance was absorbed largely to take place of K$^+$ for mediating cell turgor and osmotic equilibrium. Compared with WT, net Na$^+$ uptake rate decreased in $athkt1;1$ under low K$^+$ plus severe salt stress (Fig. 4A), implying that AtHKT1;1 mediated Na$^+$ uptake firstly in $A. thaliana$ roots. However, once Na$^+$ accumulation in the shoot exceeded the capacity of the $A. thaliana$ mesophyll cells to compartmentalize ions, then AtHKT1;1 would function to restrict the rapid accumulation of Na$^+$ in the shoot by retrieve Na$^+$ from the xylem. In this condition, loss-of-function of AtHKT1;1 disturbed redistribution of Na$^+$ in plant, which led to the more shoot Na$^+$ concentration and the less root Na$^+$ concentration (Fig. 4B, C), and finally caused inhibition of growth, especially, the restriction of shoot growth in $athkt1;1$ (not shown). These findings showed that AtHKT1;1 was the determiner of salt tolerance by mediating both Na$^+$ uptake in the root and unloading Na$^+$ in the xylem under low K$^+$ plus severe salt stress.

In this study, under the low K$^+$ plus mild or severe salt
stress, net Na\(^+\) uptake rate had no difference between atsos1 and WT (Fig. 3A, Fig. 4A), but shoot Na\(^+\) concentration of atsos1 decreased significantly (Fig. 3C, Fig. 4C), suggesting that the important role of AtSOS1 was loading Na\(^+\) into xylem under the low K\(^+\) condition. It was known that plants would absorb a part of Na\(^+\) substitute for K\(^+\) to perform function in keeping the balance of osmotic potential and cell turgor under K\(^+\) deficiency condition (Flowers and Lüchel 1983; Rodriguez-Navarro and Rubio 2006). Horie et al. (2007) demonstrated that OsHKT2;1 mediated beneficial Na\(^+\) uptake to alleviate hurt of K\(^+\) deficiency in rice roots. In A. thaliana, AtHKT1;1 firstly mediated Na\(^+\) uptake under the low K\(^+\) plus severe salt stress, and then AtSOS1 fulfilled the function to load Na\(^+\) into the xylem for controlling delivery to the shoot and storage in leaf mesophyll cells. Nevertheless, Na\(^+\) quick uptake and accumulation would cause hurt to plant. At this moment, AtHKT1;1 had to retrieve Na\(^+\) from the xylem, whereas AtSOS1 restricted Na\(^+\) accumulation in the root. This process would not only help to limit the rapid accumulation of Na\(^+\) in the shoot, but also alleviate double damage of the low K\(^+\) and high Na\(^+\).

Taken together, our results propose the model in which coordination of AtHKT1;1 and AtSOS1 facilitates Na\(^+\) and K\(^+\) homeostasis in A. thaliana under salt stress. Under the normal K\(^+\) plus mild or severe salt stress, AtHKT1;1 and AtSOS1 is leading in unloading Na\(^+\) and Na\(^+\) exclusion, respectively, to keep the low Na\(^+\) level in plants. Under the low K\(^+\) plus mild or severe salt stress, AtHKT1;1 is a determinant on Na\(^+\) uptake and AtSOS1 is loading Na\(^+\) into the xylem to insure Na\(^+\) instead of K\(^+\) to keep the osmotic equilibrium and cell turgor to compensate for K\(^+\) deficiency.

Materials and Methods

Plant Materials and Growth Conditions

*Arabidopsis thaliana* materials used were EMS-mutagenized sos1 (Shi et al. 2002), T-DNA mutagenized knockout mutant hkt1;1 and wild type (WT) control plants (Rus et al. 2004), which were kindly provided by M. Tester. Seeds of A. thaliana WT, hkt1;1 mutant (atht1;1) and sos1 mutant (atssol) were sterilized for 3 min with 75% (v/v) ethanol and 5% (v/v) bleacher, respectively, and rinsed 5 times with distilled water, soaked in distilled water at 4°C for 2 d. Then seeds were sown on substrate containing 1.2% (w/v) agar and 0.5% (v/v) sucrose dissolved in modified Hoagland nutrient solution (2 mM KNO\(_3\), 0.5 mM KHPO\(_4\), 0.5 mM MgSO\(_4\)-7H\(_2\)O, 0.25 mM Ca(NO\(_3\))\(_2\)-4H\(_2\)O, 1.25 mM CaCl\(_2\)-2H\(_2\)O, 0.06 mM Fe-citrate, 50 μM H\(_2\)BO\(_3\), 10 μM MnCl\(_2\)-4H\(_2\)O, 1.6 μM ZnSO\(_4\)-7H\(_2\)O, 0.6 μM CuSO\(_4\)-5H\(_2\)O, 0.05 mM Na\(_2\)MoO\(_4\), 2H\(_2\)O) for sterile culture. After three weeks, all the seedlings were washed three times with distilled water, soaked in distilled water at 4°C for 2 d. Then seeds were sown on substrate containing 1.2% (w/v) agar and 0.5% (v/v) sucrose dissolved in modified Hoagland nutrient solution (2 mM KNO\(_3\), 0.5 mM KHPO\(_4\), 0.5 mM MgSO\(_4\)-7H\(_2\)O, 0.25 mM Ca(NO\(_3\))\(_2\)-4H\(_2\)O, 1.25 mM CaCl\(_2\)-2H\(_2\)O, 0.06 mM Fe-citrate, 50 μM H\(_2\)BO\(_3\), 10 μM MnCl\(_2\)-4H\(_2\)O, 1.6 μM ZnSO\(_4\)-7H\(_2\)O, 0.6 μM CuSO\(_4\)-5H\(_2\)O, 0.05 mM Na\(_2\)MoO\(_4\), 2H\(_2\)O) for sterile culture. After three weeks, all the seedlings were washed three times with distilled water, soaked in distilled water at 4°C for 2 d.

**Treatments**

Six-week-old plants were used for the following treatments, respectively. (i) Plants were grown at the modified Hoagland nutrient solution for 7 d, then they were treated with modified Hoagland nutrient solutions supplemented with additional 25 and 100 mM NaCl for 4 d and 3 d, respectively. (ii) Plants were subjected to 0.01 mM K\(^+\) for 7 d (the modified Hoagland nutrient solution deprived of KNO\(_3\)) and K\(_2\)HPO\(_4\) 2 mM KNO\(_3\) was substituted by 2 mM H\(_2\)PO\(_4\), 0.5 mM KH\(_2\)PO\(_4\) was substituted by 0.5 mM H\(_2\)PO\(_4\), and 0.01 mM K\(^+\) was provided by 0.01 mM KCl, pH was adjusted to 5.7 by 1 M Tris), then they were treated with above modified Hoagland nutrient solutions containing 0.01 mM K\(^+\) supplemented with additional 25 and 100 mM NaCl for 4 d and 3 d, respectively. Treatment solutions were changed every d to maintain a constant ion concentration.

**Na\(^+\) and K\(^+\) Concentrations Determination**

At the end of the treatments, plant roots were washed three times for a total of 9 min in deionized water containing 20 mM CaCl\(_2\) to exchange cell wall-bound Na\(^+\) and the shoots rinsed in deionized water to remove surface salt. Roots and shoots were separated and blotted; FW was determined immediately and samples were dried in an oven at 70°C for 3 d to obtain DW. Na\(^+\) and K\(^+\) were extracted from dried plant tissue in 100 mM acetic acid at 95°C for 2 h and ion analysis was performed using a flame spectrophotometer (2655-00, ColeParmer Instrument Co., USA) (Flowers and Hajibagheri 2001; Gong et al. 2004).

**Net Na\(^+\) and K\(^+\) Uptake Rate Measurements Calculation**

Net uptake rate of Na\(^+\) and K\(^+\) were calculated according to the following equation described by Wang et al. (2007, 2009): Using the control data as an example, as (Na\(^+\) content in whole plant before NaCl treatments)/(fresh weight of control root × treatment time) and expressed as nmol gRFW\(^{-1}\) min\(^{-1}\).

**Statistical Analysis**

Results of ion concentration and net Na\(^+\) and K\(^+\) uptake rate are presented as means with SEs (n = 6) and data analysis was performed by ANOVA using SPSS statistical software (Ver.13.0, SPSS Inc., Chicago, IL, USA). Duncan’s multiple range test was used to detect a difference between means at a significance level of P < 0.05.

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Author Contributions

QW and SMW designed research; QW and CG performed research; QW, CG and SMW analyzed data and wrote the paper. All the authors agreed on the contents of the paper and post no conflicting interest.
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