Journal of Plant Physiology 169 (2012) 255–261

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

SOS1 gene overexpression increased salt tolerance in transgenic tobacco by maintaining a higher K^+/Na^+ ratio

Yuesen Yue [1], Mingcai Zhang [1], Jiachang Zhang, Liusheng Duan, Zhaohu Li *

State Key Laboratory of Plant Physiology and Biochemistry, Department of Agronomy, Centre of Crop Chemical Control, College of Agronomy and Biotechnology, China Agricultural University, 2#, Yuanmingyuan Xilu, Haidian District, Beijing 100193, PR China

ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 6 October 2011
Accepted 13 October 2011

Keywords:
K^+
Na^+
Transgenic
Tobacco
AtSOS1 overexpression
Salt tolerance

ABSTRACT

Crop productivity is greatly affected by soil salinity, so improvement in salinity tolerance of crops is a major objective of many studies. We overexpressed the *Arabidopsis thaliana SOS1* gene, which encodes a plasma membrane Na^+/H^+ antiporter, in tobacco (*Nicotiana tabacum* cv. *Xanthi*-nc). Compared with non-transgenic plants, seeds from transgenic tobacco had better germination under 120 mM (mmol L^{-1}) NaCl stress; chlorophyll loss in the transgenic seedlings treated with 360 mM NaCl was less; transgenic tobacco showed superior growth after irrigation with NaCl solutions; and transgenic seedlings with 150 mM NaCl stress accumulated less Na^+ and more K^+. In addition, roots of *SOS1*-overexpressing seedlings lost less K^+ instantaneously in response to 50 mM NaCl than control plants. These results showed that the *A. thaliana SOS1* gene potentially can improve the salt tolerance of other plant species.

Introduction

Soil salinity is a major abiotic stress of crop productivity because it decreases crop yields and limits expansion of agricultural land. Mineral nutrients are required to maintain crop growth and development. However, growth of crops is restrained by excessive soluble ions, such as sodium and chloride.

Salt stress involves cellular osmotic stress, ion toxicity and their consequent secondary stresses (nutritional deficiency and oxidative stress) (Zhu, 2001). Toxic effects of Na^+ include inhibition of enzyme activity (Hasegawa et al., 2000) and disruption of K^+ nutrient acquisition (Zhu, 2003). Thus, it is essential for crops that cells must be able to transport or compartmentalize Na^+ to maintain non-toxic levels of cytosolic Na^+ (Manabe et al., 2008).

Plants are thought to prevent excessive Na^+ accumulation in the symplast by restricting influx, increasing efflux, and increasing vacuolar sequestration of Na^+ (Tester and Davenport, 2003). Na^+/H^+ antiporters located in both the plasma and vacuolar membranes are ubiquitous membrane proteins that catalyze the exchange of Na^+ for H^+ across membranes; they play major roles in removing Na^+ from the cytosol or compartmentalizing it in vacuoles for maintenance of a low Na^+ concentration (Apse et al., 1999; Shi et al., 2002), energized by electrochemical H^+ gradients generated by H^+-pumps in the plasma membrane, i.e., H^+-ATPase, and the tonoplast, i.e., H^+-ATPase and H^+-PP_iase (Wang et al., 2007). Manipulating genes responsible for Na^+/H^+ antiporters to maintain ionic homeostasis in plants is an important strategy to deal with salt stress.

NHX1, a vacuolar Na^+/H^+ antiporter, was isolated first from *Arabidopsis* (Gaxiola et al., 1999). Overexpression of *NHX1* improved the salt tolerance of *Arabidopsis* (Apse et al., 1999), *Brassica napus* (Zhang et al., 2001), tomato (Zhang and Blumwald, 2001), maize (Yin et al., 2004), wheat (Xue et al., 2004) and rice (Chen et al., 2007a). Consequently, a primary strategy for improving plant salt tolerance is through overexpression of genes that are either induced by stress and/or required for normal levels of tolerance (Yang et al., 2009).

The salt-overly-sensitive (SOS) signal-transduction pathway is important for ion homeostasis and salt tolerance in plants (Hasegawa et al., 2000; Zhu, 2003). The SOS pathway in *Arabidopsis* is defined by three main protein components, *SOS1*, *SOS2*, and *SOS3*. Salt stress elicits a transient increase of Ca^{2+} that is sensed by *SOS3*, a myristoylated calcium-binding protein, which interacts with and activates *SOS2*, a serine/threonine protein kinase. The *SOS2*/*SOS3* kinase complex phosphorylates and activates the *SOS1* protein (Qiu et al., 2002; Zhu, 2003).

The *SOS1* gene was identified initially as a genetic locus required for salt tolerance in *Arabidopsis* (Wu et al., 1996). A few years later, the *AtSOS1* gene was cloned, and further studies suggested that *SOS1* in *Arabidopsis* is a plasma membrane Na^+/H^+ antiporter (Shi

Abbreviations: WT, wild type; MS, Murashige-Skoog; SIET, scanning ion-selective electrode technique.

* Corresponding author. Tel.: +86 10 62733427; fax: +86 10 62733427.
E-mail address: lizhaohu@cau.edu.cn (Z. Li).

[1] These authors contributed equally to this work.

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doi:10.1016/j.jplph.2011.10.007

et al., 2000). The *SOS1* gene in *Arabidopsis* is most strongly expressed in epidermal cells at the root tip and in parenchyma cells surrounding the vascular tissues (Qiu et al., 2002; Shi et al., 2002). *SOS1* in *Arabidopsis* under severe salt stress is assumed to mediate Na^+ efflux at the root epidermis and long-distance transport from roots to shoots while protecting individual cells from Na^+ toxicity.

Recently, a series of *SOS1*-like Na^+/H^+ antiporter coding genes have been identified and cloned from many other plants, including *Populus euphratica* (Wu et al., 2007), rice (Martínez-Atienza et al., 2007), wheat (Xu et al., 2008), tomato (Olias et al., 2009), *Thellungiella salsuginea* (Oh et al., 2009), and the moss *Physcomitrella patens* (Fraile-Escanciano et al., 2010). *SOS1* is most commonly considered to drive the expulsion of sodium from plants.

Overexpression of *AtSOS1* increased salt tolerance of transgenic *Arabidopsis* by limiting Na^+ accumulation in the xylem and stem (Shi et al., 2003). However, Yang et al. (2009) reported that salt tolerance of transgenic *Arabidopsis* overexpressing *SOS1* under salt stress conditions was improved, but accumulation of Na^+ and K^+ in transgenic plants did not change much compared to the control plants. For our research, the *SOS1* gene under control of a strong constitutive promoter was transferred into the tobacco genome by *Agrobacterium*-mediated transformation. Salt tolerance of transgenic plants and accumulation of Na^+ and K^+ in transgenic plants with salt treatment were evaluated.

Materials and methods

Constructs and plant transformation

SOS1 cDNA was cloned as an *Xba*I–*Kpn*I fragment downstream of a super promoter and consisted of three copies of the octopine synthase enhancer in front of the manopine synthase promoter in the pCAMBIA 1300 binary vector containing a hygromycin-resistant selectable marker (Yang et al., 2009). The recombinant plasmid was introduced into *Agrobacterium tumefaciens* strain *EHA105*, and transformation of tobacco (*Nicotiana tabacum* cv. *Xanthi*-nc) by *A. tumefaciens* was performed as described by Shikanai et al. (1998).

The infected leaf sections were cultivated on Murashige-Skoog (MS) co-culture medium (MS medium supplemented with 0.2 mg L^{-1} naphthalene acetic acid and 3 mg L^{-1} 6-benzylaminopurine, pH 5.8) for 2 d. Then, the explants were transferred to a selection medium (MS co-culture medium supplemented with 20 mg L^{-1} hygromycin and 500 mg L^{-1} cefotaxime) for 4–5 weeks to induce shoot development in a greenhouse at 22 °C with light intensity of 50 μmol m^{-2} s^{-1} and 70% relative humidity under a long-day photoperiod (16 h light, 8 h dark). The well-grown shoots (4–6 cm long) were excised carefully and transferred onto rooting medium (MS medium supplemented with 0.05 mg L^{-1} indole-3-butyric acid and 250 mg L^{-1} cefotaxime) and 47 independent transformed lines were raised.

No differences were observed in growth or morphology between wild-type (WT) plants and each transgenic line. Transgenic plants harboring *SOS1* were screened on MS agar medium containing 50 mg L^{-1} hygromycin. The presence and integrity of the transgene were further confirmed by PCR amplification using primers specific for *SOS1*. We selected two dominant lines (SOS1-12 and SOS1-20) that exhibited the greatest root bending in MS medium containing 150 mM NaCl, and harvested homozygous T_3 progeny for further studies.

RNA hybridization

T_3 seeds of transgenic tobacco were sown on MS agar medium containing hygromycin (50 mg L^{-1}), and seeds of the WT were sown on MS agar medium without hygromycin. Seedlings 3-week-old were transferred to MS solution either without NaCl or containing 150 mM NaCl and treated for 12 h; the control was treated with MS solution only. Total RNA isolation and RNA gel blot analysis were performed as described by Chen et al. (2005). The partial *SOS1* fragment (657 bp) was amplified by PCR with the forward 5′-CTGACTTACTCGCACTCA-3′ and reverse 5′-ACGCAGCAGAAATGTAGC-3′ primers as probes.

Analysis of salt-stress tolerance

A germination test was initiated with seeds sown on MS agar medium containing 120 mM NaCl. Seedlings 6-d-old were transferred to 0.5-strength MS nutrient solution containing 360 mM NaCl. The control seedlings were treated with 0.5-strength MS solution without NaCl. Seedlings were photographed on day 10.

For another salt-tolerance experiment, T_3 seeds of transgenic tobacco were grown on MS agar medium containing hygromycin (50 mg L^{-1}) for 1 week, and WT seeds were sown on MS agar medium lacking hygromycin. The hygromycin-resistant and WT seedlings were transferred to MS agar medium for another week. Then the seedlings were transferred to 15-by-15-cm pots containing roseite and perlite (1:1, v/v) for 4 weeks. Each pot had three transgenic tobacco and three WT plants, and there were five replicates. The experiment was conducted three times.

All transgenic tobacco and WT plants were cultured in a growth chamber under a long-day cycle (14 h light, 10 h dark) with 450 μmol m^{-2} s^{-1} light intensity, 30% relative humidity, and day/night temperatures of 26/20 °C. The plants were irrigated with a constant supply of nutrient solution (2.93 mM KNO_3, 0.73 mM KH_2PO_3, 1.6 mM $Mg_2SO_4{\cdot}7H_2O$, 0.75 mM $(NH_4)_2SO_4$, 2.86 mM $Ca(NO_3)_2{\cdot}4H_2O$, 82 μM NaFeEDTA, 1.05 μM H_3BO_3, 0.78 μM KC1, 0.27 μM $MnSO_4{\cdot}2H_2O$, 20 nM $ZnSO_4$, 4.7 nM $(NH_4)_6MO_7O_{24}{\cdot}4H_2O$, and 11.25 nM $CuSO_4{\cdot}5H_2O$) as needed.

After growth in pots for 4 weeks, the plants were fully irrigated with NaCl solutions of increasing concentration from 50, 100, 150, 200, 250 to 300 mM for 4 d at each concentration, and thus plants were treated with NaCl solution for 24 d total. After the treatment, the plants were grown for another 6 weeks and watered as needed. Then, they were irrigated again with regular nutrient solution as described above. After 3 weeks growth, photosynthesis rate and chlorophyll content were measured, and the height and fresh weight of plants were recorded.

Measurement of photosynthesis rate and chlorophyll content

Photosynthesis rate was measured with a portable photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA). Leaves were placed in 6 cm^2 chambers, and the photon flux density was set at 1000 μmol m^{-2} s^{-1} photosynthetically active radiation. Chlorophyll was extracted with 80% acetone and quantified using ultraviolet spectrophotometry.

Measurement of Na^+ and K^+ contents

To measure Na^+ and K^+ contents in plant tissues, 6-d-old tobacco seedlings were transferred to MS medium either without NaCl or with MS medium containing 150 mM NaCl and treated for 6 d in the greenhouse at 22 °C. After salt treatment, the plants were harvested, rinsed with three quick-dips in deionized water and blotted with filter paper. Then, they were dried at 85 °C for 24 h, ashed in a muffle furnace at 575 °C for 5 h, and dissolved in 0.1 M HCl. The Na^+ and K^+ contents of the samples were measured by atomic absorption spectrophotometry (Xu et al., 2006). The experiment was conducted three times with three replicates in each experiment.

Measurement of K^+ flux

Seeds of transgenic tobacco and WT plants were germinated on MS medium at 22 °C with light intensity of 50 μmol m^{-2} s^{-1}. Seedlings 6-d-old were used for net K^+ flux measurements. The K^+ flux was measured by non-invasive scanning ion-selective electrode technique (SIET) as described previously (Sun et al., 2010) at the Xuyue Science and Technology Co., Beijing, China. Briefly, the microelectrode was constructed from a silanized borosilicate glass capillary front-filled with an ion-selective cocktail (K^+: Fluka 60398, Fluka Chemie GmbH, Buchs, Switzerland). An Ag/AgCl wire electrode holder was inserted into the back of the electrode to make electrical contact with the electrolyte solution.

The ion-selective electrode was calibrated in a set of standard solutions (K^+: 0.1, 0.5, 1.0 mM, K^+ was 0.5 mM in the measuring solution) before and after use. Root segments were incubated in 2 mL of measuring solution (0.2 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.5 mM NaCl, 0.3 mM MES, 0.2 mM Na_2SO_4, pH 6.0, 5.5 mM mannitol; −0.02 MPa). The measuring site, in which a vigorous flux of K^+ was usually observed, was 500 μm from the root apex. Steady-state ion fluxes were measured for 5–20 min, then the test treatment was applied and the transient ion flux kinetics was measured for a further 10–20 min. The data recorded during the first minute were discarded because of diffusion effects from addition of the stock. The flux data were recorded with MageFlux developed by Yue Xu (http://xuyue.net/mageflux; Sun et al., 2010).

Statistical analysis

Data were analyzed using one-way analysis of variance and the means were separated using Duncan's multiple range tests at the 5% level of significance.

Results

RNA gel blot analysis of SOS1 expression in transgenic tobacco plants

The expression levels of *SOS1* transcripts were assayed in 3-week-old seedlings. RNA gel blot analysis showed that transcripts were highly expressed in SOS1-12 and SOS1-20 transgenic lines with or without salt treatment, but not in the WT plants (Fig. 1). And the result also showed that the expression of SOS1-12 became lower after salt stress.

Salt tolerance of transgenic plants

Salt tolerance during seed germination and early seedling development was determined for homozygous SOS1-12 and SOS1-20 transgenic lines germinated on MS medium containing 0 or 120 mM NaCl. On MS medium without salt, the transgenic lines and the control (WT) were similar in germination and early growth (Fig. 2A). However, on MS medium with salt, most seeds of the transgenic lines could germinate and grow, whereas the control seeds barely germinated.

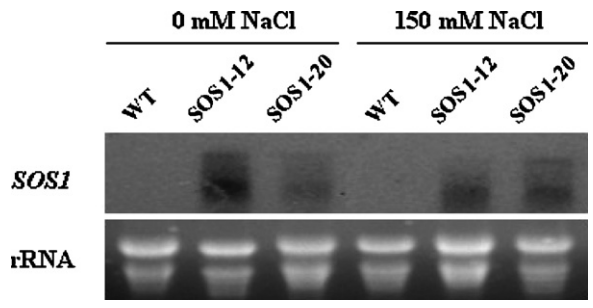

Fig. 1. RNA gel blot analysis of two *SOS1*-overexpressing transgenic tobacco lines (SOS1-12 and SOS1-20) and wild-type (WT) plants in the presence or absence of NaCl. Seedlings 3-week-old were transferred to MS medium without NaCl or containing 150 mM NaCl for 12 h. Total RNAs were extracted and used for RNA gel blot analysis.

In another salt-tolerance assay, 6-d-old seedlings of transgenic and WT plants were transferred to 0.5-strength MS liquid medium lacking NaCl or supplemented with 360 mM NaCl for 10 d. Salt treatment quickly restrained the growth of transgenic and WT seedlings and decreased chlorophyll contents, but chlorophyll loss was less in transgenic plants than WT plants (Fig. 2B).

In the third salt-tolerance assay, transgenic and WT seedlings were grown in the same pots for 4 weeks, and their growth were similar (Fig. 3A). Then the young plants were continuously treated with six increasingly higher NaCl concentrations over 24 d. During the first days of NaCl treatment, both transgenic and control seedlings grew normally, but gradually their growth rates became slower and no new expanded leaves developed after 3 weeks. After 24 d of irrigating with salt solution, the growth of all plants was inhibited (Fig. 3B). However, after rewatering with nutrient solution, the transgenic plants almost recovered normal development, whereas the WT control plants were damaged too severely to recover and some plants died (Fig. 3C).

After rewatering with nutrient solution for 3 weeks, photosynthesis rate and chlorophyll content of plant leaves were taken and plants were harvested. The biomass of transgenic plants was higher than that of WT plants. The height of transgenic plants was about 1.5 times higher and fresh weight was about 0.57 times higher than those of the WT plants (Fig. 4). In addition, the chlorophyll content of transgenic plants was 0.70 times higher than that of the WT plants, and the photosynthesis rate 1.4 times.

Na^+ and K^+ accumulation and K^+ flux

To determine if overexpression of *SOS1* reduces Na^+ accumulation in tobacco, the Na^+ content of tobacco seedlings in response to 150 mM NaCl treatment for 6 d was examined. Prior to salt treatment (0 d), Na^+ and K^+ content were similar between transgenic and WT plants (Fig. 5A and B). However, with 6-d salt treatment transgenic seedlings accumulated less Na^+ and more K^+ than WT plants. Also, the K^+/Na^+ homeostasis ratios in transgenic plants overexpressing *SOS1* were 0.45 times higher than that of the control plants under salt stress conditions (Fig. 5C).

Further analyses of K^+ flux using SIET yielded results consistent with the above findings. NaCl treatment caused a K^+ efflux in tobacco roots both for WT and transgenic plants (Fig. 6A). Stress with 50 mM NaCl induced responses from SOS-12 and SOS-20 roots that were qualitatively similar. However, roots of *SOS1*-overexpressing plants lost less K^+ instantaneously than did WT plants. NaCl treatment increased the mean rate of K^+ flux for both WT and transgenic plants (Fig. 6B). However, the mean rates of K^+ flux for the two transgenic lines were smaller than WT plants without or with NaCl treatments.

Discussion

SOS1 driven by a super promoter

Overexpression of *SOS1* in transgenic *Arabidopsis* was driven by the constitutive CaMV 35S promoter, and salt treatment could increase the levels of *SOS1* transcript (Shi et al., 2003). In this study, *SOS1* was driven by a super promoter, and high levels of *SOS1* transcripts were detected in the transgenic plants with or without salt treatment, but not in WT controls (Fig. 1). In response to

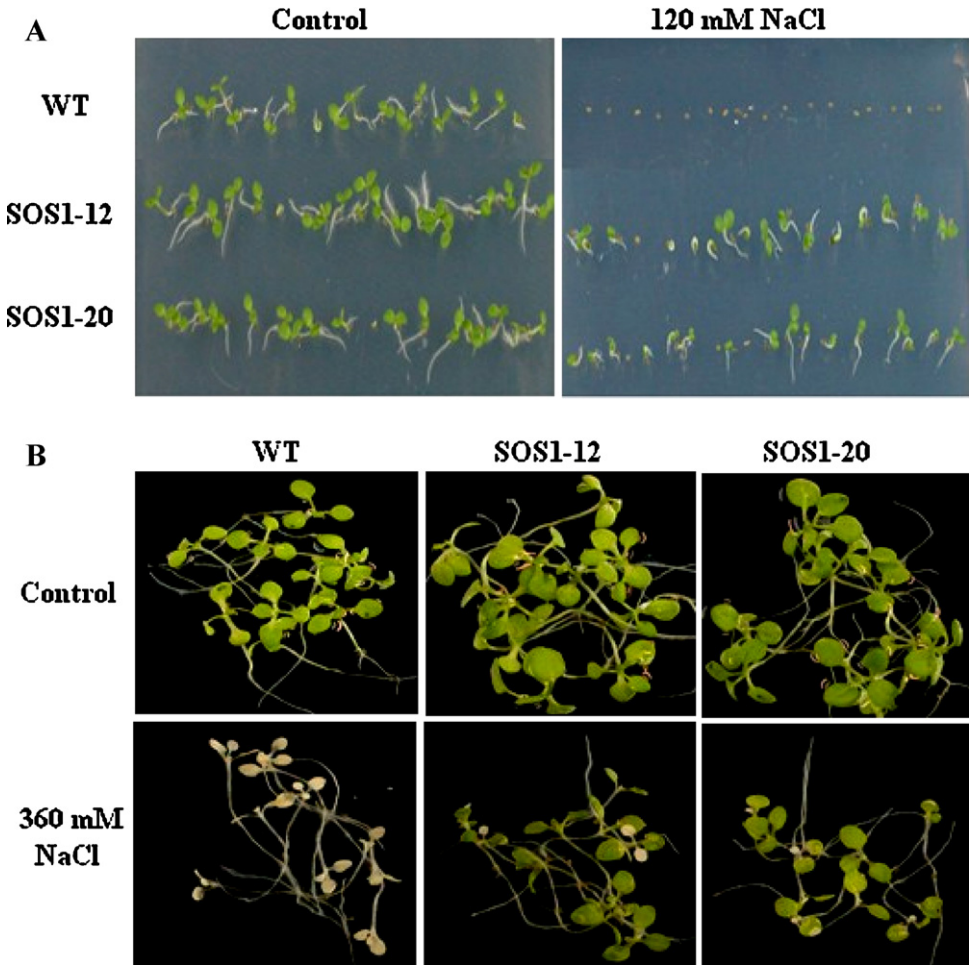

Fig. 2. Enhanced salt tolerance of two *SOS1*-overexpressing tobacco lines (SOS1-12 and SOS1-20) and wild-type (WT) plants during seed germination and early seedling development. (A) Seeds were germinated on MS medium and MS medium containing 120 mM NaCl and grown for 8 d. (B) Salt-tolerance assay of plants grown in 0.5-strength MS nutrient solution with or without 360 mM NaCl for 10 d.

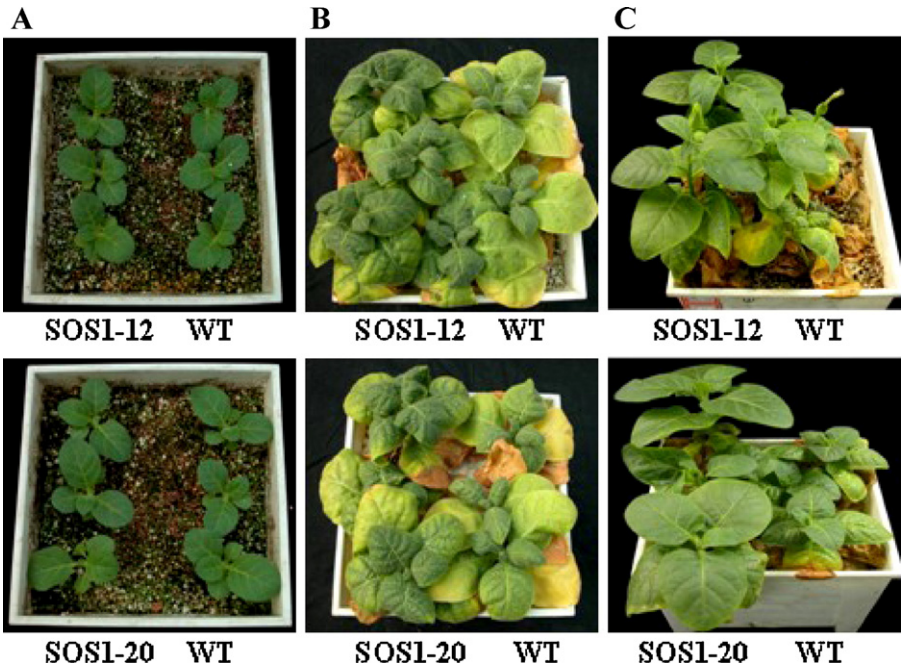

Fig. 3. Enhanced salt tolerance of *SOS1*-overexpressing tobacco seedlings. For two transgenic plants (SOS1-12 and SOS1-20) and wild-type (WT) plants: (A) Grown in the same pots for 4 weeks without salt treatment. (B) After treatment for 24 d with six increasingly higher concentrations of NaCl (50, 100, 150, 200, 250 and 300 mM for 4 d each), followed by 6 weeks of growth without NaCl. (C) Irrigated with normal nutrient solution to restore normal growth after Step B followed by 3 weeks of growth.

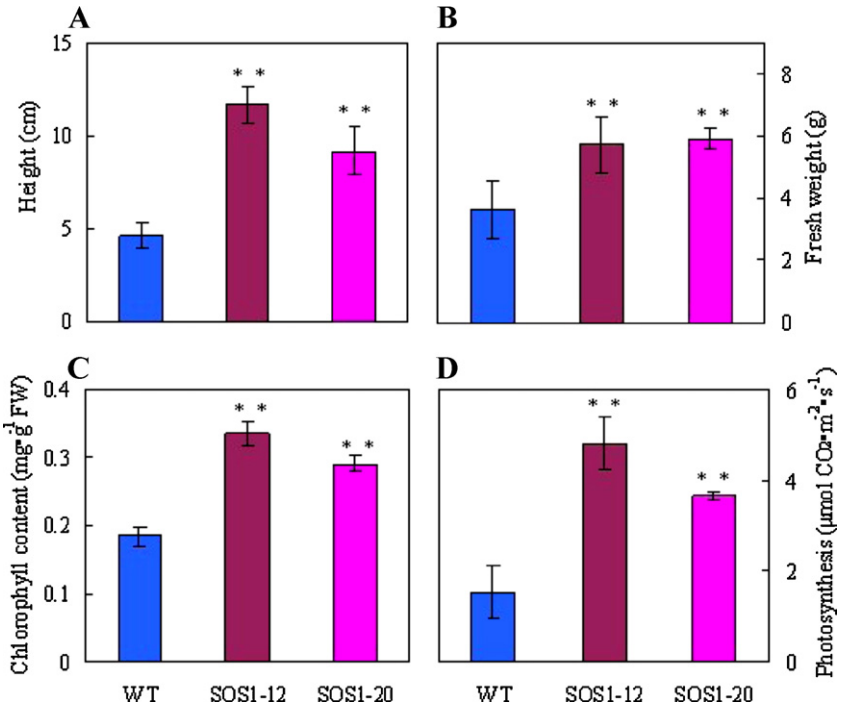

Fig. 4. Plant height, fresh weight, leaf chlorophyll content and photosynthesis rate of two *SOS1*-overexpressing tobacco lines (SOS1-12 and SOS1-20) and wild-type (WT) plants grown in pots for 9 weeks after the salt treatment. FW, fresh weight. Values are means ± SE; $^{*}P < 0.05$, $^{**}P < 0.01$.

salt treatment, a significantly elevated level of *SOS1* was observed in SOS1-20 plants, which was consistent with the results described earlier (Yang et al., 2009). The reduction of the RNA transcription of SOS1-12 plants under the salt treatment could be explained by RNA threshold model (Gallie, 1998). SOS1-12 plants showed a high level transcription of *SOS1* in the absence of salt. Salt further induced expression level of *SOS1*, activated a sequence-specific RNA degradation mechanism and resulted lower expression of SOS1-12 (Baykal and Zhang, 2010). Furthermore, tobacco plants may also have some other transporter(s) which is (are) functionally similar to *SOS1*. However, further studies are needed to verify this.

Increased salt tolerance of transgenic plants

Overexpression of *SOS1* improved the salt tolerance of transgenic *Arabidopsis*, and transgenic *Arabidopsis* showed enhanced early seedling development and increased root growth under salt stress compared to control plants (Shi et al., 2003). In our study, transgenic tobacco overexpressing *SOS1* had improved salt tolerance in three salt stress assays. Germination of seeds under salt stress was markedly increased for transgenic tobacco when compared to control seeds (Fig. 2A). Also, transgenic seedlings, when supplemented with 0.5-strength MS liquid medium containing 360 mM NaCl (Fig. 2B) or treated with six increasing NaCl concentrations (Fig. 3) showed superior growth compared with WT plants.

Salinity stress caused inhibition of growth and development, reduced rates of photosynthesis, respiration, and protein synthesis, and disturbed nucleic acid metabolism (Hasegawa et al., 2000). Also, salt induced a decrease in total chlorophyll and changes in the chlorophyll *a*/*b* ratio (Singh and Dubey, 1995). Our *SOS1*-overexpressing transgenic tobacco showed higher chlorophyll content than control plants in response to increasing NaCl concentration (Fig. 4C). Thus, transgenic plants had higher photosynthesis rates leading to greater production of assimilation products (Fig. 4D).

Redondo-Gómez et al. (2007) reported that salt stress induced a decline in stomatal conductance that led to a reduction in intercellular CO_2 concentration, which would limit carboxylation and decrease the photosynthetic assimilation rate. Increasing salinity for tomato was accompanied by significant reductions in shoot weight, plant height, and number of leaves per plant, root length, and root surface area per plant (Mohammad et al., 1998). Also, increasing salinity for cotton resulted in a significant decrease in root, shoot, and leaf growth biomass and an increase in the root/shoot ratio (Meloni et al., 2001). Similar results were obtained in our study, where transgenic plants had higher biomass than wild plants under salt stress (Fig. 4A and B).

Na^+, K^+ accumulation and K^+ flux

Under severe salt stress, *SOS1* functions were to limit the transportation of Na^+ thereby limiting accumulation of Na^+ in *Arabidopsis* (Shi et al., 2002). And that was further verified in transgenic *Arabidopsis* overexpression of *AtSOS1* at the cellular level or whole plant level (Shi et al., 2003). Recent studies also proved that *SOS1* play an important role in mediating cellular Na^+ efflux through gene silencing in tomato (Olias et al., 2009), *Thellungiella salsuginea* (Oh et al., 2009), and *Physcomitrella patens* (Fraile-Escanciano et al., 2010). Olias et al. (2009) further revealed that the critical importance of *SOS1* in preventing Na^+ from reaching the photosynthetic tissues by partitioning Na^+ in plant organs and retaining Na^+ in the stems of tomato. Similar to those results, we found that *SOS1*-overexpressing transgenic tobacco accumulated less Na^+ than WT plants (Fig. 5A) under salt stress (150 mM NaCl). Based on the model of Na^+ loading into or retrieval from the xylem in *Arabidopsis* proposed by Shi et al. (2002), *SOS1* might function in loading Na^+ into the xylem via which Na^+ is transported from root to shoot under mild salt stress (25 mM NaCl), but it plays a critical role in retrieving Na^+ from the xylem to prevent overaccumulation in the xylem transpirational stream under severe salt stress (100 mM NaCl). And the role of *SOS1* controlling long-distance Na^+ transport from xylem stream was

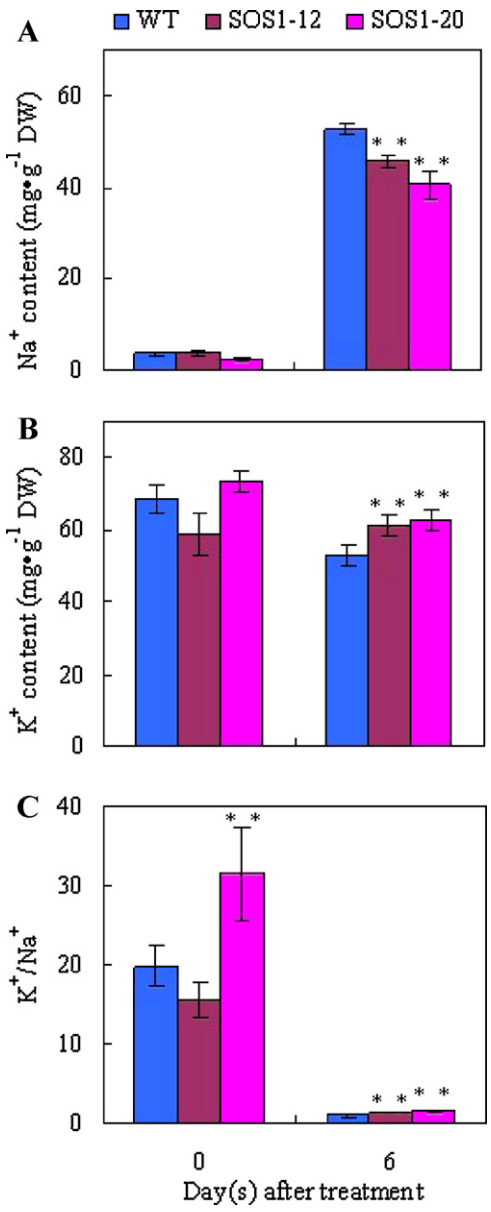

Fig. 5. Accumulation of Na^+ (A) and K^+ (B) in two *SOS1*-overexpressing tobacco lines (SOS1-12 and SOS1-20) and wild-type (WT) plants after treatment with 150 mM NaCl for 6 d. (C) K^+/Na^+ ratio. DW, dry weight. Data were obtained from experiments repeated three times and are means ± SE; $^*P < 0.05$, $^{**}P < 0.01$.

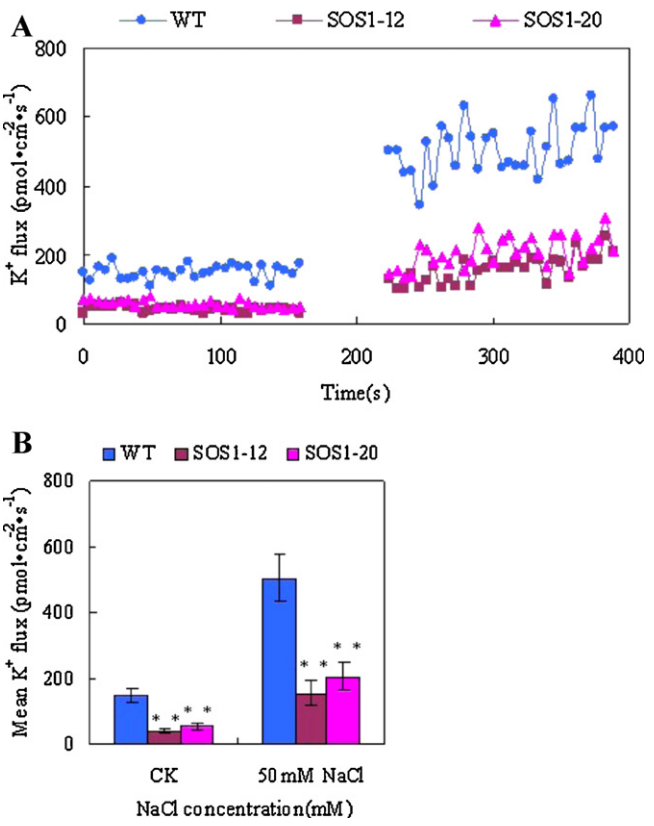

Fig. 6. Effect of salinity (50 mM NaCl) on net K^+ flux (influx negative) measured in seedling roots of two *SOS1*-overexpressing tobacco lines (SOS1-12 and SOS1-20) and wild-type (WT) plants. (A) K^+ fluxes for WT, SOS1-12 and SOS1-20 prior to salt treatment. Each point represents the mean of five to eight seedlings. (B) The mean rate of K^+ flux during the period of salt-stress treatment. Values are means; $^*P < 0.05$, $^{**}P < 0.01$.

further verified in transgenic *Arabidopsis* overexpressing *SOS1* (Shi et al., 2003). Therefore, *AtSOS1* in transgenic tobacco maybe share the same role in controlling long-distance Na^+ transport under the salt stress conditions. However, this is first time to overexpress *AtSOS1* in plant other than *Arabidopsis*, and the role of *SOS1* should be further confirmed by taking advantage of tobacco with a more convenient anatomy in future studies.

Although the *SOS1* phenotype was first identified by using a root-bending assay based on salt stress in *Arabidopsis*, *SOS1* was initially suggested to be primarily involved in high affinity K^+ transport (Wu et al., 1996). Further studies showed that there was a correlation between salt tolerance of *atsos1*, *atsos2* and *atsos3* mutants and their K^+ tissue contents (Zhu et al., 1998). *SOS1* protected the *AKT1* K^+ channel, which mediated K^+ influx in presence of increased Na^+ (Qi and Spalding, 2004). Shabala et al. (2005) observed that K^+ efflux from *atsos1* roots in presence of salt was greater than in WT plants in both the apical or mature regions and suggested that *SOS1* played an important role in controlling K^+ transport. Consistent with those reports, our results showed that K^+ efflux from the elongation region of roots of transgenic tobacco overexpressing *SOS1* was smaller than that from WT plants (Fig. 6). However, there was a difference in K^+ efflux between the control and transgenic plants under non-salt treatment. This could be the reason of difference in root zone at the same distance (500 μm) between them. It is interesting to examine whether there is a difference in the root growth and development between the transgenic and control seedlings in further studies.

K^+ is an essential macronutrient that is required for diverse cellular processes such as osmotic regulation, maintenance of membrane potential, enzyme activity, protein and starch synthesis, respiration and photosynthesis (Hauser and Horie, 2010). A high K^+/Na^+ ratio in the cytosol was essential for normal cellular functions of plants (Chinnusamy et al., 2005). A higher K^+/Na^+ ratio could minimize Na^+ toxicity under salt stress, and it was generally accepted that maintenance of K^+/Na^+ homeostasis was an important aspect of salt tolerance (Tester and Davenport, 2003; Volkov et al., 2004; Kronzucker et al., 2006; Chen et al., 2007b; Hauser and Horie, 2010). *SOS1*-overexpressing plants exposed to 150 mM NaCl accumulated more K^+ than WT plants (Fig. 5B), and the K^+/Na^+ ratio was higher in transgenic plants than WT tobacco (Fig. 5C and D).

Our results demonstrated that *SOS1* overexpression in tobacco improved salt tolerance of transgenic plants by maintaining a higher K^+/Na^+ ratio than in WT tobacco. Since salt tolerance in plants is a complex trait that involves multiple physiological and biochemical mechanisms expressed by numerous genes, whether the plasma membrane Na^+/H^+ antiporter *SOS1* of *Arabidopsis*

thaliana has a similar salt-tolerance function in other crops needs to be investigated.

Acknowledgments

This research was supported by the National Nature Science Foundation of China (No. 30825028). The authors thank Dr. Zhizhong Gong and Dr. Yan Guo, Professors, College of Biological Sciences, China Agricultural University, and Dr. Jiankang Zhu, Professor, Department of Botany and Plant Sciences, Institute for Integrative Genome Biology, 2150 Batchelor Hall, University of California, for technical advising. The authors also thank Dr. Calvin G. Messersmith, Professor Emeritus, Department of Plant Sciences, North Dakota State University, Fargo, for technical improvement of the manuscript.

References

Apse MP, Aharon GS, Snedden WS, Blumwald E. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Science 1999;285:1256–8.

Baykal U, Zhang Z. Small RNA-mediated gene silencing for plant biotechnology. In: Catalano AJ, editor. Gene silencing: theory, techniques and applications. Hauppauge: Nova Science Publishers; 2010. p. 255–69.

Chen H, An R, Tang JH, Cui XH, Hao FS, Jia C, et al. Over-expression of a vacuolar Na^+/H^+ antiporter gene improves salt tolerance in an upland rice. Mol Breeding 2007a;19:215–25.

Chen Z, Hong X, Zhang H, Wang Y, Li X, Zhu JK, et al. Disruption of the cellulose synthase gene, AtCesA8/IRX1, enhances drought and osmotic stress tolerance in Arabidopsis. Plant J 2005;43:273–83.

Chen Z, Zhou M, Newman I, Mendham N, Zhang G, Shabala S. Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance. Funct Plant Biol 2007b;34:150–62.

Chinnusamy V, Jagendorf A, Zhu JK. Understanding and improving salt tolerance in plants. Crop Sci 2005;45:437–48.

Fraile-Escanciano A, Kamisugi Y, Cuming AC, Rodriguez-Navarro A, Benito B. The *SOS1* transporter of *Physcomitrella patens* mediates sodium efflux in planta. New Phytol 2010;188:750–61.

Gallie DR. Controlling gene expression in transgenics. Curr Opin Plant Biol 1998;1:166–72.

Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR. The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. Proc Natl Acad Sci USA 1999;96:1480–5.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 2000;51:463–99.

Hauser F, Horie T. A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant Cell Environ 2010;33:552–65.

Kronzucker HJ, Szczerba MW, Moazami-Goudarzi M, Britto DT. The cytosolic Na^+:K^+ ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using $^{42}K^+$ and $^{24}Na^+$. Plant Cell Environ 2006;29:2228–37.

Manabe Y, Bressan RA, Wang T, Li F, Koiwa H, Sokolchik I, et al. The *Arabidopsis* kinase-associated protein phosphatase regulates adaptation to Na^+ stress. Plant Physiol 2008;146:612–22.

Martínez-Atienza J, Jiang XY, Garciadeblas B, Mendoza I, Zhu JK, Pardo JM, et al. Conservation of the salt overly sensitive pathway in rice. Plant Physiol 2007;143:1001–12.

Meloni DA, Oliva MA, Ruiz HA, Martinez CA. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. J Plant Nutr 2001;24:599–612.

Mohammad M, Shibli R, Ajouni M, Nimri L. Tomato root and shoot responses to salt stress under different levels of phosphorus nutrition. J Plant Nutr 1998;21:1667–80.

Oh DH, Leidi E, Zhang Q, Hwang SM, Li Y, Quintero FJ, et al. Loss of halophytism by interference with *SOS1* expression. Plant Physiol 2009;151:210–22.

Olias R, Eljakaoui Z, Li J, De Morales PA, Marin-Manzano MC, Pardo JM, et al. The plasma membrane Na^+/H^+ antiporter *SOS1* is essential for salt tolerance in tomato and affects the partitioning of Na^+ between plant organs. Plant Cell Environ 2009;32:904–16.

Qi Z, Spalding EP. Protection of plasma membrane K^+ transport by the salt overly sensitive1 Na^+–H^+ antiporter during salinity stress. Plant Physiol 2004;136:2548–55.

Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK. Regulation of *SOS1*, a plasma membrane Na^+/H^+ exchanger in *Arabidopsis thaliana*, by *SOS2* and *SOS3*. Proc Natl Acad Sci USA 2002;99:8436–41.

Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos E, Luque T, et al. Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. Ann Bot 2007;100:555–63.

Shabala L, Cuin TA, Newman IA, Shabala S. Salinity-induced ion flux patterns from the excised roots of *Arabidopsis sos* mutants. Planta 2005;222:1041–50.

Shi H, Ishitani M, Kim C, Zhu JK. The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter. Proc Natl Acad Sci USA 2000;97:6896–901.

Shi H, Lee B, Wu SJ, Zhu JK. Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat Biotechnol 2003;21:81–5.

Shi H, Quintero FJ, Pardo JM, Zhu JK. The putative plasma membrane Na^+/H^+ antiporter *SOS1* controls long-distance Na^+ transport in plants. Plant Cell 2002;14:465–77.

Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa K, Yokota A, et al. Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts. FEBS Lett 1998;428:47–51.

Singh AK, Dubey RS. Changes in chlorophyll a and b contents and activities of photosystems 1 and 2 in rice seedlings induced by NaCl. Photosynthetica 1995;31:489–99.

Sun J, Wang MJ, Ding MQ, Deng SR, Liu MQ, Lu CF, et al. H_2O_2 and cytosolic Ca^{2+} signals triggered by the PM H^+-coupled transport system mediate K^+/Na^+ homeostasis in NaCl-stressed *Populus euphratica* cells. Plant Cell Environ 2010;33:943–58.

Tester M, Davenport R. Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 2003;91:503–27.

Volkov V, Wang B, Dominy PJ, Fricke W, Amtmann A. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. Plant Cell Environ 2004;27:1–14.

Wang WQ, Li Y, Zhang YY, Yang CP, Zheng NY, Xie Q. Comparative expression analysis of three genes from the *Arabidopsis* vacuolar Na^+/H^+ antiporter (*AtNHX*) family in relation to abiotic stresses. Chin Sci Bull 2007;52:1754–63.

Wu SJ, Ding L, Zhu JK. *SOS1*, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 1996;8:617–27.

Wu YX, Ding N, Zhao X, Zhao MG, Chang ZQ, Liu JQ, et al. Molecular characterization of *PeSOS1*: the putative Na^+/H^+ antiporter of *Populus euphratica*. Plant Mol Biol 2007;65:1–11.

Xu HX, Jiang XY, Zhan KH, Cheng XY, Chen XJ, Pardo JM, et al. Functional characterization of a wheat plasma membrane Na^+/H^+ antiporter in yeast. Arch Biochem Biophys 2008;473:8–15.

Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, et al. A protein kinase, interacting with two calcineurin b-like proteins, regulates K^+ transporter *AKT1* in *Arabidopsis*. Cell 2006;125:1347–60.

Xue ZY, Zhi DY, Xue GP, Zhang H, Zhao YX. Enhanced salt tolerance of transgenic wheat (*Tritivum aestivum* L.) expressing a vacuolar Na^+/H^+ antiporter gene with improved grain yields in saline soils in the field and a reduced level of leaf Na^+. Plant Sci 2004;167:849–59.

Yang Q, Chen ZZ, Zhou XF, Yin HB, Li X, Xin XF, et al. Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic *Arabidopsis*. Mol Plant 2009;2:22–31.

Yin XY, Yang AF, Zhang KW, Zhang JR. Production and analysis of transgenic maize improved salt tolerance by the introduction of *AtNHX1* gene. Acta Bot Sin 2004;46:854–61.

Zhang HX, Blumwald E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotechnol 2001;19:765–8.

Zhang HX, Hodson JN, Williams JP, Blumwald E. Engineering salt tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc Natl Acad Sci USA 2001;98:12832–6.

Zhu JK. Plant salt tolerance. Trends Plant Sci 2001;6:66–71.

Zhu JK. Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 2003;6:441–5.

Zhu JK, Liu J, Xiong L. Genetic analysis of salt tolerance in *Arabidopsis*: evidence for a critical role of potassium nutrition. Plant Cell 1998;10:1181–91.