Identification \textit{MdeSOS1} in \textit{Magnolia denudata} and its function in response to salt stress

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\textbf{ABSTRACT}

In this study, annual \textit{Magnolia denudata} seedlings were treated with a 200 mmol L$^{-1}$ NaCl solution. Na$^+$ content in its stems increased by more than about 24 times after 72 h and K$^+$ content in leaves basically maintained in a steady state. \textit{MdeSOS1}, isolated from \textit{M. denudata}, comprised a 3453-bp ORF and included 12 transmembrane structures within its N terminal and a hydrophilic tail in its C-terminal. Its protein shared the identity of 68.3% and 62.7% at the peptide level with the homologue \textit{PeSOS1} and \textit{AtSOS1} respectively. The \textit{MdeSOS1} was significantly induced to up-regulation in various tissues exposed to salt stress and improved the salt tolerance of \textit{Arabidopsis}. Our results also revealed that the \textit{MdeSOS1-GFP} fusion protein was located on the plasma membrane and \textit{MdeSOS1} encoded a salt-inducible plasma membrane Na$^+/H^+$ antiporter, which provides a reference to improve the salt tolerance of \textit{Magnolia} species by transgenic approaches.

\textbf{Introduction}

Salt stress has a significant effect on plant growth and development that is mainly restrained by excessive soluble ions, such as sodium and chloride ions (Yue et al. 2012). Intracellular elevated Na$^+$ concentrations not only cause single salt toxicity but also trigger a variety of physiological metabolic disorder in plants. Excessive Na$^+$ competes with and replaces K$^+$, disrupting the balance of the K$^+$/Na$^+$ ratio (Deinlein et al. 2014). The decrease in intracellular K$^+$ concentration further affects the activity of enzymes, influences multiple enzymatic reactions and then produces toxic effects in cells (Olias et al. 2009). It is essential for plants to transport and exclude Na$^+$ from cells to maintain ion balance under salt stress. In addition, the influx of Na$^+$ is a passive process, which mainly depends on differences in the negative potential of the plasma membrane and low concentrations of Na$^+$ in the cytosol, while Na$^+$ compartmentation and exclusion are indirect and active processes (Deinlein et al. 2014).

In high salinity medium, Na$^+$ of high concentrations enters the roots and is transported to the leaves. The excessive Na$^+$ in the leaves is translocated to vacuoles or exude from cells through Na$^+/H^+$ antiporter proteins located in both the plasma and the tonoplast (Shi et al. 2002). The H$^+$-adenosine triphosphatase (H$^+$-ATPase) in the membrane will generate transmembrane electrochemical H$^+$ gradient potential to provide energy for Na$^+/H^+$ antiporters (Gao et al. 2016). Salt overly sensitive (SOS) signal pathway is a major mechanism at the cellular level to control Na$^+$ efflux and transport. Three main protein components SOS1, SOS2, and SOS3 were found in the model plant \textit{Arabidopsis thaliana} (Shi et al. 2000). The gene for the first plasma membrane Na$^+/H^+$ antiporter, SOS1, was initially demonstrated by positional cloning in \textit{A. thaliana} (Shi et al. 2000). Its salt tolerance was demonstrated in \textit{AtSOS1}-overexpressing lines and \textit{35S:AtSOS1} lines were correlated with decreased Na$^+$ accumulation compared with that in control plants grown in MS medium with 50 mmol L$^{-1}$ NaCl (Shi et al. 2003). Additionally, SOS3 activates and binds SOS2, and the SOS3-SOS2 protein kinase complex regulates the expression and activity of the ion transporter SOS1 (Qi et al. 2002). To date, the gene encoding the SOS1 protein has been cloned from a variety of plants, including edible plants such as tomato (Olias et al. 2009), wheat (Xu et al. 2008) and cucumber (Wang et al. 2012), ornamental plants such as \textit{Helianthus tuberosus} (Yan et al. 2007), \textit{Dendranthema crissum} and \textit{Artemisia japonica} (Song et al. 2012), and the woody plants \textit{Bruguiera gymnorrhiza} and \textit{Populus euphratica} (Wu et al. 2007). SOS1 mediates Na$^+$ distribution in plant organs (Chung et al. 2008) and can be expressed preferentially in the root epidermis and the parenchyma cells of the xylem (Shi et al. 2002). In \textit{P. euphratica}, the expression of \textit{PeSOS1} enhanced plant salt tolerance by exuding Na$^+$ from cells (Wu et al. 2007).

\textit{Magnolias} (\textit{Magnolia} spp.) are an excellent ornamental tree species in early spring with wide distribution in China. Most of the present studies concentrate on their phylogenetic evolution, flooding or drought stress (Wu et al. 1991; Xu and Rudall 2006; Shi et al. 2014), while few reports on their salt tolerance. It was found that \textit{M. virginiana} did not exhibit a remarkably reduction in shoot dry mass, root dry mass or leaf surface area during flooding or drought (Nash and Graves 1993), while other researchers investigated the physiological activities of \textit{M. wufengensis} under drought stress and the changes of the photosynthesis parameters.
of *M. sinostellata* under flooding and shading stress (Sang et al. 2011; Yu et al. 2019).

In saline areas, magnolias tend to grow poorly and hard to form excellent visual effects. *Magnolia denudata* is widely used as a rootstock in the propagation of magnolias due to its strong resistance. In this study, the Na⁺ and K⁺ contents in different tissues (root, shoot and leaf) of *M. denudata* annual seedlings were detected under salt stress. The SOS1 homolog *MdeSOS1* isolated from *M. denudata* was analyzed, and its expression was further detected under virous abiotic stress. The subcellular localization experiment suggested that *MdeSOS1* was located on the plasma membrane. Furthermore, the *MdeSOS1* gene in *A. thaliana* was transformed into wild-type (WT) and mutant (*at sos1-1*) to assess the contribution of *MdeSOS1* to salinity tolerance.

**Materials and methods**

**Plant materials and growth conditions**

Annual *M. denudata* seedlings were maintained in the germplasm repository for magnolias (located at 30°15’14” N/119° 43’39” E) at Zhejiang Agriculture and Forestry University, Hangzhou, China. All of the materials were grown in incubator (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night cycle, 27°C day/25°C night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m² (PGX-350B, Saifu, Ningbo) under a 14 h day/10 h night temperature, light intensity of 200 W/m². The seedlings were selected and transferred to liquid culture containing half-strength Hoagland solution (Hewitt 1952), and NaCl was dissolved in it at 200 mmol L⁻¹. 20% polyethylene glycol (PEG) 6000 and 50 mmol L⁻¹ CdCl₂ was configured in the same way. The seedlings were transferred into the climatic chamber (Premium ICH insect chamber; Snijders Labs, Tilburg, Netherlands) for the low temperature of 4°C, and the light and relative humidity remain the same as before. After 0, 1, 3, 6, 12 and 24 h of treatment, samples induced by abiotic stress were extracted for analysis of gene expression.

**Determination of Na⁺ and K⁺ content in different tissues in *M. denudata***

To detect the ion content and distribution, different tissues (leaf, stem and root) were separately harvested under various stresses. The harvested leaves are middle mature leaves, and three leaves are taken from each plant. Experiments in each tissue sample were repeated three times. The samples were oven-dried at 70°C (3 d) and then ground into powder with a mortar. A total of 0.2 g of the powder was accurately weighed and digested with 18 mL of a solution (nitric acid: perchloric acid=5:1) on an electric heating plate until the white smoke appeared. Finally, 20 mL of deionized water was added after cooling that was then filtered and transferred to a 100 mL bottle. The Na⁺ and K⁺ contents were determined by atomic absorbance spectrophotometry (AA6650F; Shimadzu). Each measurement was repeated three times. The significance of the differences was analyzed using Duncan’s multiple range tests with SPSS v 17.0 software.

**RNA extraction and cDNA synthesis**

RNA was extracted from the tissues of plants exposed to 200 mmol L⁻¹ NaCl using an RNA Prep Plant Kit (DP441; Tiangen) following the manufacturer’s protocol. The first strand of the cDNA was synthesized according to the instructions of the Prime Script TM RT Master Mix (RR036A; Takara) reagent and used as a template for PCR amplification.

**MdeSOS1 isolation and sequence analysis**

According to the transcriptome database (NCBI: SRR6407153) constructed in the early stage of our research, we screened out the sequence (CL13.Contig3_1N) related to *MdeSOS1* gene and designed specific primers (R_F/R_R) with PRIMER 5.0 software (Table 1). The PCR products were cloned into the pMD19-T vector using T₄ DNA Ligase (2011A; Takara). Then, the recombinant vector was transferred into *Escherichia coli* (E. coli) DH5α competent cells and grown on Luria–Bertani (LB) medium with 50 mg·mL⁻¹ kanamycin. Positive bacterial colonies were detected by PCR using specific primers. The bacteria solution was sent to Sangon Biotech (Shanghai, China) for sequence verification. Open reading frames (ORFs) were identified using the ORF-FINDER program (https://www.ncbi.nlm.nih.gov/orffinder). The physical and chemical parameters of the *MdeSOS1* protein were analyzed by the ProtParam tool (https://web.expasy.org/protparam/). Protein signal peptides were predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). Conserved domains within proteins were obtained from NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Protein structure–activity analysis was carried out using SWISS-MODEL (https://swissmodel.expasy.org/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/).

**Specific expression of MdeSOS1 in different tissues under abiotic stress**

Real-time quantitative PCR (qPCR) was employed to detect the specific expression of *MdeSOS1*. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes. The qPCR primers were designed by PRIMER 5.0 and are shown in Table 1. The GBP (GTP-binding protein), *UBQ* (Polyubiquitin protein) and *UBC* (Ubiquitin-conjugating enzyme) were used as the reference genes.

**Table 1. The sequences of primers used for PCR.**

| Primer name | 5′-3′ sequence | Usage |
|-------------|----------------|-------|
| R_F         | TGGTTTTTGTGGTTAGACGAG | RT-PCR for ORF |
| R_R         | TTGTAGCAGCTGTCGGAAGA | RT-PCR for ORF |
| S_F         | CGGGGTTCCCCCTGTCGCCGGTGCGAAGG | Subcellular localization |
| S_R         | ACCGCTGCAGCTGCTGATCCTCCTGGATCGG | Subcellular localization |
| T_F         | CGGGGTACCCTGCGTGCGCGGTGCAGAAGG | Transformation of *A. thaliana* |
| T_R         | CGGGCGCTGCGATCCTGATCCTCCTGGATCGG | Transformation of *A. thaliana* |
| q_F         | CATCAGAGTGCACCAAAATAAA | Real-time fluorescence quantitative PCR |
| q_R         | AGGCACCTCAAGACATTT | Real-time fluorescence quantitative PCR |
| GBP_F       | TTCTGCCTAGCTGTCGGA | Reference gene |
| GBP_R       | CGCGAGACCGCAGATACCA | Reference gene |
| UBC_F       | CGAATCTCCCTGCGAATTCT | Reference gene |
| UBC_R       | TCAGCTGGCCTGCGACTC | Reference gene |
| UBO_F       | TCTATGCCCTTAAGGCGAAA | Reference gene |
| UBR_O       | AATGACAGAGGGTGCTGCT | Reference gene |
reference genes (Chang et al. 2018). Each 20 μL reaction contained 10 μL TB Green™ Premix Ex Taq™ II (RR820L; Takara), 2 μL cDNA, 0.8 μL of each primer (10 μmol·L⁻¹) and 6.4 μL ddH₂O. The amplification program comprised an initial denaturation (95°C/30 s) followed by 40 cycles of 95°C/5 s, 61°C/30 s and 72°C/20 s. A melting curve was from 60°C to 95°C, with each cycle increased by 0.5°C for 0.05 s to obtain the melting temperature, and the fluorescence signal was collected. Relative transcript levels were calculated using the 2⁻ΔΔCt method (Livak and Schmittgen 2001). Each sample was repeated three times.

Subcellular localization of MdeSOS1

A PCR-generated KpnI and SalI fragment containing the MdeSOS1 ORF was cloned in-frame to the C-terminal end of green fluorescent protein (GFP) in the 35S-GFP-nos plasmid to generate MdeSOS1-GFP and used to define the subcellular location of MdeSOS1. The primers (S_F/S_R) were designed by PRIMER 6.0 (Table 1). GFP was located behind the inserted MdeSOS1 gene, and the terminator was removed from the downstream primer sequence so that GFP could be expressed. A recombinant expression vector was used to transform E. coli DH5α grown on LB medium with kanamycin (50 mg·mL⁻¹). Positive bacterial colonies were selected for and amplified to extract recombinant plasmid and then verified by PCR and double enzyme restriction. The recombinant plasmid was transformed into A. tumefaciens GV3101, cultivated overnight at 28°C and harvested at an OD600 of 1.5–2.0 in YEB medium supplemented with 100 mg·mL⁻¹ rifampicin and 25 mg·mL⁻¹ kanamycin. Finally, Agrobacterium cells were diluted to an OD600 of approximately 0.1 for the infiltration of onion epidermal cells (Xu et al. 2014). Images of epidermal cells expressing GFP were obtained by using a laser scanning confocal microscope (LSM-510, Carl Zeiss AG).

Constructs and plant transformation in WT and mutant Arabidopsis

The ORF of MdeSOS1 was amplified by PCR using the primers with restriction enzyme site (T_F/T_R) (Table 1). The PCR products were ligated to pCAMBIA1301-35S-MdeSOS1 downstream of the CaMV 35S promoter. Then, the recombinant plasmid was transformed into A. tumefaciens GV3101 by the liquid nitrogen freeze-thaw method. A. thaliana transformation was performed using the fluorescence infection technique. Positive transgenic lines (WT 35S: MdeSOS1 and atsos1-135S:GmsSOS1) were selected on MS medium (HB8469, Hope, Qingdao) plus 50 mg·mL⁻¹ hygromycin and advanced to the T3 generation to obtain homozygous lines for the next experiment. Seeds from the WT, atsos1-1, WT 35S:MdeSOS1, and atsos1-1 35S:MdeSOS1 plants were sown in MS agar medium supplemented with 100 mmol L⁻¹ NaCl or no NaCl. After vernalization for 3 days at 4°C, the medium was moved to a phytotron under a controlled environment with 23°C /14 h of light and 20°C /10 h of darkness. Seedling root growth and seed germination were observed and determined after 15 days of incubation.

Statistical analysis

Data are reported as the means ± SEs of three replicates and were analyzed using Duncan’s multiple range tests with P<0.05 indicating significance.
Figure 3. Multiple sequence alignment analysis of MdeSOS1, AtSOS1 (NP_178307.2), PeSOS1 (ABF60872.1) and BgSOS1 (ADK91080.1). The 12 putative transmembrane domains are underlined and numbered 1 through 12. Residues conserved in at least three proteins are highlighted in pink and blue. Nhap, an Na\textsuperscript{+}/H\textsuperscript{+} exchanger domain spanning the transmembrane region.
Results

Phenotypes and ion contents were changed by salt stress

The phenotype of M. denudata following 200 mmol L\(^{-1}\) NaCl treatment was investigated, and the margin of the bottom leaves of plants exhibited wilting and yellowing at 48 h. These symptoms became more pronounced as the treatment time was extended. After 72 h, the tip of the middle leaves also appeared to scorched and the bottom leaves edges were showed necrotic phenotype (Figure 1).

Na\(^+\) was significantly increased in roots and shoots after 72 h, while K\(^+\) was obviously reduced in roots and stems (but not in leaves). Compared with the expression at 0 h, Na\(^+\) increased respectively by 71.06%, 2425.09% and 1502.98% in the roots, stems and leaves after 72 h, while K\(^+\) contents reduced by 29.15% and 37.60% in the roots and stems (Figure 2). Under normal physiological conditions, the plants maintained a high ratio of K\(^+\)/Na\(^+\), which decreased when the plants were exposed to salinity stress. The ratio of K\(^+\)/Na\(^+\) declined markedly in all tissues of M. denudata exposure to the salinity and showed biggest drops in the stem due to the large amount of Na\(^+\) was accumulated under salt stress.

Sequence analysis of MdeSOS1

The length of the MdeSOS1 ORF encoding a protein of 1150 aa residues is 3453 bp. The molecular formula of the gene activator protein-e spanning the Nhap transmembrane region, a catabolite drops in the stem due to the large amount of Na\(^+\) was accumulated (Figure 1). ORF encodeing a protein of 1150 amino acid sequences revealed highly conserved sequences: the identity between MdeSOS1 and AtSOS1 was 62.7%, and that between MdeSOS1 and PeSOS1 was 68.3% (Figure 3). The phylogenetic tree showed that MdeSOS1 occupies a single branch in the evolutionary process of SOS1 proteins (Figure 4).

Transcription profiling of MdeSOS1 under salt and other abiotic stresses

Under salinity stress, MdeSOS1 gene was induced to express in all M. denudata tissues, although response time in roots was slightly later than in stems and leaves. The transcript level in roots and stems also showed a trend of increasing firstly and then decreasing under PEG stress, but in leaves, PEG negatively regulated MdeSOS1 expression. Low temperature increased the abundance of MdeSOS1 transcripts in roots and reduced in stems or leaves though there was a minor and short-lived spike in expression at 12 h. Additionally, all tissues MdeSOS1 transcript level decreased below the pre-treatment condition under CdCl\(_2\) stress.

Subcellular localization of MdeSOS1

The MdeSOS1 subcellular location was examined by monitoring GFP fluorescence in living onion epidermis cells transformed with both the MdeSOS1 construct (MdeSOS1-GFP) and the control (35S-GFP). GFP in the cells transformed with MdeSOS1-GFP was exclusively observed at the plasma membrane while 35S-GFP fluorescence in the control plasmid-transformed cells was detected in the entire cellular region, including the nucleus and cytoplasm (Figure 7). Therefore, we speculated that MdeSOS1 was located on the plasma membrane to control sodium ion movement in cells.

MdeSOS1 overexpression enhances salt tolerance in transgenic Arabidopsis plants

WT, WT 35S:MdeSOS1, atsos1-1 and atsos1-1 35S:MdeSOS1 seeds germinate normally when grown in MS medium with no NaCl, but the root length of the atsos1 seedlings was significantly decreased compared to that of the other plants (P<0.05). When 100 mmol L\(^{-1}\) NaCl was added to the medium, the seedling root length from the four types of
Figure 5. Transcription profiling of the MdeSOS1 gene in response to abiotic stress. (A) NaCl stress; (B) PEG6000 stress; (C) Low temperature stress; (D) CdCl₂ stress. The relative expression in all tissues and all time points was first compared to that of the reference gene in each species and then calculated using the expression at the initial time (0 h) in roots. *, **: differ significantly from levels in the control treatment (P<0.05 and <0.01, respectively).

Figure 6. Variation in Na⁺, K⁺ contents and K⁺/Na⁺ ratio in tissues after PEG6000, low temperature and CdCl₂ stress. (A)-(C) Na⁺ contents. (D)-(F) K⁺ contents. (G)-(I) K⁺/Na⁺ ratio. Data are presented as the mean ± SE (n=3).
A. thaliana plants was obviously reduced compared to that of the control ($P<0.05$), especially for the atsos1-1 mutant, in which the root length decreased by 83.27% (Figure 8D). However, when the plants were transformed with MdeSOS1, the root lengths of WT 3SS:MdeSOS1 and atsos1-1 3SS:MdeSOS1 plants increased respectively by 49.98% and 103.29% compared to non-transgenic plants.

**Discussion**

Na$^+$ content of the stems increased by more than about 24 times after 72 h of salt stress compared with that at 0 h and the amount of increase was significantly higher than that in roots and leaves. It is plausible that the stem of *M. denudata* had strong ability to intercept Na$^+$ transport. The distribution of ions in different organs significantly affects the salt tolerance of plants (Guan et al. 2010). During salt stress process, the accumulation of Na$^+$ in stems increased significantly, which effectively reduced Na$^+$ stress on other tissues. The result was consistent with ion variation in the woody plant *Salix saposhnikovii* (Zhou and Zhang 2017). As an important inorganic cation in cells, K$^+$ plays a critical role in regulating osmotic pressure and maintaining the integrity of the cell membrane. Salinity had little effect on the level of K$^+$ in leaves and it may be that K$^+$ was transported from the plant stems and roots into leaves for struggling to sustain normal physiological function of leaves, further alleviating the damage caused by Na$^+$ accumulation. Under normal physiological conditions, plants contain a lower Na$^+$ content and a higher K$^+$ content, that is, a higher ratio of K$^+$/Na$^+$. Salt stress disrupts ion balance and reduces the ratio of K$^+$/Na$^+$. As stress time progresses, the ratio of K$^+$/Na$^+$ in all *M. denudata* tissues displayed obvious decrease. The regulation of the ion steady state has been explicated with some success as a means to discriminate between salt-sensitive and salt-tolerant plants (Song et al. 2012; Gao et al. 2016). In this study, *M. denudata* is a woody plant with a tough and strong stem and a well-developed vascular organization, that has large biomass and vigorous physiological metabolic activity. The stem part of *M. denudata* seemed to give full play to the interception function for Na$^+$ as a response to salt stress. In addition, maintaining the stable state of K$^+$ in leaves through the transport of stem vascular tissue also facilitates to protect photosynthesis tissues.

The SOS1 gene controls the transport of Na$^+$, including its loading and retrieval into or out of the xylem (Shi et al. 2002). Several studies have demonstrated that the SOS1 antipporter is not only essential in maintaining ion homeostasis under sal-

![Figure 7. Step verifications of MdeSOS1-GFP construction. (A) The amplification of MdeSOS1 cDNA is shown in line 1; (B) The verification of MdeSOS1-GFP by double enzyme restriction is shown in line 2; (C) Colony PCR of MdeSOS1-GFP is shown in line 3; (D) Model of the constructed vector; (E) The subcellular localization of MdeSOS1-GFP and 3SS-GFP. M represents the DNA marker. Bar=100 μm.](image-url)
inity but also critical for the partitioning of Na⁺ between plant organs (Olías et al. 2009; Gao et al. 2016). Thus, we isolated the *MdeSOS1* gene from *M. denudata*. It contains 12 transmembrane domains in hydrophobic N-terminal portion and a long hydrophilic cytoplasmic tail in its C-terminal portion, which is a typical feature of the *SOS1* proteins and considered to contact with other proteins in the cell (Zhu 2000). In addition to Nhap domain, it also contains CAP_ED, Crp and cNMP. Nhap is a conserved functional domain spanning the transmembrane region that is primarily responsible for inorganic ion transport and metabolism. Further research revealed that Nhap has important functions in Na⁺ efflux in *Pseudomonas aeruginosa* (Utsugi et al. 1998). CAP_ED and Crp are both present in ion channels, and cNMP partakes cell signal transduction (Zeng et al. 2016). Similar domains also exist in *AtSOS1* (Shi et al. 2000), *PeSOS1* (Wu et al. 2007), and *SlSOS1* (Olías et al. 2009). The presence of these domains also confers certain salt tolerance to *M. denudata*. *Magnolia* spp. is an original angiosperm and has a primitive position in phylogenetic evolution, so *MdeSOS1* is located in a primitive position in the branch of the phylogenetic tree corresponding to dicots. Physcomitrella patens did not evolve a vascular bundle and belonged to a relatively low-level plant on phylogenetic tree and its *SOS1* sequence became an independent branch. From the phylogenetic tree, *MdeSOS1* is closely related to *CnSOS1*, while the *Populus euphratica PeSOS1* and *Bruguiera gymnorrhiza BgSOS1* (also the *SOS1* sequence of woody plants) have a distant relationship with *MdeSOS1*.

It was reported that *SOS1* proteins was significantly up-regulated in the roots than in either stems or leaves (Gao et al. 2016), while other papers had been found that salt stress induced up-regulated expression of *SOS1* genes in leaves but not in roots (Wu et al. 2007; Cosentino et al. 2010). The first

![Figure 8](image_url)

**Figure 8.** *MdeSOS1* overexpression enhance *A. thaliana* salt tolerance. (A) PCR products of transgenic *A. thaliana* using upstream primer of 35S promoter and downstream primer of *MdeSOS1*; the templates for T1-1-T8 were from *WT 35S:MdeSOS1*, and those for lanes t1-1-t1-8 from *atsos1-1 35S:MdeSOS1*; (B) *WT* and *WT 35S:MdeSOS1* seedlings grown on MS medium plus 100 mmol L⁻¹ NaCl after 15 days; (C) *atsos1-1* and *atsos1-1 35S:MdeSOS1* seedlings grown on MS medium plus 100 mmol L⁻¹ NaCl after 15 days; (D) Comparison of the root lengths of four *Arabidopsis* plants.
case suggests that the SOS1 protein exudes superabundant Na⁺ from the root cell to protect it from damage in a high-salt environment, while in the second, it has been suggested a function of antiporters as the maintainer of a low cytosolic Na⁺ concentration in the leaf structure to protect photosynthetic tissues (Ma et al. 2014). In this study, the MdeSOS1 is significantly up-regulated in all tissues following the imposition of salinity stress, providing that it may play a key role in the process of salt tolerance. However, the expression in leaves and stems showed a decreasing trend in the late induction period, and these results may be related to the stability of SOS1 mRNA (Chung et al. 2008). The similar phenomena can also be observed in Chrysanthemum crassum (Song et al. 2012). Salt stress usually reduces the ability of plants to absorb water, so it often implies drought stress. Under PEG treatment, the content of Na⁺ in all seedling tissues showed an upward trend at 24 h of stress, which would drive the expression of MdeSOS1 gene to play a transport function. The results showed that MdeSOS1 expression was induced in the stems and leaves after PEG treatment for 24 h (Figure 5). However, the gene in leaves did not appear to be up-regulated, and there may be other mechanisms to regulate ion homeostasis, which can explain K⁺/Na⁺ ratio of leaves is relatively stable during the PEG stress period (Figure 6). In C. crassum and A. thaliana, low temperature does not induce the expression of GmSOS1 and AtSOS1 (Song et al. 2012; Shi et al. 2000). We also found that low temperature and cadmium chloride stress severely damaged the physiological state of seedlings, and the ion content in the tissues showed irregular variation, which corresponded to the disordered expression of the MdeSOS1. Under CdCl₂ stress, the abundance of MdeSOS1 transcripts was significantly lower than that of untreated conditions. In conclusion, MdeSOS1 gene can fully respond to the induction of salt stress in various tissues and its protein was located on the plasma membrane, which means MdeSOS1 may be a salinity-inducible plasma membrane Na⁺/H⁺ antiporter (Figures 7 and 8).

Overexpression of SOS1 significantly improved the salt tolerance of A. thaliana and tobacco (Shi et al. 2003; Yue et al. 2012). Compared with those in the control, transgenic plants accumulated fewer sodium ions in the shoots to improve salt tolerance (Shi et al. 2003). GmsSOS1 in soybean increased transgenic Arabidopsis salt tolerance by promoting seedling root growth in a saline environment. In the woody plant P. euphratica, the expression of PeSOS1 suppressed the salt-sensitive phenotypes of the EP432 bacterial strain, which lacks the activity of two Na⁺/H⁺ antiporters, EcNhaA and EcNhaB (Wu et al. 2007). In this study, when MdeSOS1 was overexpressed in WT and sos1-1 mutant, the root length and seed germination rate of transgenic plants outperformed those of non-transgenic plants under 100 mmol L⁻¹ NaCl stress (Figure 8 (c–e)). These results further demonstrate that MdeSOS1 can enhance the salt tolerance of Arabidopsis. The transgenic atsos1-1 35S:MdeSOS1 plants obviously recovered but did not reached the level of the WT plants. Similarly, the integration of soybean GmsSOS1 into the Arabidopsis sos1-1 mutant increased mutant salt tolerance (Nie and Yu 2015) and its complementary phenotypes were superior compared to that of the sos1-1 mutant complemented by rice OsSOS1 and durum wheat TdSOS1. This is because soybean is a moderately salt-tolerant crop plants while rice and wheat are relatively salt-sensitive species. In general, M. denudata is also considered as a relatively salt-sensitive species, so the phenotype of the Arabidopsis sos1-1 mutant complemented with MdeSOS1 did not reach that of the WT plants.

Conclusion

The stems of M. denudata had a strong interceptive effect on Na⁺ and leaves can maintain the steady state of K⁺. MdeSOS1 was significantly induced in all tissues under salt stress and it may be a salinity-inducible Na⁺/H⁺ antiporter, whose overexpression in A. thaliana WT and sos1-1 mutant also enhanced plant salt tolerance. Hence, our results provide a reference for improving the salinity tolerance of Magnolia plants by transgenic approaches and will be useful in the adaptive application of M. denudata as a rootstock in the saline soil.

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