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ORIGINAL ARTICLE

Heterologous expression of Arabidopsis SOS3 increases salinity tolerance in Petunia

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Abstract

Background Salinity stress is one of the most important rising problems worldwide. It significantly reduces plant growth and development, mainly by provoking excessive uptake of ions such as Na^+. The *Salt Overly Sensitive* (SOS) machinery is a well-known signaling pathway that help plants to maintain ion homeostasis by reducing Na^+ accumulation in plant cells. Overexpression of key components of this pathway has been reported to increase salinity stress tolerance in some plant species.

Methods and results In this study, SOS3 cDNA isolated from Arabidopsis seedlings was transferred into the Petunia genome by two common plant transformation methods, *Agrobacterium* and biolistic gun. Transgene integration and expression in putative lines were evaluated by PCR and RT-PCR techniques. In vitro and greenhouse evaluation of transgenic plants for salt tolerance showed that, compared to the wild type, transgenic plants overexpressing *AtSOS3* gene exhibit enhanced salt tolerance in response to high NaCl concentrations.

Conclusions These results not only demonstrate the potential of SOS pathway components to improve salt tolerance in Petunia, but also provide more evidence for functional conservation of the SOS salt tolerance signaling pathway among different plant families.

Keywords AtSOS3 · *Petunia hybrida* · Salinity stress · Salt Overly Sensitive · Salt tolerance

Introduction

Water and soil salinity is a major factor negatively affecting productivity of cultivated soils [1]. Various factors such as climate change and global warming [2, 3], intrusion of saline water into wells and groundwater [4], aquifers colliding with mineral sediments [5], and direct use of salt in some cases, such as treatment of road freezing [6], cause increasing salinity of soil and water. Therefore, in addition to development of mechanisms to reduce salinity, improvement of methods for the optimal use of saline soils is critical for agriculture and gardening [7]. In addition, the use of different methods to increase plant tolerance to salinity is very important [8]. These methods mainly include the use of beneficial soil microorganisms to improve salinity tolerance in plants [9], production of salt-tolerant plants through conventional breeding and selection systems using molecular markers [10], and production of salt-tolerant plants through genetic engineering methods, customarily in combination with traditional breeding methods [11].

The development of techniques for identifying, isolating, and transferring key genes into the plant genome, molecular breeding through genetic engineering can lead to significant progress in introducing important agronomic traits such as salinity tolerance. Salt-stress-related genes can be divided into two categories, including effector genes (mainly genes encoding ion channels and transporters, protective proteins, and enzymes involved in osmolyte and antioxidant biosynthetic pathways) and regulatory genes (including salinity-related transcription factors, protein phosphatases, kinases, and proteases) which are involved in regulation of transcriptional, post-transcriptional, and

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signaling processes [12]. The *Salt-Overly-Sensitive* (SOS) signaling machinery is the principale salt excretion system and is important in protecting plants against salt stress [13]. This system, which helps plants to reduce the accumulation of sodium ion (Na^+) in plant cells, consists of the SOS1 sodium-transport protein and two regulatory proteins, SOS2 and SOS3.

High levels of Na^+ ions in cells, in addition to affecting water absorption and osmotic response, prevent the entry of vital K^+ ions, which play a key role in the activity of many enzymes [14]. In salt-tolerant plants, Na^+ levels in the cytoplasm are kept low. In most plants, this is achieved in three ways: reducing the influx, increasing the efflux and vacuolar compartmentation of Na^+ ions [15].

The SOS pathway is a well-known salt signaling transduction pathway that excludes Na^+ from the cytosol [16]. In this process, first, the calcium sensing protein SOS3, is activated by a temporary Ca^{2+} surge by the plant after perceiving a salt signal [17, 18]. It is believed that SOS3 physically interacts with the SOS2 protein kinase to form an SOS2/SOS3 complex, which in turn engages with the plasma membrane to activate SOS1 by phosphorylation. SOS1, a Na^+/H^+ antiporter, is responsible for excluding Na^+ from the cytosol to the apoplast and the soil environment [19].

Singular or combinatory overexpression of genes encoding the SOS pathway components has been reported to increase salt tolerance significantly in several plant species such as *Arabidopsis thaliana* [20], *Oryza sativa* [21], *Nicotiana tabacum* [22], *Ipomoea batatas* [23], *Festuca arundinacea* [24], hybrid poplar, *Populus tremula* × *Populus tremuloides* [25], and *Vitis vinifera* [26]. Also, overexpression of enhancers of SOS genes is reported to increase salt tolerance by promoting Na^+ exclusion from the cytosol [27].

Petunia × *hybrida* (Hook.f.) Vilm. (2n = 14) is a gametophytic self-incompatible ornamental plant which is sensitive to salt accumulation, especially sodium chloride, in the root zone [28]. Due to their broad range of variety and flower color, Petunias are among the most valuable and popular ornamental plants worldwide. In addition, the genus *Petunia* is a model plant in much horticultural and biological research [29]. Poor-quality saline water containing even relatively low concentrations of sodium chloride (40 mM) reduces Petunia plant growth and marketability [28]. A transcriptome analysis of Petunia in response to salt stress using RNA sequencing methods has identified several gene groups with differential expression in response to salt stress, including regulatory genes of reactive oxygen species, transport, and signal transduction as well as a few undescribed transcripts, although differential expression of Na^+/H^+ antiporters could not be detected [30]. Recently, the SOS pathway has been reported in additional plant species, such as wheat [31], *Brassica juncea* [32], *Kochia scoparia* [33] and Barley [34], but the mechanisms contributing to Na^+ compartmentation outside of the cytoplasm in Petunia plant cells is largely unknown.

In this work, we aimed to generate Petunia transgenic lines expressing *SOS3* transgene cDNA from *Arabidopsis thaliana*. These results, in addition to being important in Petunia breeding programs to increase salinity tolerance, can provide important information about the role of the SOS regulatory pathway in this plant.

Methods

Seed sterilization and establishment of sterile plant material

Seeds of *Arabidopsis thaliana* Col-0 and *Petunia* × *hybrida* (Wave® Pink hybrid) were sterilized using 70% ethanol for 30 s, and then 6% sodium hypochlorite solution for 2 min. After 3–5 times washing with sterile distilled water, the seeds were cultured in glass jar vessels containing MS basal medium. The cultures were then placed in a growth chamber with a diurnal cycle of 16 h of light (50 µE) and 8 h of darkness at 25 °C. One-week-old Arabidopsis plants were used for total RNA extraction experiments. Three-week-old petunia plants were used for transformation experiments.

Total plant nucleic acid extraction

Total RNA extraction from appropriate tissues of Arabidopsis and Petunia plants was performed using the Trizol™ Plus RNA Extraction Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. For total genomic DNA extraction from Petunia leaf tissues, the cetyltrimethylammoniumbromide (CTAB) method [35] was used. The quality and quantity of isolated nucleic acids were determined by spectrophotometric and agarose gel electrophoresis analysis.

Isolation of AtSOS3 cDNA by RT-PCR

RT-PCR was used to isolate Arabidopsis SOS3 cDNA. To prepare cDNA, 14 µl of reaction mixture containing 5 µg of RNA extracted from 7-day-old seedlings, 10 pM poly T oligonocleotide and 10 mM dNTPs mix, was placed at 65 °C for five min, followed by transfer to ice. After one minute, 4 µl of 5 × reverse-transcription buffer, 1 µl of 0.1 M DTT, 1 µl of 50 mM $MgCl_2$ and 0.5 µl of Super Script III reverse transcriptase (5 U/µl) were added to the reaction solution. Synthesis of the first strand of cDNA was carried out for 1 h at 50 °C. A pair of *AtSOS3*-gene-specific primers (PF-*Nco*I/*Xba*I: 5′- AT CCATGG TCTAGA GG ATG GGC TGC TCT GTA TCG AA-3′; and PR-*Bgl*II/*Eco*RI: 5′-AT GAATTC AGATCT TTA GGA AGA TAC GTT TTG CAA-3′) designed using a previously reported *AtSOS3*

mRNA sequence (Accession No. NM_122333.6), were used to amplify *AtSOS3* cDNA copies of 699 bp size (669 bp *AtSOS3* cDNA + 30 nucleotides containing the bordering restriction endonuclease sites as indicated in the above primer structures). The PCR program included a 4-min stage at 95 °C; then 30 cycles, each consisting of 30 s at 95 °C, 30 s at 58 °C and 60 s at 72 °C; and a final extension step at 72 °C for 10 min. PCR products were then separated by 1% agarose gel electrophoresis to isolate the *AtSOS3*-specific band.

Construction of AtSOS3 transformation vector

To generate a suitable transformation vector, we used the pBIN61 *Agrobacterium* transformation vector, a derivative of pBIN19 [36, 37]. The purified *AtSOS3* cDNA fragment produced by RT-PCR, was digested with *Xba*I and *Bgl*II restriction endonucleases (whose recognition sites had been incorporated in the forward and reverse primers, respectively). After purification from a low melting point agarose gel, the fragment was ligated with the purified pBIN61 vector fragment digested with *Xba*I and *Bam*HI, to build a suitable expression vector. Note that the *Bam*HI site is present in the *AtSOS3* structure and therefore, the *Bgl*II restriction enzyme was used to produce similar overhangs as *Bam*HI to proceed with the ligation experiment. With this procedure, the *AtSOS3* cDNA was cloned between the strong 35S promoter and terminator from Cauliflower mosaic virus (CaMV) to generate the pBIN61-SOS3 transformation vector (Fig. 1a).

Agrobacterium-mediated transformation procedure

The pBIN61-SOS3 vector was transferred to *Agrobacterium tumefaciens*, strain GV2260, by electroporation. The colonies obtained, after confirmation of their structure by PCR, were used to transfer the *SOS3* gene into the Petunia genome by the co-cultivation method. Briefly, 10 ml of *Agrobacterium* overnight culture was prepared from confirmed colonies using LB liquid medium containing 50 mg/l each of kanamycin and rifampicin. The main culture was then prepared by adding 100 µl of overnight culture on 250 ml of liquid LB medium, followed by incubation at 28 °C with 180 rpm shaking. When OD_{600} reached 0.6, the bacteria were sedimented by centrifugation for 10 min at 4000 rpm. The bacterial pellet was dissolved in 25 ml of liquid half-strength MS medium [38] containing 0.1 mM Acetosyringone, and used for inoculation of 3 mm^2 pieces of young sterile Petunia leaves. After 20 min, excess liquid was removed from explants by placing them on a sterile filter paper for a few sec, followed by co-culturing of inoculated explants on antibiotic-free MS medium in the dark at 23 °C for two days. The leaves were then transferred to selective shoot regeneration medium (MS basal medium

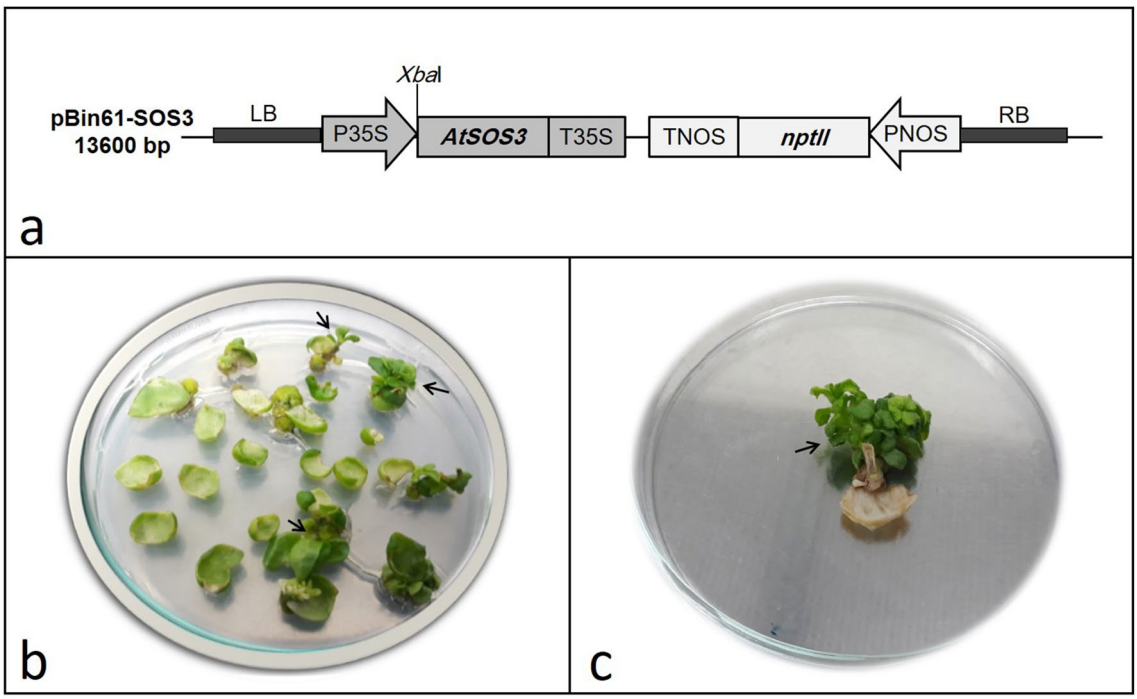

Fig. 1 (**a**) Physical map of the pBIN61-SOS3 *Agrobacterium*-mediated transformation vector constructed for *AtSOS3* cDNA integration into Petunia genome. (**b, c**) Shoot regeneration on selective media from leaf explants transformed by *Agrobacterium* (**b**) or gene gun (**c**) methods, after eight weeks. Examples of regenerated shoots are shown by arrows

complemented with 1 mg/l IAA and 1 mg/l BAP) containing 35 mg/l kanamycin and 250 mg/l cefotaxime, for double selection (Agrobacterial removal and transgenic cell line selection). The cultures were placed in a growth chamber with a diurnal cycle of 16 h of light (50 µE) and 8 h of darkness at 25 °C. Regenerated shoots on selective medium were transferred to MS medium containing 50 mg/l kanamycin for shoot elongation.

Biolistic transformation method

As an alternative to *Agrobacterium*-mediated transformation, the biolistic method was used to transfer the *AtSOS3* transgene to young leaf cells of Petunia plants using the BioRad PDS-1000/He™ gene gun system. In summary, the pBIN61-SOS3 plasmid DNA was precipitated on gold particles of 0.6 µm in diameter and bombarded to the surface of young sterile leaves with 1100 psi pressure. The leaves were then cut into 3 mm^2 pieces and transferred to the above-mentioned shoot regeneration medium containing 35 mg/l kanamycin, followed by incubation in the growth chamber for selection of transgenic cell lines. Regenerated shoots were then transferred to 50 mg/l kanamycin-containing MS medium for further growth.

Analysis of AtSOS3 integration and expression in transgenic plants by PCR and RT-PCR methods

To evaluate successful integration of the *AtSOS3* transgene into the genome of selected transgenic candidates, PCR analysis was performed using the same program as described above for the *AtSOS3* cDNA isolation procedure. Also, *AtSOS3* expression in transgenic plants at the transcriptional level was analyzed by the Reverse Transcription-PCR (RT-PCR) technique, as described above.

Evaluation of transgenic plants for salt tolerance

To evaluate salinity tolerance of transgenic plants, in vitro and greenhouse experiments were conducted. For the in vitro test, cuttings of regenerated plants of similar size and developmental stage from transgenic lines as well as wild-type plants were placed on MS medium containing 25 and 50 mM NaCl respectively for primary adaptation to NaCl addition. Note that all of the cuttings showed similar gowth pattern on unsalinized medium. After 3 days on each medium, the plants were transferred to MS medium containing either 100, 200 and 300 mM NaCl. After 40 days, various characters including leaf number, and root and stem length were recorded.

For greenhouse tests, two-week-old greenhouse-adopted plants were irrigated with two different concentrations of NaCl solution (100 and 200 mM) for 4 weeks. The plants were then photographed to perceive their reaction to salinity.

Software and statistical data analysis

The data were statistically analyzed using MSTAT-C software, and mean comparison was performed with Duncan's multiple range test. The NCBI BLASTn online program was used for similarity searches in nucleotide databases. The EBI CLASTAL OMEGA multiple sequence alignment program was applied to generate sequence alignments.

Results

Isolation and cloning of AtSOS3 cDNA to generate transformation vector

In order to prepare a suitable construction vector to introduce Arabidopsis *SOS3* gene into Petunia genome, the *AtSOS3* cDNA was isolated by RT-PCR method from Arabidopsis, and cloned in the pBin61 *Agrobacterium* vector between the CaMV 35S promoter and terminator (Fig. 1a). The resulting 13.6 kb vector contains the *neomycin phosphotransferase* (*nptII*) gene in the T-DNA region under control of the nopaline synthase promoter and terminator as a marker gene for selection of transgenic plant cell lines after transformation.

Petunia transformation and selection of transgenic candidates

Two common plant transformation methods – *Agrobacterium*-mediated transformation and a gene gun [39]—were used to transfer *AtSOS3* gene into the Petunia genome. In both methods, several independent resistant lines were regenerated on selective medium (Fig. 1b, 1c). By transferring regenerated shoots on MS medium containing higher levels of kanamycin (50 mg/l), 5 plant lines produced by the *Agrobacterium* method and 6 lines produced by the gene gun method were selected. These lines were subjected to further experiments to demonstrate *AtSOS3* integration and expression.

Confirmation of AtSOS3 integration in selected plants by PCR

PCR analysis using *AtSOS3*-specific primers amplified the expected 699 bp fragment from 4 *Agrobacterium*-transformed candidates (A1, A3, A4 and A5) and 5 biolistic gun-derived transgenic lines (B1, B2, B3, B5 and B6) (Fig. 2a, 2b). The results show that the two-step selection procedure with increased selective agent concentration at the second step eliminates escapes and false transformants efficiently.

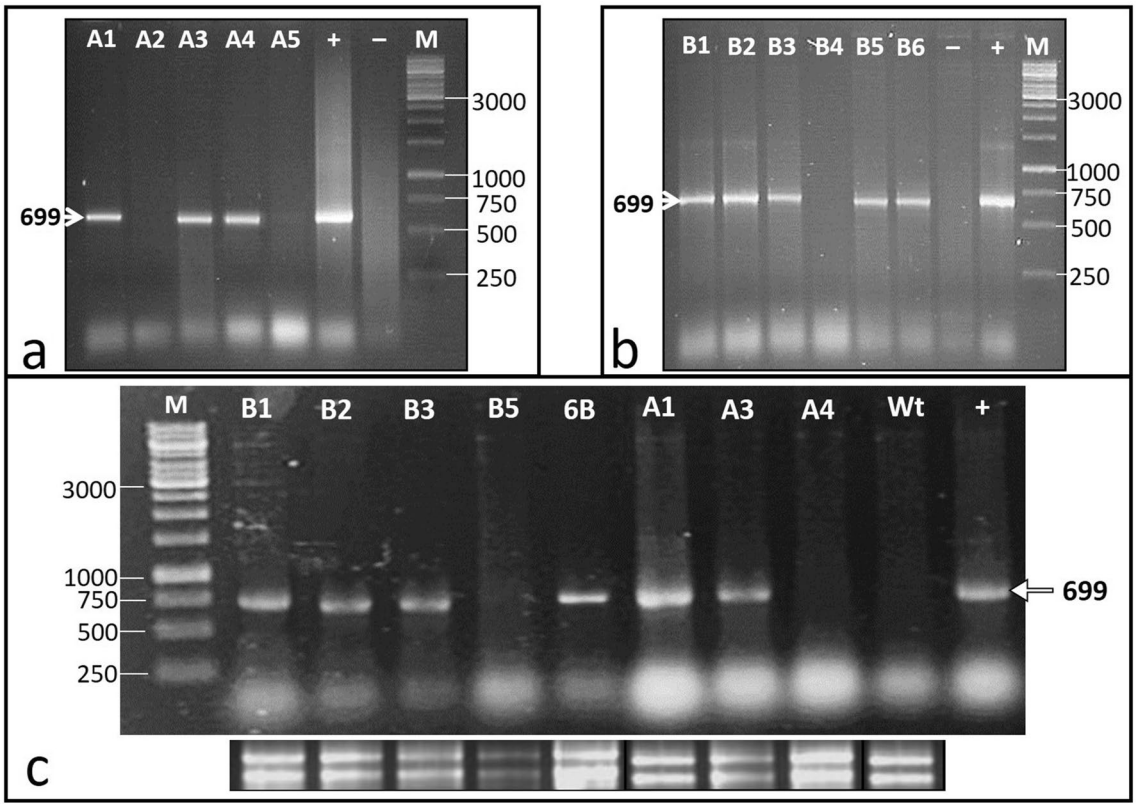

Fig. 2 Agarose gel electrophoresis of PCR products on genomic DNA of Petunia *AtSOS3* transgenic candidates selected by the *Agrobacterium* (**a**) and biolistic gun (**b**) transformation methods. The *AtSOS3*-specific 699 bp band was observed in some of the selected plants. (**c**) Analysis of PCR products on the cDNA synthesized from PCR-positive selected plants. Candidates marked as A1 and A3 produced by the *Agrobacterium* transformation method, and B1, B2, B3 and B6 produced by the gene gun method, showed the desired band which indicates the presence of *AtSOS3* gene transcripts in these plants

PCR-positive plant lines were used for further experiments. Note that the plants from the line A5, which showed a weak signal in the PCR experiment, were lost and were hence excluded from further analysis.

Evaluation of AtSOS3 expression in transgenic candidates by RT-PCR

The 699 bp *AtSOS3*-specific PCR fragment was amplified from cDNA samples of at least 6 transgenic lines out of 8 PCR-positive lines (Fig. 2c). These results demonstrate that the *AtSOS3* is successfully transcribed in 75% of the transformants.

In vitro *assessment of salt tolerance in transgenic lines*

All of the remaining PCR-positive lines were subjected to high levels of NaCl in in vitro conditions to evaluate their salinity tolerance at early stages of plant development, when the plants are generally more sensitive to salinity stress [40]. The results, some of which are presented in Fig. 4, showed that a concentration of 100 mM NaCl is suitable to differentiate plant lines with higher salt tolerance than the wild-type in Petunia. At this concentration, unlike wild-type plants, the transgenic lines produced roots efficiently (Fig. 2). An NaCl concentration of 200 mM had deleterious effects on all of the resistant lines, and therefore, is not recommended for selection purposes in future experiments. The 300 mM NaCl concentration was fatal to all plant lines, and was therefore excluded from further analysis.

Statistical analysis of data obtained for three characters (leaf number, stem length and root length) in the in vitro experiments indicated that 4 out of 6 RT-PCR-confirmed transgenic lines (A1, A3, B2 and B6) have significantly better performance in comparison to the wild type (Fig. 4). The differences were particularly noticeable for root length at 100 mM NaCl concentrations (Fig. 3 and 4). Two RT-PCR-positive lines including B1 and B3, as well as two lines (B5 and A4) that were not positive in the RT-PCR experiment (Fig. 3c), showed similar performance to the wild type in most cases, especially in root formation. These results suggest that the transgenic lines A4 and B5 do not support *AtSOS3* transcription, but that in the B1 and B3 lines

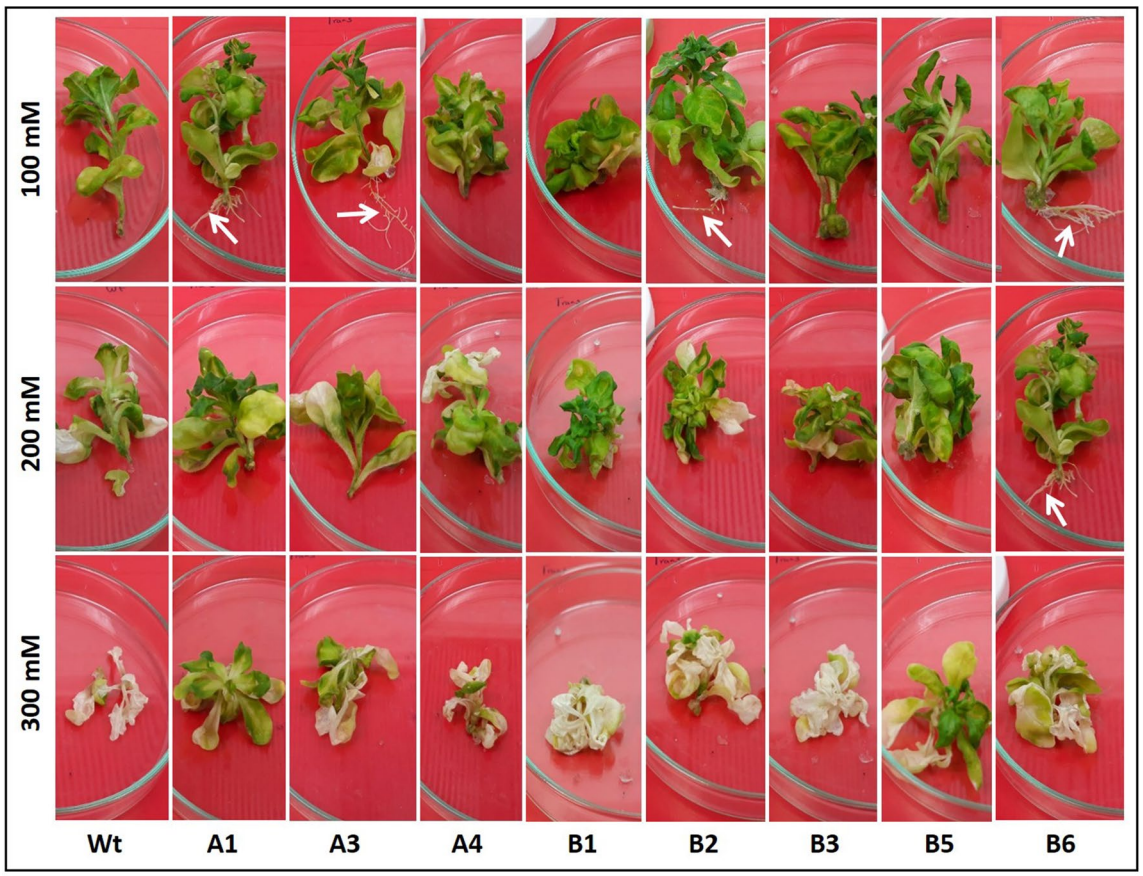

Fig. 3 Evaluation of tolerance of selected lines to different concentrations of NaCl in in vitro condition after 40 days. As indicated on the picture, root formation in 100 mM NaCl concentration appeared to be a good indicator for detecting plant lines with higher salt tolerance compared to control plants in in vitro conditions. The 200 mM NaCl concentration was inhibitory for root formation even in transgenic plants. None of the plant lines were able to survive at the 300 mM NaCl level

production of functional AtSOS3 is blocked post-transcriptionally. Although the results from data analysis for 200 mM NaCl are largely in agreement with the data observed for 100 mM NaCl, the differences are diminished by application of the more stringent NaCl concentrations. Taken together, our results and other considerations [41, 42], indicate that evaluation of root formation at 100 mM NaCl concentrations is a highly efficient indicator for salt tolerance in Petunia in vitro (Fig. 4). It has been previously shown that roots of transgenic plants overexpressing SOS1, lose less K^+ in response to NaCl than control plants [43], allowing their growth and development in higher salt concentrations.

Growth of transgenic lines under salinity stress in greenhouse

The 4 transgenic lines which showed superior performance in the in vitro salt tolerance experiments (A1, A3, B2 and B6) were subjected to greenhouse salt-tolerance experiments. The results showed that in comparison to the wild type, 3 out of 4 lines (A1, A3 and B6) perform better in growth and development in soil that was irrigated with either 100 or 200 mM NaCl solution (Fig. 5). The line B2, on the other hand, did not perform better than the wild type, probably because of its relatively weak root system (see Fig. 4).

Discussion

Salinity is one of the major factors limiting efficient crop production in agricultural fields. Petunia is a model plant of the *Solanaceae* family that is increasingly used in studies on salinity stress. It can tolerate up to 80 mM salt concentration, at which slight wilting and yellowing of the leaves occurs, but the plants can continue to grow [44]. In recent years, development of molecular methods for genetic transformation in plants has led to production of salt-tolerant transgenic lines using genetic engineering techniques [39]. In this study, we aimed to produce petunia transgenic lines expressing Arabidopsis *SOS3* cDNA. To this end, we first

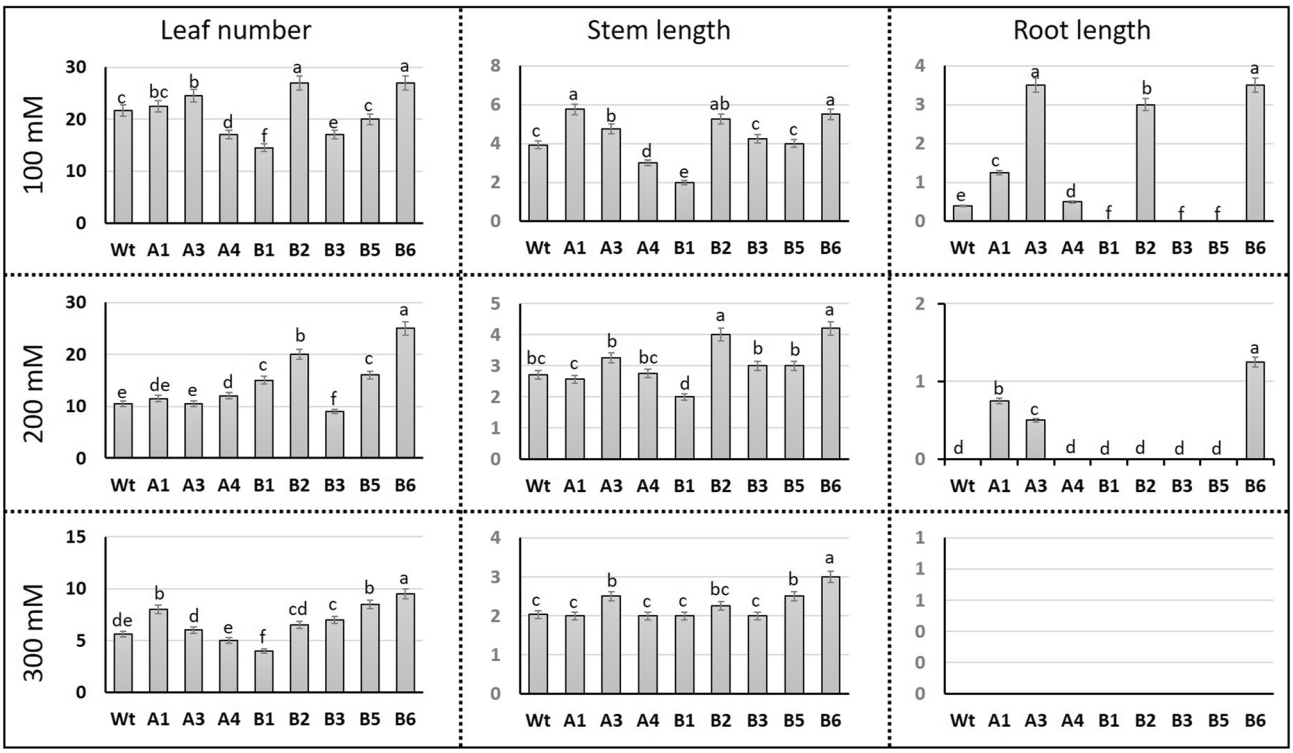

Fig. 4 Diagrams of mean comparison for leaf number, stem length and root length characters in transgenic lines after 40 days culture in different concentrations of NaCl. Different letters on columns show significant differences based on Duncan's multiple range test for the corresponding feature

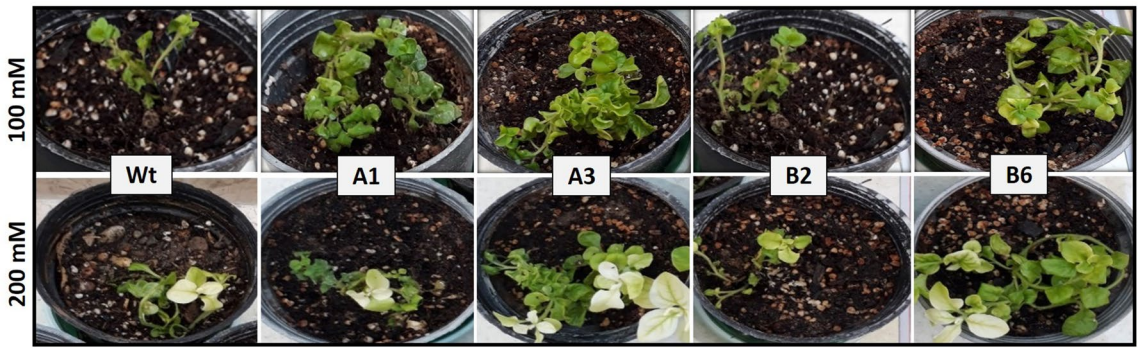

Fig. 5 Transgenic lines after 4 weeks of irrigation with 100 or 200 mM NaCl solution in greenhouse. Transgenic lines A1, A3 and B6, displayed higher NaCl tolerance in comparison to the wild type control plants

isolated *SOS3* cDNA from *Arabidopsis thaliana* seedlings by RT-PCR, followed by cloning in the pBIN61 vector to generate the transformation vector pBIN61-SOS3 (Fig. 1a). We then used *Agrobacterium* and biolistic transformation methods to integrate *AtSOS3* into the Petunia genome and selected a number of resistant plants on selective media (Fig. 1b, 1c). *Agrobacterium*-mediated transformation of leaf discs has been previously reported in *Petunia*×*hybrida* [45]. The biolistic transformation method, on the other hand, has been mainly used in Petunia for plastid transformation experiments [46, 47]. Here, we have successfully generated transgenic Petunia plants expressing Arabidopsis *SOS3* cDNA, using both methods with comparable efficiency (Fig. 2). Four transgenic lines (2 from each method) showed significantly higher salt tolerance in comparison to the wild-type plants, as demonstrated by in vitro and greenhouse analysis (Figs. 3, 4, 5). The results show that the SOS signaling machinery has great potential to improve salt tolerance in Petunia by overexpression of key factors involved in this pathway, such as the SOS3 calcium-sensing protein.

So far, little information has been reported about the SOS pathway in Petunia plants. In a recent study by Villarino et al. using the RNA-seq method to study the Petunia transcriptome response to NaCl stress, no overexpression of Na^+/H^+ antiporters was observed [30]. However, the 24 h of salinity stress used in these experiments may not have been sufficiently long to trigger the SOS pathway. Nevertheless, overexpression of a vacuolar Na^+/H^+ antiporter (AtNHX1) from Arabidopsis (*Brassicaseae*) in tomato plants (*Solanaceae*) resulted in a significant increase in salinity resistance [48]. This suggests that similar antiporter-based salinity tolerance mechanisms may be present in *Solanaceous* plants. For more evidence, we performed a Blast analysis to search for sequences with high similarities to *AtSOS3* in the *Solanaceae* plant family. The results showed that the calcineurin B-like proteins detected in a number of plants of the *Solanaceae* family are very similar to *AtSOS3* (Fig. 6 and Supplementary File 1). However, in the primer binding sites used in this study for *AtSOS3* amplification, especially the reverse primer, significant differences were observed between Arabidopsis and *Solanaceae* plants. This may explain why the heterologous SOS3-related cDNA fragments were not amplified from wild-type Petunia plants (Fig. 2). We detected a lysine-rich sequence in the 5′ region of the *Arabidopsis SOS3* gene, which was not found in the *Solanaceae* species (Fig. 6). This led us to incorporate three other *Brassica* plant species (*Brassica napus*, *Capsella rubella* and *Camelina sativa*) in the alignment experiments. The results show that this lysine-rich region is found in most plants of the *Brassicaceae* family (Fig. 6 and Supplementary Files 1 and 2). However, the number of lysine repeats in *B. napus* was less than the other two species (Fig. 6). Interestingly, the lysine genetic code in this region was exclusively "aag" in *Brassicaseae* species, possibly generated by duplication events, whereas the *Solanaceae* species contain only a single lysine amino acid in this region with the "aaa" codon (Fig. 6. and Supplementary File 1). We also observed similar results for a tomato calcineurin B-like protein, described as *SOS3* with the accession number AB675686.1 (Fig. 6. and Supplementary Files 1 and 2).

The use of SOS genes belonging to a different plant family to develop lines resistant to salinity stress has been reported previously. In *Festuca arundinacea* (*Poaceae*), for example, transgenic plants with higher salt tolerance have been produced by co-expressing Arabidopsis SOS genes [24]. Overexpression of an SOS2-like protein from apple (*Malus domestica*; *Rosaceae*) also conferred salt tolerance in tomato plants [49]. The *Brassica juncea SOS3* functionally complemented *Atsos3* mutants in Arabidopsis [32], demonstrating a strong functional conservation of SOS pathway in *Brassicaceae*. Similarly, heterologous expression of SOS1, SOS2 and SOS3 from the woody plant *Populus trichocarpa* (*Salicaceae*), could rescue salt-sensitive phenotypes of the corresponding Arabidopsis *sos* mutants [50]. Among

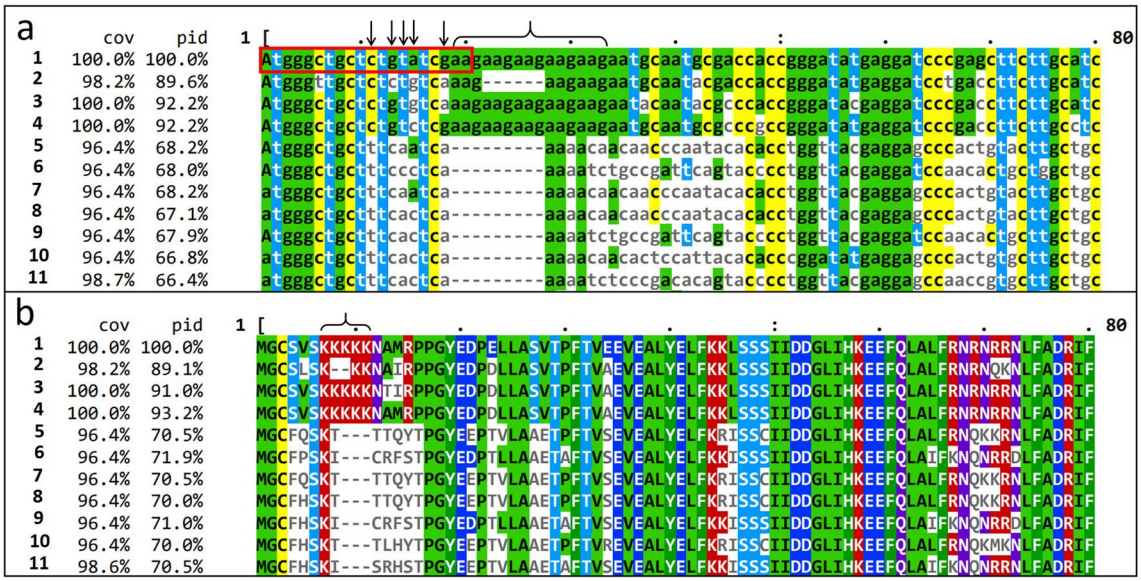

Fig. 6 A part of nucleic acid (**a**) and protein (**b**) alignment outputs for 7 examples of calcineurin B-like protein sequences from *Solanaceae* species, including *Nicotiana tabacum* (5), *Solanum lycopersicum* (6), *N. sylvestris* (7), *S. attenuata* (8), *S. pennellii* (9), *S. tuberosum* (10), and *Capsicum annum* (11), which show high similarity to *AtSOS3* (1), as revealed by blast analysis. For better comparison and output reliability, three more examples of calcineurin B-like protein sequences from *Brassicaseae* including *Brassica napus* (2), *Capsella rubella* (3) and *Camelina sativa* (4) were included. A lysine-rich region was identified in the N-terminal part of proteins belonging to the *Brassicaceae* species (Brackets). The *AtSOS3*-specific forward primer nucleotide sequence is indicated by a red box. Several nucleotides showed mismatches (black arrows) in this area, especially between the two plant families

Solanaceae species, *Nicotiana tabacum* overexpressing the *SOS1* gene from *A. thaliana* has been shown to perform well against salt stress [43]. In this study, we demonstrated that the overexpression of *SOS3* gene from *A. thaliana* can improve the salt tolerance in *Petunia* × *hybrida*. Our results together with previously reported documents show that, despite structural differences among DNA and protein sequences of SOS genes in different plant species, the SOS pathway is functionally highly conserved among plant families to trigger salt stress signaling response.

Conclusion

In this study, we produced Petunia transgenic plants expressing heterologous *SOS3* gene from Arabidopsis. Even though relatively high nucleic acid sequence variation was observed for *SOS3* mRNA among the *Solanaceae* and *Brassicaseae* plant species, the AtSOS3 protein appeared to be functional in Petunia and resulted in increased salt stress tolerance in transgenic plants. Our results, in addition to being important in petunia breeding programs to increase salinity tolerance, provide further evidence for conservation of the SOS salt stress response pathway among different plant families.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07495-x.

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Author Contributions KM: experimental work; MA: design and supervision, manuscript preparation and review; MP: co-supervision.

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Declarations

Competing interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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