Lcsain3, A Novel Gene From Sheepgrass, Regulates Arabidopsis Seed Germination And Seedling Growth Under Salt Stress

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Original Article

Keywords: Sheepgrass, Chloroplast, Salt stress, Seed germination

Posted Date: February 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-169077/v1

Abstract

Sheepgrass is a perennial native grass species with an aggressive and vigorous rhizome system, and it can tolerate high levels of salt stress. Many salt stress-responsive genes have been identified in sheepgrass. Here, we identified and characterized a novel salt-induced gene, *LcSAIN3* (*Leymus chinensis* salt-induced 3), from sheepgrass. Expression analysis confirmed that *LcSAIN3* is induced by PEG, ABA and salt stress. Subcellular localization analysis indicated that the LcSAIN3 protein is mainly localized in the chloroplasts. The heterologous of *LcSAIN3* in *Arabidopsis* increases seed germination under various stress conditions. More importantly, the seedling survival, plant height and weight of the transgenic plants are higher than those of the WT plants under salt stress. The overexpression of *LcSAIN3* causes a relatively high accumulation of free proline; enhances SOD activity; and leads to the upregulated expression of several stress-responsive genes, such as *AtRAB26*, *AtRD29B*, *AtSOS1* and *AtP5CS1*. Our results suggest that *LcSAIN3* may be a useful gene for the molecular breeding to improve plants salt stress tolerance.

Key Message

Heterologous expression of a novel chloroplast protein gene LcSAIN3 from sheepgrass enhances tolerance to salt stress in Arabidopsis

Introduction

Soil salinization is a severe problem that affects plant growth, development and productivity worldwide (Munns and Tester 2008). The effects of salt on plants lead to osmotic, ion toxicity and oxidative stress (Deinlein et al. 2014; Isayenkov and Maathuis 2019; Munns and Tester 2008). Changes in physiological, biochemical, cellular and molecular processes in plants under salt stress have been investigated by many researchers (Deinlein et al. 2014; Ma et al. 2020; Reddy et al. 2011; Xiong et al. 2002; Zhao et al. 2019), while genetic sources for salt tolerance development in crops were also studied (Isayenkov 2019). Extensive numbers of transcription factor-encoding genes in response to salt stress have been identified, including DREB, bZIP, NAC, and MYB family genes (Bo et al. 2020; Hu et al. 2008; Liang et al. 2017; Wang et al. 2017; Yang et al. 2012), and overexpressing these genes can enhance salt stress tolerance in transgenic plants (Deinlein et al. 2014; Hao et al. 2011; He et al. 2012; Peng et al. 2011; Zhao et al. 2019). Previous studies suggest that the transcription factors can activate many stress-induced genes, such as LEA genes (*RD26*, *RD29A*, *RD29B*, and *RAB18*) and proline biosynthesis genes (*P5CS*), and the LEA proteins are mainly involved in protection to desiccation by acting as cellular dewatering protectants under stress conditions (Zheng et al. 2019). Proline plays an important role in osmoregulation, and can be also used as an active oxygen scavenger to stabilize protein and membrane structure under pressure (Deinlein et al. 2014; Szabados and Savoure 2010).Molecular regulatory networks related to salt stress are complex and have not been fully explored (Xu et al. 2019); thus, mining key and novel salt tolerance-related genes is required for developing breeding strategies to enhance salt stress tolerance in crops.

Sheepgrass (*Leymus chinensis* (Trin.) Tzvel) is a perennial gramineous plant species belonging to the *Leymus*, Triticeae, and Poaceae classification groups and is widely distributed on the eastern Eurasian steppe (Lu et al. 2019). This species can survive when the soil moisture content is less than 6% in the dry season, and grows well in environments of 600 mmol/L NaCl and 175 mmol/L Na_2CO_3 (Chen et al. 2013; Gao et al. 2016; Nevo and Chen 2010). Many stress-induced genes have been identified and characterized in sheepgrass using transcriptome sequencing, including *LcDREB2*, *LcDREB3a*, *LcDREB21*, *LcMYB1*, *LcWRKY5*, *LcP5CS*s, and *LcSAMDC*s (Cheng et al. 2013; Liu et al. 2017; Ma et al. 2014; Peng et al. 2011). In addition, several novel genes were discovered; *LcSAIN1* and *LcSAIN2* genes have been identified to improve the greening rate of cotyledons, root elongation, plant height, and survival under salt stress in the transgenic plants (Li et al. 2013; Li et al. 2013); and ectopic expression of *LcFIN1* and *LcFIN2* significantly increased freezing stress tolerance in transgenic Arabidopsis and rice (Gao et al. 2016; Li et al. 2019).

In this study, we characterized a novel gene, *LcSAIN3* from *s*heepgrass, and the expression of *LcSAIN3* was induced by salt stress. We found that the *LcSAIN3* gene can improve salt tolerance in Arabidopsis, and thus propose that *LcSAIN3* plays an important role in regulating salinity tolerance.

Materials And Methods

Plant materials, growth conditions and stress treatment

Sheepgrass variety Zhongke No. 1 (released from Institute of Botany, the Chinese Academy of Sciences) seeds were grown in a mixture of peat moss and vermiculite (2:1, v/v) in an incubator at 28 °C/16 °C under a 16 h light/8 h dark photoperiod. For analysis of specific expression in different tissues, the roots, stems, leaves, and seeds were collected from two-year-old sheepgrass plants under normal condition. For salt stress analyses, 4-week-old sheepgrass seedlings were immersed in the solution of 400 mM NaCl. Seedlings were treated with 100 mM abscisic acid (ABA) and 20% PEG6000 for drought stress treatment, respectively. A total of 40 plants were sampled at 0, 1, 3, 5, 12, and 24 h after various stresses treatments, and three replicates of each sample were collected, immediately frozen in liquid nitrogen and stored at -80 °C (Li et al. 2013; Li et al. 2019).

Arabidopsis thaliana (ecotype Columbia (Col-0)) and Tobacco (*Nicotiana benthamiana*) seeds were grown in a greenhouse under a 16 h light/8 h dark photoperiod with an average temperature of 23 °C.

Cloning and sequence analysis of the LcSAIN3 gene

In our previous study, a number of candidate salinity-induced transcripts were identified using transcriptome analyses of sheepgrass seedlings subjected to salinity stress or not (Chen et al. 2013; Li et al. 2013; Li et al. 2013). Among them, one transcript designated *LcSAIN3* was encoded by an unknown functional gene and was significantly induced by salt stress treatment. To obtain the full-length cDNA of *LcSAIN3*, 4-week-old sheepgrass seedlings under 400 mM NaCl stress for 12 h were harvested.

Total RNA was isolated using a TRIzol kit (Invitrogen, Carlsbad, CA, USA), and first-strand cDNA synthesis was performed with a SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA) according to the manufacturers' instructions. Full-length *LcSAIN3* cDNA was amplified using the primers 5'-GTAGCCCGTGAGGAAGTT – 3' and 5'- CACTAGAAGGGCCCCGAA – 3', and the cDNA of 5' RACE was used as a template. The amplification conditions were as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. All of the PCR products were cloned into a pMD19-T vector and sequenced at Sangon Biotech (Shanghai Co., Ltd., China). The *LcSAIN3* sequences were analyzed using the BLAST program of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), and subcellular localization was predicted using the Plant-mPLoc program (Chou and Shen 2010).

RNA extraction and qRT-PCR analysis

Total RNA from Arabidopsis and sheepgrass seedlings was extracted using a TRIzol kit (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized, and qRT-PCR was conducted following the manufacturer's instructions using a PrimeScript™ PCR Kit (TaKaRa, Dalian, China). The cDNA template was amplified with a qRT-PCR system (Roche Light Cycler 480 II, Switzerland), and the data were quantified using the comparative $2^{-\Delta\Delta CT}$ method after the PCR program was run (Livak and Schmittgen 2001). The primer sequences for qRT-PCR are listed in Table S1. *LcActin* and *AtActin2* were used as internal controls for assessing the expression levels in sheepgrass and Arabidopsis, respectively.

Subcellular localization of LcSAIN3

The open reading frame (ORF) of the *LcSAIN3* was recombined into a pMDC45 vector (containing GFP) using the Gateway cloning while the construct was introduced into *Agrobacterium tumefaciens* (EHA105) cells. The intact leaves of 4-week-old wild-type tobacco (*N. benthamiana*) plants were injected with *A. tumefaciens* strain EHA105 harboring pMDC45 and pMDC45-LcSAIN3 (35S::GFP-LcSAIN3) and the infiltration was performed with a 2 ml syringe without niddle. The transgene-derived expression was monitored 2 to 3 d after infiltration by confocal microscopy on a Leica TCS SP5 microscope (Leica Microsystems, Wetzlar, Germany) (Li et al. 2019). Fluorophores were excited using an argon laser at 488 nm (GFP) and 382 bright-field images were collected using the transmitted light detector.

Construct creation and plant genetic transformation

The ORF of *LcSAIN3* was inserted into a pSN1301 vector under the control of the Cauliflower mosaic virus (CaMV) 35S promoter via the *BamH*I and *Kpn*I sites, after which the construct pSN1301-*LcSAIN3* plasmid was transformed into *Arabidopsis* using the floral dip method (Clough and Bent 1998). T1 transgenic *Arabidopsis* seeds were subsequently sterilized in 30% (v/v) bleach for 15 min, rinsed five times with sterile water, and selected on Murashige and Skoog (MS) agar supplemented with 30 μg ml^{-1} hygromycin, and the seedlings were confirmed by PCR analysis using the gene-specific primers.

Stress treatment for the transgenic plants

For the seed germination of transgenic Arabidopsis plants under stress treatments, T3 homozygous seeds of three transgenic plants (line 5, line 6 and line 8) were incubated at 4 °C for 2 d to break dormancy, after which they were germinated on half-strength MS media (sucrose concentration 15 g l^{-1}, agar concentration 5 g l^{-1} and pH 5.8) supplemented with different concentrations of ABA (1 and 2 μM), mannitol (200 and 300 mM), and NaCl (100, 125, 150, and 200 mM). The germination rate was scored daily for 7 d by observing radical protrusion, and at least 120 seeds from each transgenic line were evaluated. To test the salt tolerance of transgenic Arabidopsis, 3-week-old plants were treated with 200 mM NaCl for 3 weeks at 3-day intervals (Zhao et al. 2019).

Measurement of proline and (superoxide dismutase) SOD

Proline was measured as previously described (Shan et al. 2007), and total superoxide dismutase (SOD) activity was measured using nitro blue tetrazolium (NBT) reduction as previously described (Durak et al. 1993; Li et al. 2019).

Statistical analysis

The data concerning *Arabidopsis* seed germination and seedling growth parameters, proline content and SOD activity were subjected to ANOVA using the SPSS 21.0 program (IBM, Chicago).

Results

Isolation and expression analysis of LcSAIN3

Our previous studies identified many stress-induced genes using transcriptome sequencing techniques (Chen et al. 2013). Among those transcripts, the full length of stress-induced gene designated *LcSAIN3* was obtained by the RACE technique while its function was unknown.. The *LcSAIN3* (GenBank ID: MN901606) gene is 847 bp long and encodes a protein comprising 198 amino acids, which has high homology (72%) with a wheat cDNA clone, WT004_K04 (GenBank ID: AK331493), but rice does not have an endogenous homolog of *LcSAIN3.* Furthermore, the amino acid sequence of *LcSAIN3* shows 52% homology with predicted protein product of *Triticum turgidum* subsp. *Durum*, suggesting that *LcSAIN3* is a novel protein with unknown function.

The expression of *LcSAIN3* transcripts under control conditions was highly expressed in the stems, but less expressed in the leaves, seeds, and roots (Fig. 1a). To investigate the effects of stress conditions on the expression of *LcSAIN3*, sheepgrass seedlings were exposed to various abiotic stresses. QRT-PCR was performed using the total RNA extracted from 4-week-old sheepgrass plants subjected to stress treatments at different time intervals. As shown in Fig. 1, the transcript levels of *LcSAIN3* were significantly increased beginning at 3 h and reached highest at 5 h after NaCl treatment (Fig. 1b). Similar to the treatment with salt stress, treatment with PEG and ABA also led to a significant increase in expression levels at 5 h (Fig. 1c). These results indicate that salt, ABA and PEG treatments significantly induce the expression of *LcSAIN3* in sheepgrass seedlings.

Subcellular localization of LcSAIN3

The *LcSAIN3* protein was predicted by the Plant-mPLoc program (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) being a chloroplast protein. To determine the actual subcellular localization of *LcSAIN3 in vivo*, the ORF sequence was inserted into a pMDC45 vector fused to a GFP reporter gene under the control of the CaMV 35S promoter, after which the construct was infiltrated into tobacco (*Nicotiana tabacum*) leaf cells. As shown in Fig. 2 and Figure S1, the fluorescent signals from the 35S::GFP-LcSAIN3 fusion protein and autofluorescent signals of chloroplasts were merged together, and demonstrating that *LcSAIN3* is a chloroplast-localized protein.

Overexpression of LcSAIN3 **regulates seed germination under salt stress**

To confirm the function of *LcSAIN3* in response to salt stress, the *LcSAIN3* gene was transferred to Arabidopsis. Three lines homozygous (line 5, line 6, and line 8) with higher expression levels of *LcSAIN3* were selected for further analysis, and confirmed by qRT-PCR (Figure S2). The salt tolerance of the WT and transgenic *LcSAIN3* plants was then tested at seed germination stage. WT and transgenic seeds were germinated on MS media supplemented with different concentrations of NaCl (100, 125, 150, 175, and 200 mM) after 2 d of stratification, and no obvious differences were detected between the WT and transgenic plants on the MS media. However, the germination rates of the *LcSAIN3*-overexpressing lines were significantly higher than those of the WT plants in the presence of NaCl (Fig. 3a). As shown in Fig. 3b, the three transgenic lines (L5, L6, and L8) showed significantly higher germination rates (92%, 86%, and 81%, respectively) than did the WT plants under 150 mM NaCl (~ 55%) (Fig. 3b). Thus, *LcSAIN3* overexpression in Arabidopsis reduces sensitivity to salt stress at seed germination stage.

LcSAIN3 **overexpression in Arabidopsis enhanced seed germination under ABA and osmotic stress**

Our previous qRT-PCR analysis showed that the expression of the *LcSAIN3* gene was significantly induced by ABA and drought stress. To determine whether *LcSAIN3* also increases tolerance to ABA, the tolerance of transgenic lines and WT was examined. Under 2 μM ABA treatment, the germination rate of WT seeds was only 57%, while the germination rates of the three *LcSAIN3* overexpression lines, i.e. L5, L6 and L8 seeds were 79%, 87% and 75% respectively (Fig. 4a and 4b). When *LcSAIN3* overexpression lines and WT were grown on MS plates containing 200 and 300 mM mannitol, the transgenic plants germinated, while the germination of the WT plants was completely inhibited (Fig. 4c and 4d). This implies that *LcSAIN3* reduces the sensitivity to ABA and osmotic stresses at seed germination stage.

LcSAIN3 **overexpression enhances tolerance to salt stress in Arabidopsis seedlings**

To determine whether enhanced salt tolerance is present in adult seedlings, 3-week-old Arabidopsis plants were treated with 200 mM NaCl for 2 weeks at 3-day intervals (Zhao et al. 2019). Under salt stress treatment, the germination of the transgenic lines and WT were all inhibited, but the sensitivity of *LcSAIN3* overexpression lines to salt stress was reduced.. For example, most of the WT seedlings were bleached and wilted after 3-week salt treatment; in contrast, most seedlings of the three *LcSAIN3* overexpression

lines survived and had both green and yellow leaves. Three transgenic lines had significantly higher survival rate (93, 87, and 98%) compared to that of the WT plants (~ 30%) (Fig. 5a, b). Furthermore, plant height and weight were significantly greater for the transgenic plants than for the WT plants (Fig. 5c, d). These results indicate that *LcSAIN3* confers salt tolerance to Arabidopsis.

LcSAIN3 regulates proline accumulation and SOD activity in response to salt stress

To further explore the possible mechanisms that may be responsible for the improved tolerance of the transgenic plants to salt stress relative to the WT plants, proline content and SOD (a major antioxidant enzyme) activity were measured. Proline content in both transgenic plants and control plants increased under salt stress conditions, but the increased levels were significantly higher in the transgenic plants than in the control plants (Fig. 6a). Further, the SOD activity was also significantly higher in the transgenic plants than in the wild-type plants under salt stress ($P < 0.01$) (Fig. 6b). Taken together, our results indicate that *LcSAIN3* overexpression in Arabidopsis increases the proline content and SOD activities under salt stress.

LcSAIN3 overexpression alters the expression of salt-related genes in Arabidopsis plants

The expression levels of several known salt stress-responsive marker genes were compared between the transgenic *Arabidopsis* lines and WT plants using qRT-PCR under salt stress condition. The functional genes *RAB26* and *RD29B* exhibited increased expression levels in the transgenic plants compared with the WT plants under salt stress (Fig. 7). Furthermore, *SOS1* and *P5CS1* also exhibited increased expression levels in the transgenic lines (Fig. 7). Altogether, these data suggest that *LcSAIN3* confers salt stress tolerance to plants by upregulating the expression of salt stress-responsive genes.

Discussion

Sheepgrass is an important forage grass as well as an environmentally friendly native grass species in China. It has high yield with high protein content, better palatability, strong regeneration ability, strong cold and drought resistance, as well as salt-alkali resistance (Chen et al. 2013; Lu et al. 2019). Our previous studies demonstrated that the novel genes *LcFIN1* and *LcFIN2* from sheepgrass enhance tolerance to low temperature in Arabidopsis and rice, while overexpressing *LcSAIN1* and *LcSAIN2* could enhance the salt stress resistance of transgenic plants compared with wild-type plants (Gao et al. 2016; Li et al. 2013; Li et al. 2013; Li et al. 2019). The isolated salt-induced gene, *LcSAIN3* from sheepgrass in present study has high homology (72%) only with a wheat cDNA clone, WT004_K04 (GenBank ID: AK331493). To further investigate the role of *LcSAIN3* in the plant response to various stresses, the gene was overexpressed in *Arabidopsis*, as genetic transformation in sheepgrass is still very difficult (Wang et al. 2009).

We found that the expression of the *LcSAIN3* gene was significantly induced by salinity, PEG, and ABA stresses, and heterologous of *LcSAIN3* gene in Arabidopsis led to an increase in tolerance to NaCl, ABA and PEG stress at germination stage. Further, the tolerance of transgenic plants to salinity was markedly

enhanced during the seedling growth stage. Moreover, our preliminary results showed LcSAIN3 is a chloroplast-localized protein (Fig. 2). Previous studies demonstrated that chloroplast proteins play a vital role in plant growth and development and participate in various abiotic stress responses (Li et al. 2019). CEST, a novel chloroplast protein, can reduce photooxidative damage and enhance tolerance to multiple environmental stresses in transgenic Arabidopsis (Yokotani et al. 2011). Heterologous expression of a chloroplast outer envelope protein from *Suaeda salsa* could enhance oxidative stress tolerance and induce chloroplast aggregation in transgenic Arabidopsis plants (Wang et al. 2012). In addition, overexpression of chloroplast-localized rice OsRH58 is involved in the stress response and can improve seed germination and seedling growth under salt stress conditions (Nawaz and Kang 2019). Thus, novel chloroplast protein, *LcSAIN3* identified in this study plays a positive role in the responses to salt stress and other abiotic stresses.

In plants under salt stress, the accumulation of proline has multiple protective functions, including osmotic protection and ROS scavenging (Zsigmond et al. 2012). SOD, a kind of antioxidant enzyme, also plays an important role in scavenging ROS and protects against oxidative stress under salt conditions (Xu et al. 2019).In this study, proline levels and SOD activities are significantly higher in the *LcSAIN3* overexpression lines than in the wild type under salt stress. SOS pathway is a key regulator of Na^+ homeostasis, for example *via* SOS1 (Isayenkov and Maathuis 2019). *P5CS1* is a key enzyme in the proline biosynthesis pathway, and it functions as a positive regulator in proline accumulation and plant responses to salt tolerance (Bo et al. 2019; Xu et al. 2018). Our results indicated that *AtP5CS1* and *AtSOS1 a*re expressed at much higher levels in the *LcSAIN3* transgenic plants than in the WT plants under salt stress. Furthermore, in transgenic Arabidopsis plants expressing *LcSAIN3*, the transcription levels of the ABA-dependent genes *AtRD26* and *AtRD29B* are higher than those in the WT plants under salt stress. The expression of these genes has been found to be induced by salinity, and they play important roles in abiotic stress. *RD29B* is involved in ABA-dependent signaling pathways (Han et al. 2019; Msanne et al. 2011). These findings indicate that the improved tolerance of the transgenic plants under salinity stress might partly result from the enhanced proline content, SOD activity, while the salt-induced gene expression levels might result from the overexpression of *LcSAIN3*.

In conclusion, we characterized a novel chloroplast-localized protein, *LcSAIN3*, and the protein plays a positive role in regulating salt stress. Constitutive expression of *LcSAIN3* in Arabidopsis accelerates seed germination and increases seedling survival when subjected to salt stress by improving proline levels and SOD activities and by regulating the expression of some stress-responsive genes.

Abbreviations

ANOVA analysis of variance

CaMV cauliflower mosaic virus

GFP green fluorescent protein

NBT nitro blue tetrazolium

ORF open reading frame

PEG polyethylene glycol

qRT-PCR quantitative real-time PCR

RACE rapid amplification of cDNA ends

ROS reactive oxygen species

SOD superoxide dismutase

WT wild type

LEA proteins late embryogenesis abundant proteins

Declarations

Author contribution statement: G.L. and X.L. planned and designed the research. X.L. and W.Y. performed the experiments. J.J., P.Z., and D.Q. made much contribution to plant material collection and experimental management. Q.L. and S.C. analyzed the data. X.L. and W.Y. wrote the manuscript. GL and L.C. edited the manuscript and gave the final approval the manuscript. All authors read and approved the final manuscript.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 32071869,31601996), the Science and Technology Major Project of Inner Mongolia Autonomous Region of China, the National Key Research and Development Program of China (Grant No. 2018YFD1001000, 2016YFC0500700), Science and technology cooperation project between Jilin Province and Chinese Academy of Sciences (2019SYHZ0035) and Kulun Banner Science and Technology Poverty Alleviation Project of Chinese Academy of Sciences.

Compliance with ethical standards

Conflict of interest: The authors declare no conflflict of interest.

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Figures

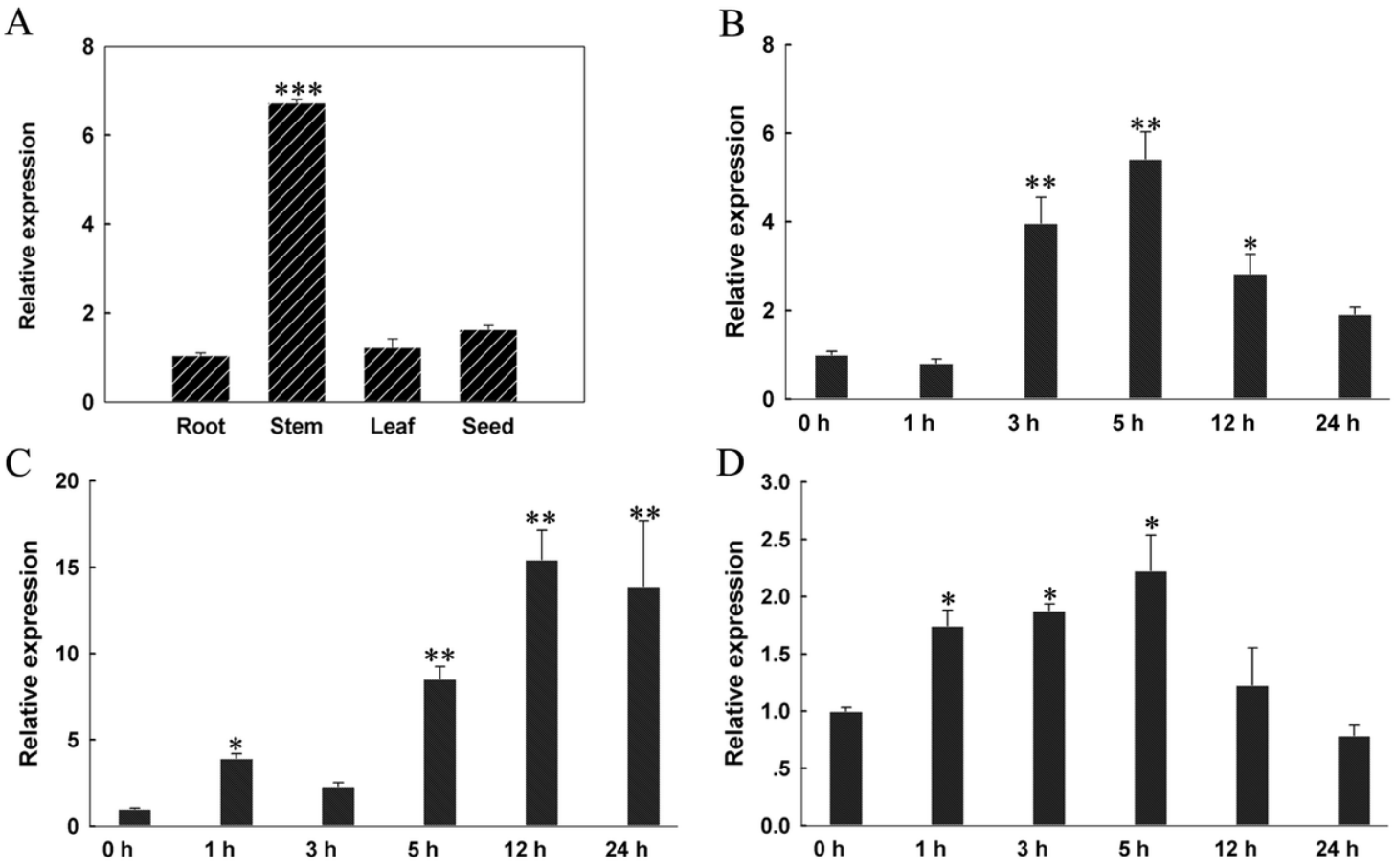

Figure 1

Expression patterns of LcSAIN3 in various tissues and abiotic stress conditions. a Expression of LcSAIN3 in roots, stems, leaves, and seeds. b Expression patterns of LcSAIN3 during salt treatments. c Expression patterns of LcSAIN3 during ABA and PEG treatments, respectively. The sheepgrass Actin gene is used as the internal reference gene for normalization and data represent means±SDs of three replicates. ***, **, and * indicate significant differences at P < 0.001, P < 0.01, and P < 0.05, respectively.

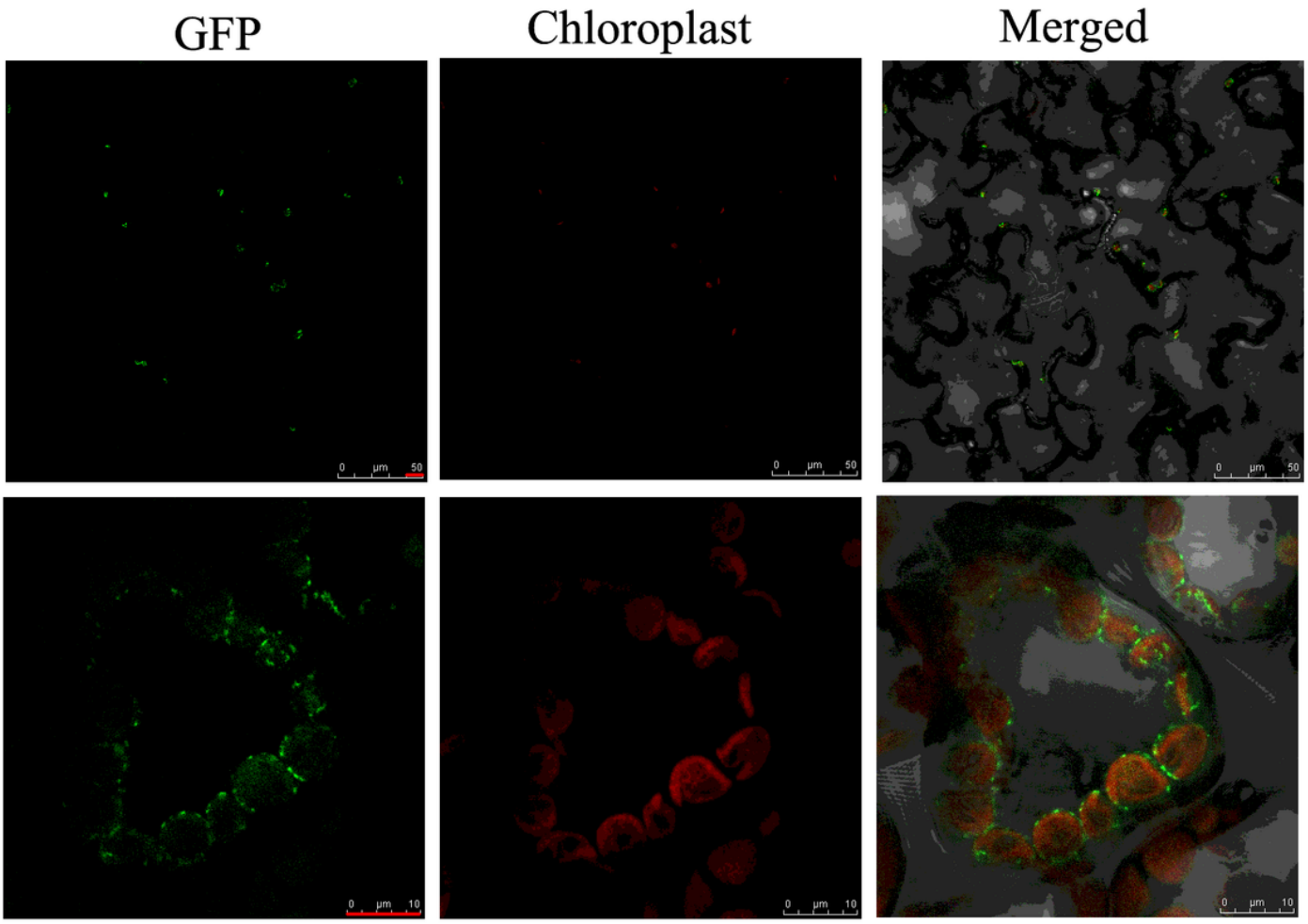

Figure 2

The subcellular localization of LcSAIN3. The GFP signals arising from the 35S::GFP-LcSAIN3 fusion protein expressed in tobacco leaves were detected using a confocal microscope. Red signals represent chloroplast auto fluorescence. Scale bar = 10 μm.

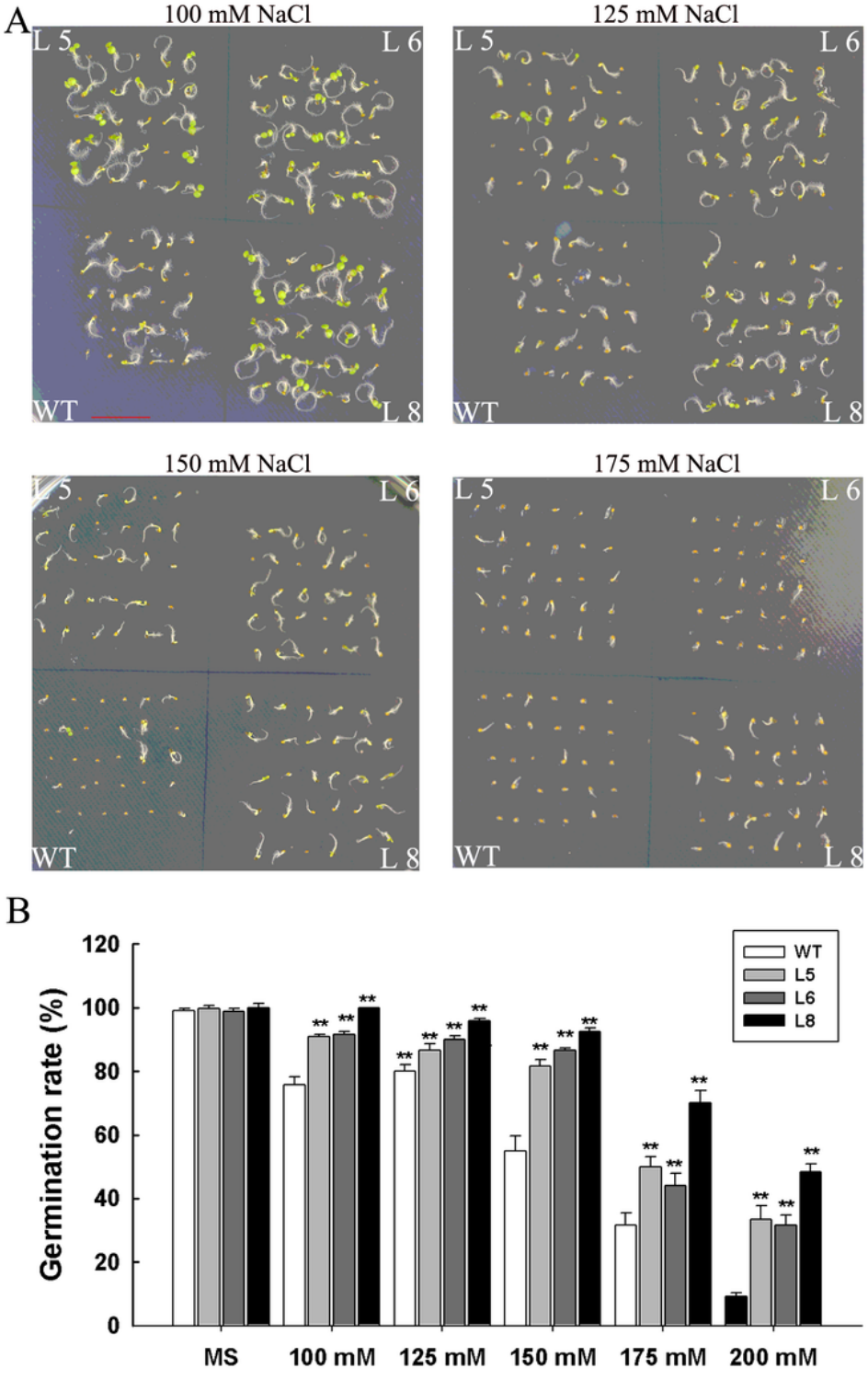

Figure 3

Salt stress tolerance of LcSAIN3 transgenic Arabidopsis plants at germination stage. (A) The WT and LcSAIN1-over-expressing seeds in the presence of various concentrations of NaCl (0, 100, 125, 150 or 175 mM). (B) Seed germination rate under salt stress. Thirty seeds were allowed to grow for 7days after sowing and each column represents an average of three replicates, and the bars indicate standard

deviations (SDs). ** indicates significant differences in comparison with the control at P < 0.01. Scale bar = 1 cm.

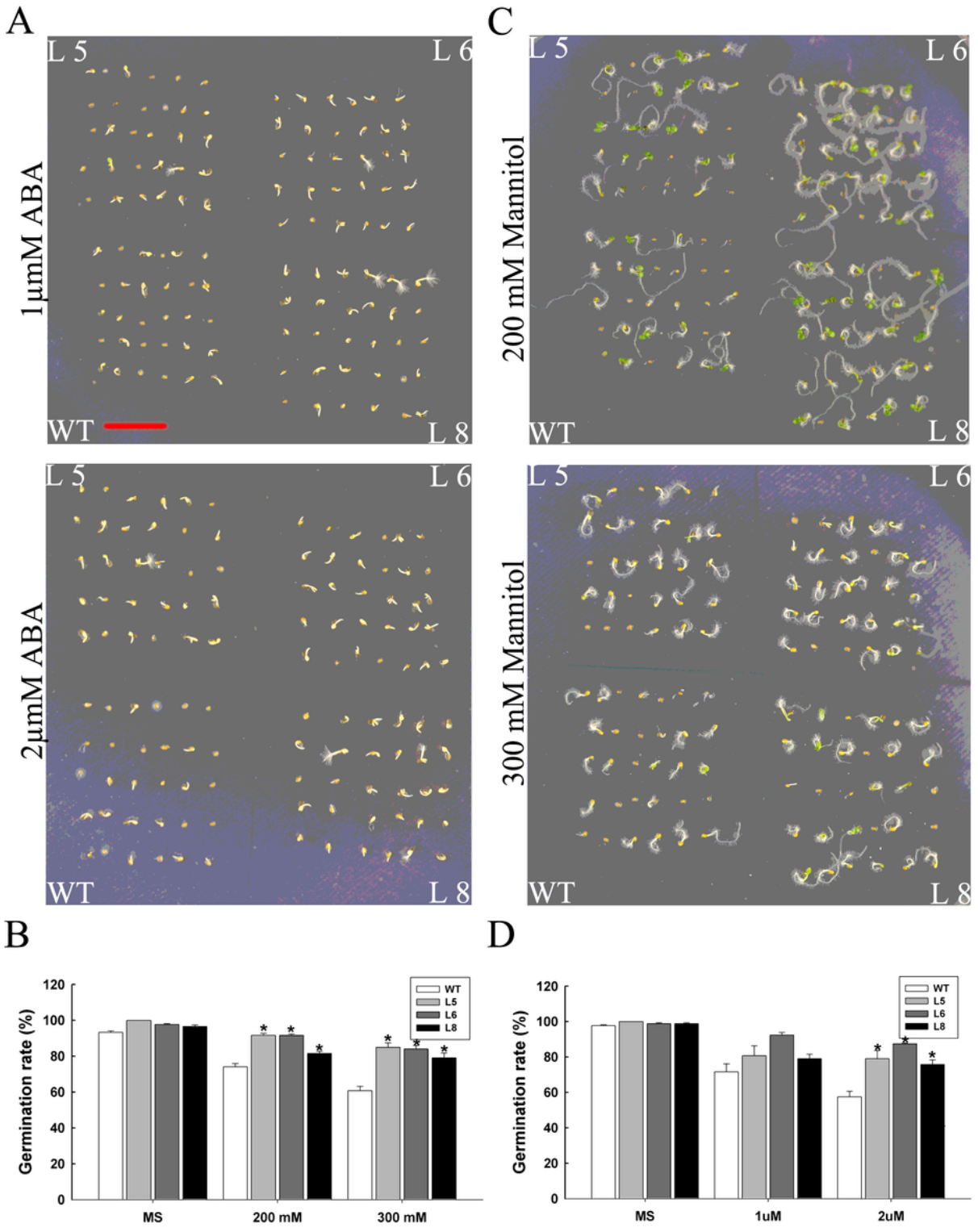

Figure 4

ABA and mannitol stress tolerance of LcSAIN3 transgenic Arabidopsis plants at germination stage. A and B seed germination rates of the WT and LcSAIN3-expressing transgenic line was measured on MS medium containing 1μmM and 2μmM ABA. C and D seed germination rates of the WT and LcSAIN3-

expressing transgenic line was measured on MS medium containing 200mM and 300mM mannitol. Thirty seeds were allowed to grow for 7days after sowing and each column represents an average of three replicates, and the bars indicate standard deviations (SDs). * indicates significant differences in comparison with the control at P < 0.05. Scale bar = 1 cm.

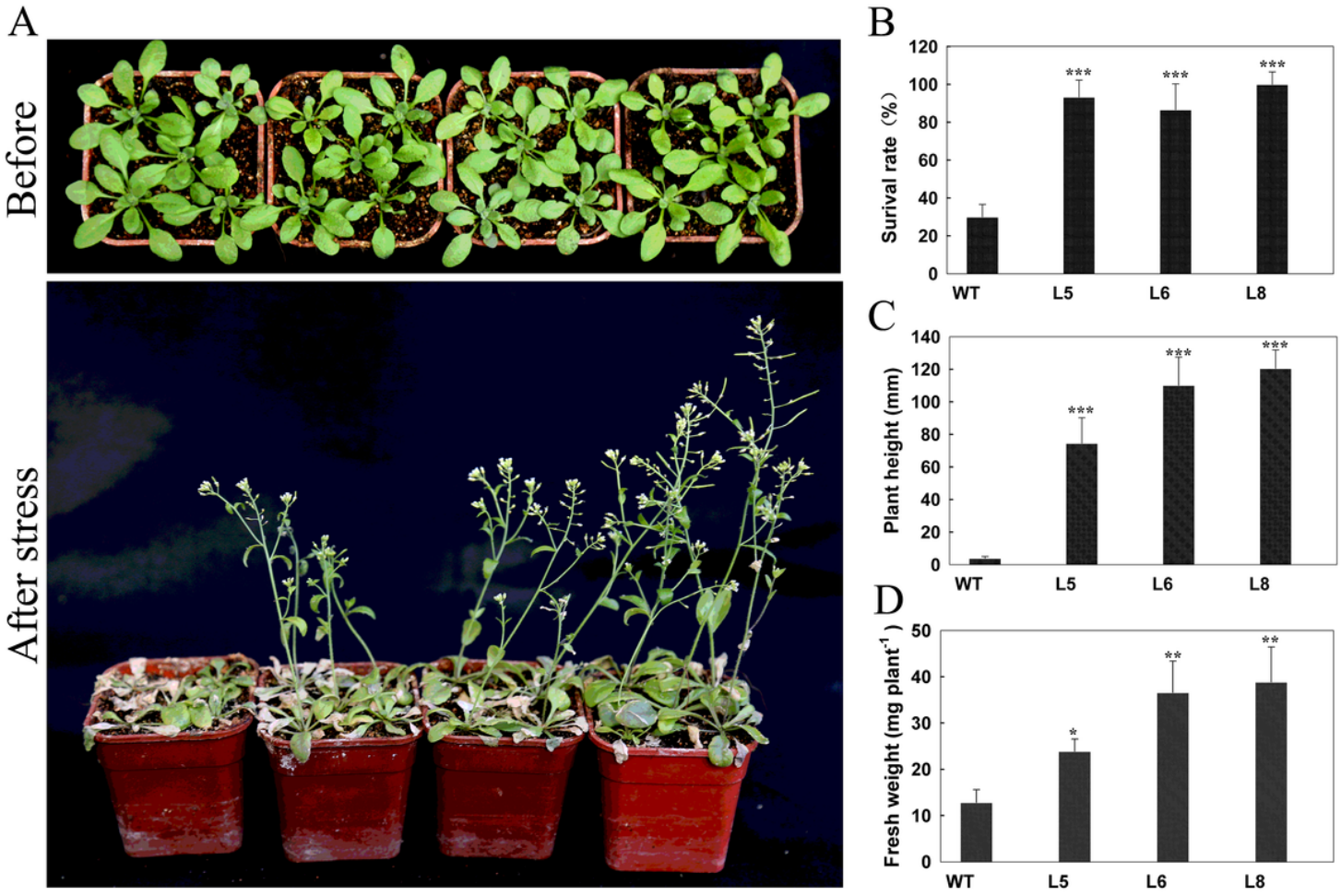

Figure 5

Enhanced tolerance to salt stress in LcSAIN3-overexpressing plants at seedling stage. A Three -week-old seedlings in soil were treated with 200 mM NaCl for 3 weeks. The seedling survival rates (B) and plant height (C) and plant weight (D) were scored after 3-week treatment. The mean and standard error were obtained from three biological replicates, and *** indicate statistically significant differences (P <0.001).

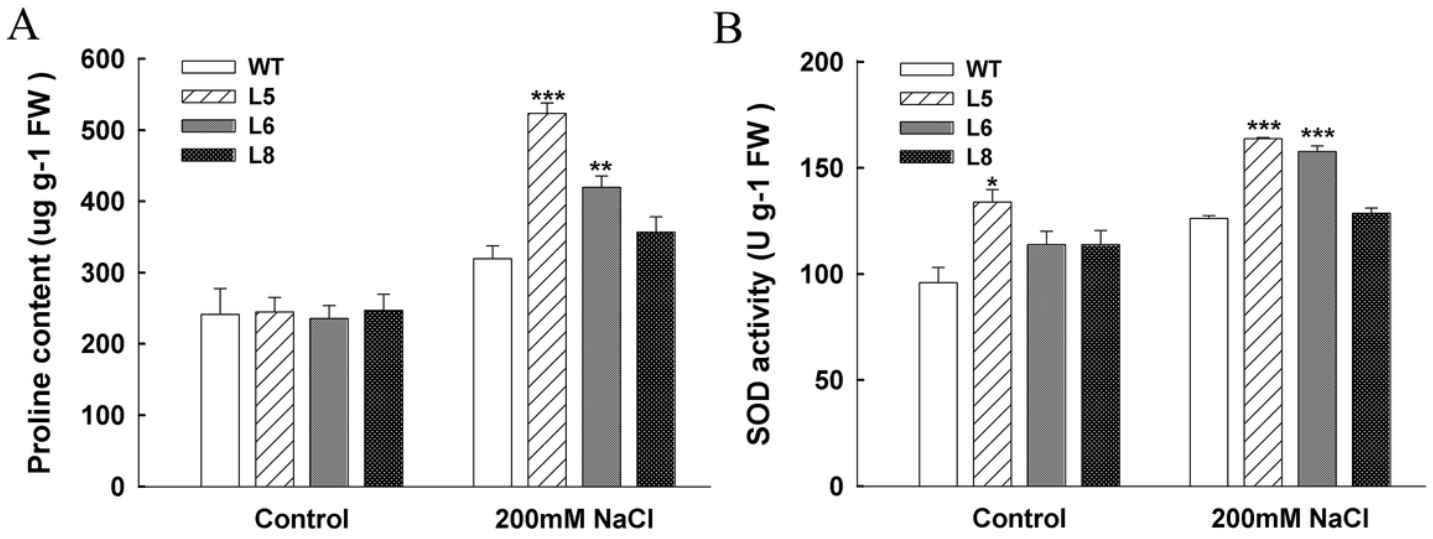

Figure 6

Physiological index analyses of the wild-type and LcSAIN3-overexpressing plants. Proline contents (A) and antioxidant enzymes levels (B) in transgenic and WT seedlings exposed to 150 mM NaCl for 2 d. The bars indicate standard deviations, and results are from three independent biological replicates. ***, **, and * indicate significant differences in comparison with the control at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

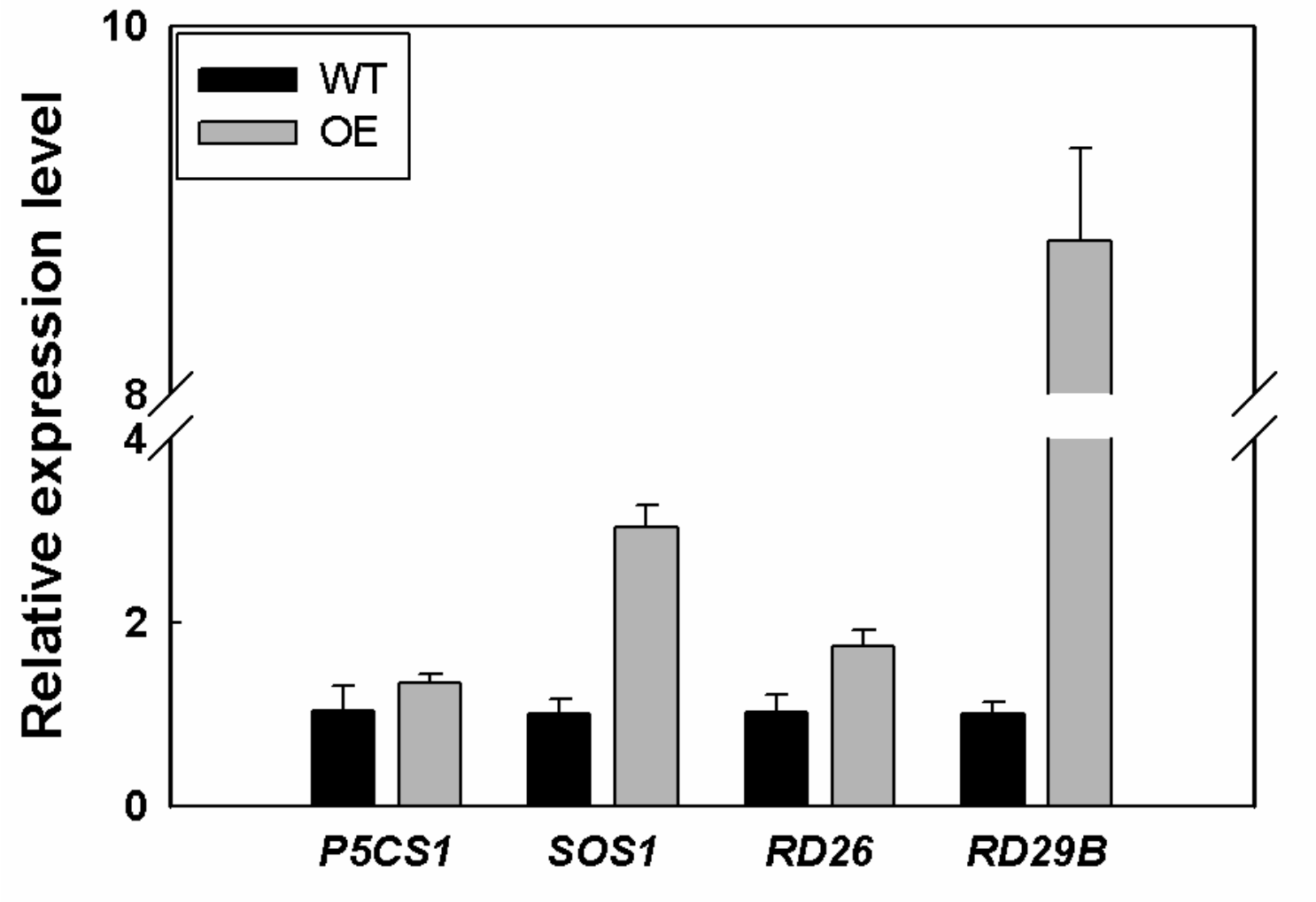

Figure 7

Relative expression levels of five stress-associated genes in the LcSAIN3-overexpressing plants compared with wild-type under salt stress. Real-time PCR analysis of six stress-induced genes in transgenic Arabidopsis plants in the presence of 150mM NaCl. AtActin2 was used as the reference gene.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementary.pdf