

Functional Characteristics of The Stelar K⁺ Outward Rectifying Channel (EeSKOR) Gene in *Elytrigia Elongata*

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Abstract

As an important nutrient, K^+ plays a crucial role in plant stress resistance. It has been reported that the stelar K^+ outward rectifying channel (SKOR) is involved in loading K^+ into the xylem for its transport from roots to shoots. *Elytrigia elongata* is a perennial, sparsely distributed, rhizome-type herbaceous plant belonging to the wheatgrass family; it has high salt tolerance. Here, we isolated *EeSKOR* from decaploid *E. elongata* and investigated its function in transgenic tobacco. The results showed that *EeSKOR* was mainly expressed in the roots and was up-regulated with increasing salinity and drought intensity. Overexpression of *EeSKOR* in plants exposed to salt stress enhanced growth performance, increased SOD activity and chlorophyll content, significantly reduced H_2O_2 and MDA content, reduced Na^+ concentration, and increased K^+ concentration in transgenic tobacco plants compared with wild-type (WT) and null vector (Vector) plants. Our findings suggest that transgenic plants overexpressing *EeSKOR* could enhance K^+ transport from the roots to the aboveground parts to maintain K^+ steady-state in the aboveground under conditions of salt stress, thereby enhancing tobacco salt tolerance.

Introduction

Plant physiological drought leads to ionic imbalance in cells, cell membrane dysfunction, attenuation of metabolic activity, plant growth inhibition, and even cell death owing to excessive soil Na^+ concentrations^{1,2}. To cope with salinity stress, the strategy adopted by plant cells to compartmentalize Na^+ into vacuoles alleviates cellular Na^+ toxicity to maintain osmotic balance using Na^+ as an osmoregulation substance, thus improving salt tolerance of plants³. Under salt stress, as the Na^+ in the root xylem catheter is continuously unloaded into the xylem parenchyma cells, parenchymal membrane depolarization occurs, the stelar K^+ outward rectifying channel (SKOR) or non-selective ion external rectifying channel protein activity is activated, and the K^+ in the parenchyma cells is loaded into the xylem sap and transported to the aboveground part of the plant^{4,5}. The *SKOR* gene has also been cloned from maize (*Zea mays*), where it was mainly expressed in the root cortex and columnar cells and was responsible for discharging K^+ from columnar cells into xylem sap⁶. Gaymard et al.⁷ cloned *AtSKOR* from *Arabidopsis thaliana* and demonstrated that *AtSKOR* could mediate the loading of K^+ from xylem parenchyma cells to xylem sap, which would then be transported to the aboveground part with xylem sap flow by transpiration pull. In our previous studies, the high-affinity K^+ transporter *EeHKT1;4* was closely linked with salt tolerance, and there was a significant positive correlation between the expression level of *EeHKT1;4* in decaploid *Elytrigia elongata* ($2n = 70$, PI276399) roots and the selective ability of K^+/Na^+ , indicating that *EeHKT1;4* was a major component in maintaining K^+/Na^+ selectivity in the root system⁸. In fact, the K^+/Na^+ selectivity of plant roots under salt stress is controlled and regulated by multiple genes. Among them, HKT1 proteins unload Na^+ from the root xylem catheter into the xylem parenchyma cells, causing depolarization of their plasma membrane, thereby activating SKOR transport activity, loading K^+ from parenchyma cells into the xylem to the aboveground parts, maintaining the K^+/Na^+ selectivity and enhancing salt tolerance⁹.

As one of the ideal herbaceous plants for improving saline-alkali soil, *Elytrigia elongata* is a perennial, sparsely distributed, rhizome-type, herbaceous plant of the wheatgrass family. It is a close relative of wheat and has become an indispensable wild gene pool for improving wheat^{10,11}. Our preliminary research results showed that the salt-tolerant decaploid *E. elongata* was screened out from thirty-four accessions collected from twenty-one countries¹². It has also been established that the root system has a stronger K^+/Na^+ selectivity, which limits the absorption of Na^+ by the root system and reduces the concentration of Na^+ in the aboveground parts to maintain the concentration of K^+ in the plant. This increases the K^+/Na^+ ratio of the *E. elongata* plant, which is the main physiological mechanism of salt tolerance¹³. At the same time, we have cloned out the *EeSKOR* gene from the salt-tolerant decaploid *E. elongata* PI276399 using real-time fluorescence quantitative PCR (qRT-PCR) and the rapid amplification of cDNA ends (RACE) methods and conducted its full-length sequence analysis. However, the role of *EeSKOR* in salt tolerance of *E. elongata* remains unclear. To assess whether the overexpression of *EeSKOR* confers improved salt tolerance in plants, we introduced the *EeSKOR* gene into tobacco to determine growth performance, physiological indices, and Na^+ and K^+ concentrations in the transgenic tobacco plants, null vector plants, and wild-type (WT) plants subjected to salinity stress. The results indicated that *EeSKOR*-mediated compartmentalization of Na^+ into the vacuoles may play a key role in the salt tolerance of plants. This would provide potential benefits for generating engineered plants to increase tolerance to saline conditions.

Results

Cloning and sequence analysis of *EeSKOR*

Degenerate primers (*EeSKOR*-1 and *EeSKOR*-2) were designed according to the conserved homologous sequences of the SKOR protein of other plants. The cDNA synthesized by total RNA reverse transcription from the young roots of *E. elongata* was used as the template, the cDNA fragment of the *EeSKOR* gene was amplified by PCR, and the core fragment was 555 bp. Sequences of the 5' and 3' ends were obtained by RACE, which yielded products of 1136 bp and 1033 bp, respectively (Figure S1).

The full-length cDNA sequence, 2402 bp of *EeSKOR*, was obtained by splicing the DNAMAN software sequence. The ORF of *EeSKOR* was 2145 bp long and encoded a polypeptide protein consisting of 717 amino acid residues. The predicted protein had an isoelectric point of 8.29 and a molecular weight of 81.15 kDa. The cDNA sequence of *EeSKOR* was submitted to GenBank under accession number MK203848. The amino acid homology of *EeSKOR* with *TaSKOR* (*Triticum aestivum*), *TuSKOR* (*Triticum urartu*), and *AetSKOR* (*A. tauschii*) were 87.67%, 87.14%, and 86.09%, respectively. Analysis using the TMpred tool indicated that *EeSKOR* contained five transmembrane regions (Figure 1). The phylogenetic tree analysis showed that *EeSKOR* was closely related to the SKOR of wheat monocotyledons of Gramineae (Figure S2). These results indicate that the *EeSKOR* gene encodes the external rectifier K^+ channel protein.

EeSKOR plasmid with a GFP signal was transferred into tobacco leaves to observe the transient expression of EeSKOR in the epidermis of tobacco leaves. The results showed that EeSKOR-GFP was significantly localized on the plasma membrane and nucleus of tobacco epidermal cells under FV10-ASW laser confocal microscopy. Thus, EeSKOR was located in the plasma membrane and nucleus (Figure S3).

Analysis of *EeSKOR* gene expression patterns under salt and drought treatments

The expression levels of the *EeSKOR* gene in the roots, sheaths, and leaves of 4-week-old *E. elongata* seedlings treated with different NaCl concentrations for 24 h (0, 25, 50, 100, 150, and 200 mmol·L⁻¹) were analyzed. The results showed that the expression level of *EeSKOR* in roots increased with an increase in NaCl concentration (25–100 mmol·L⁻¹) but decreased slightly at 150–200 mmol·L⁻¹ NaCl. With the increase in NaCl concentration, the expression level of *EeSKOR* in sheaths and leaves showed a decreasing trend compared with that in the control. On the whole, it showed an expression trend of roots > leaves > sheaths (Figure 2a). The *EeSKOR* expression patterns in 4-week-old *E. elongata* seedlings under osmotic stress for 24 h (-0.5, -1.0, and -1.5 Mpa) were analyzed. The results showed that *EeSKOR* expression was upregulated with an increase in drought stress concentration, and in general, *EeSKOR* expression level was in the order of roots > leaves > sheaths (Figure 2b). Moreover, *EeSKOR* expression was induced and regulated by salt and osmotic stress.

Construction of plant expression vector of *EeSKOR* and molecular testing of positive lines of tobacco

According to the requirements of the Clontech Infusion seamless connection technology, *Nco* I and *Bgl* II restriction sites were introduced at both ends of the upstream and downstream primers of the ORF frame of *EeSKOR*, and the PCR products were amplified by RT-PCR and detected by 1.2% gel electrophoresis (Figure S4a). Then, the restriction sites on pCAMBIA1301 were double-digested by *Nco* I/*Bgl* II, and the large fragment was recovered and named pCAMBIA1301-A. The target gene *EeSKOR* was inserted into pCAMBIA1301 to obtain the recombinant plasmid pCAMBIA1301-35S-*EeSKOR*-Nos, and a specific band of approximately 2942 bp was obtained after *Hind* III/*Bgl* II double enzyme digestion (Figure S4b). The pCAMBIA1301-35S-*EeSKOR*-Nos vector was introduced into *Agrobacterium* GV3101 by the freeze-thaw method, and the target fragments of 2154 bp and 795 bp were detected by PCR *agrobacterium* and gel electrophoresis, which confirmed that the strain was positive (Figure S4c). This indicates that we have successfully constructed the plant expression vector of the *EeSKOR* gene of *E. elongata* (Figure S5).

To investigate the potential benefits of transferring *EeSKOR* to other plant species, we identified forty-five independent *EeSKOR* transgenic tobacco lines, Vector, and WT plants by qRT-PCR amplification. We observed relatively higher expression levels in L12 and L36 (Figure S6). Therefore, L12 and L36 were used in the following assays. Western blot analysis showed that the membrane proteins of isolation markedly increased the EeSKOR protein level in L12 and L36 compared to that in WT, Vector, and lower expression lines (L1 and L11). Moreover, the protein levels in L12 and L36 were higher than those in WT (Figure S6), indicating that heterologous expression of *EeSKOR* could enhance salt tolerance in transgenic tobacco.

Analysis of salt tolerance of transgenic tobacco plants

Under normal conditions ($0 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$), the wild-type (WT) plants, Vector plants, and transgenic plants all showed good growth performance, with the increase in NaCl salt concentration, the transgenic tobacco (L12 and L36) plants showed higher salt tolerance than the WT and Vector plants (Figure 3a). Plant height and dry weight significantly decreased with increasing salt concentration compared to those observed under normal growth conditions, but the dry weight and plant height of transgenic tobacco plants under salt treatment were significantly higher than those of WT and Vector plants (Figure 3b, c). In particular, under $200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment, the dry weight of L12 increased by 35 and 42%, and that of L36 increased by 39 and 46%, respectively, compared with that of the WT and Vector tobacco plants, the plant height of L12 increased by 39 and 47%, and that of L36 increased by 38 and 45%, respectively. Thus, it was demonstrated that the overexpression of *EeSKOR* under salt treatment significantly increased the biomass and plant height of transgenic tobacco plants.

As shown in Figure 4, under the treatment of $0 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$, there was no significant difference in the contents of H_2O_2 , MDA, Chl, and SOD activity of the WT, Vector, and transgenic tobacco plants. H_2O_2 , MDA, and SOD content increased gradually with an increase in external salt concentration, but the Chl content decreased greatly, compared to the values observed following $0 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment. Further, with $200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment, H_2O_2 content in transgenic tobacco L12 decreased by 36 and 39% and that of L36 decreased by 38 and 41%, respectively (Figure 4a), compared to that in the WT and Vector plants. MDA content of L12 decreased by 18 and 22% and that of L36 decreased by 16 and 20%, respectively (Figure 4b). In the $200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment, the Chl content of L12 increased by 118 and 114%, and that of L36 increased by 101 and 97%, respectively (Figure 4c). The SOD activity of L12 increased by 47 and 49%, and that of L36 increased by 48 and 50%, respectively (Figure 4d). This showed that the overexpression of the *EeSKOR* gene under salt treatment significantly reduced H_2O_2 content and MDA in transgenic tobacco plants while increasing activity and Chl content.

As shown in Figure 5, under normal conditions ($0 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$), there was no significant change in Na^+ and K^+ concentrations in the aboveground parts and roots of the WT, Vector, and transgenic plants. With an increase in salt treatment concentration ($50\text{--}200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$), Na^+ concentration in the aboveground parts and roots of transgenic tobacco plants significantly decreased and the K^+ concentration significantly increased, compared to the concentrations in the WT and Vector plants. In particular, with $200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment, the Na^+ concentration in the aboveground part of transgenic tobacco plant L12 decreased by 59 and 58%, respectively, and the Na^+ concentration in the aboveground part of L36 decreased by 65 and 64%, respectively (Figure 5a); the Na^+ concentration in the roots of L12 decreased by 28 and 30%, respectively, and that in the root of L36 decreased by 32 and 34%, respectively (Figure 5b). With the $200 \text{ mmol}\cdot\text{L}^{-1} \text{ NaCl}$ treatment, the K^+ concentration in the aboveground part of L12 was 2.1 times and 2 times higher than that in the WT and Vector plants, respectively, and the K^+ concentration in the aboveground part of L36 was 1.9 times and 1.8 times higher than that in the WT and Vector plants, respectively (Figure 5c); the K^+ concentration in the root of L12 was twice that in the roots of the WT and Vector plants, and the K^+ concentration in the root of L 36 was 1.6 times and 1.5 times that

in the roots of the WT and Vector plants, respectively (Figure 5d). Thus, the overexpression of the *EeSKOR* gene under salt treatment not only significantly reduced the Na⁺ concentration of transgenic plants but also increased the K⁺ concentration in transgenic plants.

Discussions

The Shaker family of potassium channels is one of the most intensively studied potassium transport families in plants and plays an important role in the absorption and transport of potassium ions in plants¹⁴. Previous research has shown that there are two different families of inner rectifying K⁺ channels and outer rectifying K⁺ channels in the Shaker channel¹⁵. The first reported rectifier K⁺ channel protein AKT1 in *A. thaliana* belonged to the Shaker family, which had the characteristics of amphiphilic potassium absorption, and was mainly expressed in the root epidermal cells and cortical cells. Gaymard et al.⁷ first isolated and cloned the K⁺ channel protein gene SKOR from *A. thaliana* and found that it was specifically expressed in root columnar tissue and was inhibited by abscisic acid (ABA), which mediates K⁺ transport from root cells to the xylem. It was also found that the K⁺ selective channel protein identified in the *Arabidopsis* genome contains 2–12 transmembrane domains, forming 1–2 pore regions and multiple regulatory domains, which are located on the plasma membrane. The pore region structure of the SKOR protein of the Shaker family contains the conserved GYGD basic sequence¹⁶. Previous studies have shown that the positively charged amino acids (arginine and lysine) in the fourth transmembrane region can induce transmembrane voltage changes and play a key role in controlling the opening and closing of K⁺ channels¹⁷. Duan et al.¹⁸ reported that the *PtSKOR* gene (from *Puccinellia tenuiflora*) sequence had six highly conserved transmembrane regions (S1–S6), a highly conserved pore region TVGYG between S5 and S6, and a C-terminal containing a predicted cyclic nucleotide-binding domain and an ankyrin domain. In this study, it was found that *EeSKOR* had five transmembrane regions, among which there was a highly conserved P-ring structure (containing GYGD basic sequence) between the fourth and fifth transmembrane regions, which is also the marker sequence of K⁺ channel proteins (Figure 1). The C-terminal of *EeSKOR* contains a cyclic nucleotide-binding domain and an anchorin region, but the sites that regulate potassium channel activity are often located in the C-terminal cell of the SKOR protein. This indicates that the nucleotide-binding domain can regulate the activity of the outer rectifying K⁺ channel protein. In our studies, the phylogenetic tree analysis results showed that *EeSKOR* had high homology with monocotyledons such as TaSKOR and ZmSKOR, and low homology with internal rectifying K⁺ channel AKT1 (Figure S2). Thus, *EeSKOR* encodes the external rectifier K⁺ channel protein SKOR.

Potassium, being an essential nutrient in plants accounts for about 10% of the dry matter of plants¹⁹, and plays an important role in plant nutrition, growth, homeostasis, and osmotic regulation of the enzyme system^{20,21}. Soil salinization has become a topical environmental issue affecting global agricultural production, disturbing normal uptake of potassium in plants²². Because K⁺ and Na⁺ have similar ionic hydration radii, under salt stress, Na⁺ competes for the K⁺ binding sites in the plasma membrane of plant

roots and prevents the transport of K^+ by the roots^{23,24,25}. Therefore, the K^+ transport system plays an important role in plant resistance to salt stress²⁶. Previous studies have shown that Na^+ unloading to the parenchyma cells around the xylem would cause depolarization of the membrane and activate the external rectifier K^+ channel protein SKOR to load K^+ to the xylem and then transport it to the aboveground parts to enhance the salinity tolerance of plants^{4,5,27}. The transcriptional abundance of the *AtSKOR* gene in *A. thaliana* was significantly up-regulated under salt stress²⁸. Garcia-Mata et al.²⁹ reported that *AtSKOR* expression was upregulated under salt treatment, and K^+ concentration in *A. thaliana* also increased compared to that in the control. Thus, the increase in *AtSKOR* gene expression leads to more K^+ being loaded into the xylem and transported to the aboveground parts. Under high salt stress, *ZxSKOR* in roots and stems is well-coordinated to mediate long-distance K^+ transport and plays an important role in K^+ accumulation and homeostasis in *Zygophyllum xanthoxylum*²⁶, and SKOR genes from *Puccinellia tenuiflora* and *Lycium ruthenicum* showed similar salt tolerance mechanisms^{18,30}. Moreover, it was also confirmed that the SKOR channel protein was closely related to K^+ transport in plants³¹.

In this study, *EeSKOR* was mainly located in the cytoplasmic membrane of the epidermis of tobacco leaves, and a small part was located in the nucleus, indicating that *EeSKOR* is a membrane transporter (Figure S3). With the increase in the concentration of external salt and drought treatment, the expression levels of *EeSKOR* in the roots, sheaths, and leaves of *E. elongata* showed an increasing trend (Figure 2a,b). Moreover, the overexpression of the *EeSKOR* gene in tobacco resulted in the accumulation of more K^+ and lesser Na^+ concentrations in transgenic tobacco plants under salt stress than those observed in the WT and Vector plants (Figure 5). This may be because HKT1 proteins unload Na^+ from xylem juice into its parenchyma cells, which reduces the concentration of Na^+ in the aboveground part, and may cause the depolarization of the parenchyma membrane of the xylem^{6,7,9}. This activates the external rectification of the K^+ channel, SKOR, and results in more K^+ being loaded into the xylem and transported over long distances to the aboveground parts to maintain a constant K^+ concentration there.

At the same time, we also found that under salt stress, overexpression of *EeSKOR* significantly enhanced SOD activity and increased Chl content, and significantly reduced H_2O_2 and MDA contents in the transgenic tobacco plants (Figure 5). It has been shown that overexpression of *EeSKOR* can improve the antioxidant activity of plants, effectively removing and reducing the generation of reactive oxygen species, protecting the integrity of the membrane system and photosynthetic organs allowing normal photosynthesis and improvement of plant's salt tolerance. In addition to *EeSKOR*'s location in the plasma membrane, we also found that this protein was located in the nucleus. Therefore, it is speculated that the *EeSKOR* gene may be involved in the regulation of plant salt tolerance through other salt-tolerant pathways.

Conclusions

EeSKOR was cloned from the decaploid *E. elongata*. *EeSKOR* was mainly expressed in the roots, and its transcript abundance changed with increasing salt concentration and drought intensity. The phenotypes, dry weight, plant height, and the concentrations of Na⁺ and K⁺ in roots, sheaths, and leaves were measured in response to treatment with different NaCl concentrations and drought treatments. The transgenic tobacco plants displayed enhanced tolerance to NaCl stress compared with the Vector and WT plants. These results suggest that overexpression of *EeSKOR* in tobacco plants enhances the transport of K⁺ from the root to the aboveground part to maintain K⁺ steady-state in the aboveground under salt stress, thereby enhancing tobacco salt tolerance.

Materials And Methods

Material culture

Seeds of decaploid *E. elongata* were collected from the National Experiment Station of Precision Agriculture, Xiao Tang Shan, China, located approximately 55 km from Beijing (39°34' N, 116°28' E). Plump seeds were sterilized with sodium hypochlorite (NaClO) solution (5%, v/v) for 5 min, rinsed thoroughly with distilled water, incubated in 40°C water for 56 h, and then germinated on moistened filter paper for four days at 25°C in the dark. After plumule emergence, uniform seedlings were transferred to plastic containers (20 cm long, 10 cm wide, and 7 cm high) containing modified Hoagland's nutrient solution [2 mmol·L⁻¹ KNO₃, 0.5 mmol·L⁻¹ NH₄H₂PO₄, 0.25 mmol·L⁻¹ MgSO₄·7H₂O, 1.5 mmol·L⁻¹ Ca(NO₃)₂·4H₂O, 0.5 mmol·L⁻¹ Fe-citrate, 92 μmol·L⁻¹ H₃BO₃, 18 μmol·L⁻¹ MnCl₂·4H₂O, 1.6 μmol·L⁻¹ ZnSO₄·7H₂O, 0.6 μmol·L⁻¹ CuSO₄·5H₂O, 0.7 μmol·L⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O] for four weeks. The nutrient solution was renewed every two days. All seedlings were grown in the same chamber under a day/night cycle of 16 h/8 h at 25°C/18°C, relative humidity of 60%–80%, and a flux density of 600 μmol·m⁻²·s⁻¹.

Cloning of *EeSKOR*

Four-week-old *E. elongata* seedlings were treated with 200 mmol·L⁻¹ NaCl for 24 h. After treatment, fresh roots (200 mg) were washed in sterile water and then ground in liquid nitrogen. Total RNA was extracted with a Trizol Kit (Sangon Biotech. Co., LTD, Shanghai, China) according to the manufacturer's instructions. Primers *EeSKOR*-1/*EeSKOR*-2 were designed according to the principle of high homology and degeneracy by comparing *SKOR* gene sequences of other plants in the GenBank database (Table S1). The conserved core fragments of *EeSKOR* were amplified using PCR. The PCR amplification was calibrated at 94°C for 2 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were purified from agarose gels, ligated into the PMD19-t vector, and sequenced by Sangon Biotech Co, Ltd., (Shanghai, China). The 5'- and 3'- RACE primers were designed by referring to the core sequence of the *EeSKOR* gene and were obtained according to the corresponding methods of the RACE Kit (Table S1). The gene fragments of 5'- and 3'- RACE were amplified using the Takara Premix PrimeSTAR HS Kit (Takara Biotech Co., Ltd., Dalian, China) according to the manufacturer's instructions, and the cDNA of *EeSKOR* gene was spliced and detected by 1.2%

agarose gel electrophoresis. The recovered products were connected to the PMD19-t vector, then transformed and cloned, and the positive strain was identified and sequenced by Sangon Biotech.

DNA sequence and phylogenetic analyses

The *EeSKOR* sequence was analyzed, and the coding regions were predicted using ESPript 3 (Easy Sequencing in PostScript 3) software. *EeSKOR* sequence homology analysis and phylogenetic tree construction were performed using DNAMAN 6.0 software (Lynnon Biosoft, San Ramon, CA, USA). The isoelectric point and molecular mass were predicted using the online Compute pI/Mw tool (http://web.expasy.org/compute_pi/).

Expression analysis of *EeSKOR*

Real-Time Quantitative Reverse Transcription methods PCR were used to analyze the expression patterns of the *EeSKOR* gene in the root, sheath, and leaf of *E. elongata* subjected to different NaCl concentrations for 24 h (0, 25, 50, 100, 150, and 200 mmol·L⁻¹) and different osmotic potentials of sorbitol for 24 h (0, -0.5, -1.0, and -1.5 MPa). Under each treatment, the extraction of total RNA in the root, sheath, and leaf of *E. elongata* was carried out according to the RNA Extraction Kit instructions of Takara Biotech. The first strand of cDNA was synthesized according to the instructions for the Prime Script™ RT reagent Kit with gDNA Eraser (Takara Biotech., Co., Ltd.). The qRT-PCR forward primer for the *EeSKOR* gene was P1 (5'-TACGGAGGCTGCTCAGGTTT-3'), and the reverse primer was P2 (5'-CGCATCTCCTCGCTTCATC-3'); the PCR product length was 189 bp. The positive primer for qRT-PCR of the internal gene *Actin* was P3 (5'-CTTGACTATGAACAAGAGCTGGAAA-3') and the reverse primer was P4 (5'-TGAAAGATGGCTGGAAAAGGA-3'); the PCR product length was 139 bp. The qRT-PCR experiment was conducted using a StepOnePlus instrument (Thermo Fisher Scientific, Waltham, MA, USA). The reaction system consisted of SYBR® Premix Ex Taq II (Takara Biotech., Co., Ltd.) 12.5 L, both positive and reverse primers 1 L, cDNA 2 L, and water was added to 25 L. The PCR amplification procedures were as follows: denaturation at 95°C for 1 min, 95°C for 5 s, 60°C for 30 s, and 40 cycles. The relative expression of *EeSKOR* was calculated using the 2^{-ΔΔCT} method. Experiments were repeated at least five times to obtain similar results.

Subcellular localization analysis

The positive primers with the *Kpn* I enzyme loci (5'-GGGGACGAGCTCGGTACCATGGAGAGGGAGATTGTAGCAGAGT-3') and reverse primers with the *Xba* I enzyme loci (5'-CATGGTGTCTGACTCTAGACTGATCGGCTGCAACAGCAGCTGTA-3') were designed using Primer 5.0 software (Premier Biosoft International, Palo Alto, CA, USA). The product templates were *EeSKOR* open reading frame bacterial liquid that had been sequenced and verified. The target fragments were amplified using the Takara Premix PrimeSTAR HS Kit, and the PCR reaction conditions were 95°C for 5 min, 94°C for 30 s, 58°C for 30 s, 72°C for 1 min, 30 cycles, and a final extension at 72°C for 10 min. The pCAM35-GFP plasmid was digested using *Kpn*I and *Xba*I, and the results were detected by agarose gel electrophoresis after enzyme digestion. The recombinant plasmid pCAM35-*EeSKOR*-GFP was obtained by ligating, transforming, and identification of the target fragment and the linearized pCAM35-GFP

enzymatic digestion product using the BioTeke DNA purification recovery Kit (BioTeke Corporation, Wuxi, Jiangsu, China). The empty vectors pCAM35-GFP and pCAM35-*EeSKOR*-GFP were imported into *Agrobacterium* GV3101 using the freeze-thaw method for future use. The pCAM35-GFP and pCAM35-*EeSKOR*-GFP were transferred into the epidermal cells of *Nicotiana benthamiana* by injection, and the expression location of *EeSKOR* in the epidermal cells of tobacco leaves was observed using a Leica TCS SP8 confocal microscope (Leica Camera AG, Wetzlar, Germany) in Germany.

Construction of plant expression vector and transformation of agrobacterium

Nco I and *Bgl* II restriction sites were introduced at both ends of the upper and lower primers of the *EeSKOR* open reading frame (ORF), and the target fragment PCR products were detected by 1.2% gel electrophoresis after RT-PCR amplification. The restriction sites on pCAMBIA1301 were double-digested with *Nco* I/*Bgl* II, and the large fragments were recovered. Then, the target *EeSKOR* gene was inserted into the linear plant expression vector (pCAMBIA1301-35S-*EeSKOR*-Nos) using Clontech In-Fusion seamless connection technology (Takara Biotech Co., Ltd.) according to the manufacturer's instructions, and the specific bands were obtained by double-digestion with *Hind* III/*Bgl* II. The constructed overexpression vector was transformed into the sensing state of *Agrobacterium* GV3101 by the freeze-thaw method, and the transformed bacterial solution was uniformly coated onto YEP solid medium (50 mg·L⁻¹ kanamycin, 50 mg·L⁻¹ rifampicin, 50 mg·L⁻¹ gentamycin). After inverted culture at 28°C away from light, a single colony was grown after 2–3 days. The single colony was picked up using an inoculation ring, inoculated into 5 mL YEP medium, and incubated overnight. The plasmid was extracted and confirmed as a positive clone by PCR amplification.

Genetic transformation and molecular characterization

The above-mentioned constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 by the chemical method and then used for tobacco (*Nicotiana tabacum* cv. Wisconsin 38) transformation using the leaf disc method as described by Horsch et al.³² with minor modifications. The infected leaf sections were cultivated on Murashige and Skoog (MS) medium containing 2 mg·L⁻¹ 6-benzylaminopurine (6-BA) and 0.2 mg L⁻¹ 1-naphthaleneacetic acid (NAA) at pH 5.8–6.0 for three days. The WT tobacco leaf DNA and agrobacterium-positive bacterial liquid were used as controls. Kan-resistant tobacco leaf DNA was used as a template, *EeSKOR*-F/*EeSKOR*-R and DPF1/DPR1 were used as primers (Table S1), and the transgenic tobacco positive plants were detected by PCR amplification. For western blotting, 20 µg tonoplast proteins were separated using 12% (m/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Blots were performed according to the method described by Kumari et al.³³.

Analysis of salt tolerance of transgenic tobacco plants

T1-generation seeds of wild-type tobacco (*N. tabacum* cv. Wisconsin 38), null vector (Vector), and overexpressed *EeSKOR* transgenic tobacco (L12 and L36) plants were sterilized with 5% (v/v) sodium hypochlorite (NaClO) solution for 5 min and then washed several times in distilled water. The moist seeds

were evenly seeded in a petri dish covered with absorbent paper, and dark cultured at 25°C for four days. After germination and rooting, the seeds were transferred into a black culture box (20 cm×10 cm×7 cm) and seeded with Hoagland nutrient solution. Light culture was conducted for 16 h (day)/8 h (night) at a light intensity of about 600 mol·m⁻²·s⁻¹ with the Hoagland nutrient solution replaced after two days; air relative humidity was kept at 60%–80%. The WT, Vector, and transgenic tobacco (L12 and L36) plants were subjected to twenty-one days of stress in Hoagland nutrient solution with NaCl concentrations of 0, 50, 100, 150, and 200 mmol·L⁻¹, respectively. The plant height, biomass, H₂O₂, MDA, and chlorophyll (Chl) content, SOD activity, and Na⁺ and K⁺ accumulation concentrations in the aboveground part and root of the plant were measured. After fresh weight measurements, roots, sheaths, and leaves were oven-dried at 80°C to a constant weight, and the dry weight of each organ was recorded. The MDA and Chl contents were determined using the method of Zhou³⁴, and the SOD activity was determined by the nitroblue tetrazolium (NBT) method. Na⁺ and K⁺ concentrations in the roots, sheaths, and leaves were measured using a flame emission spectrophotometer.

Statistical analysis

Each treatment was repeated five times independently with three seedlings per replicate. All data are presented as mean ± standard deviation (SD). Duncan's multiple range tests were performed using statistical software (v.13.0, SPSS Inc, Chicago, IL, USA).

Declarations

Acknowledgements

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Author Contributions

L. Meng conceived and designed the experiments, wrote and revised the manuscript. Y. T. Zhou, X. X. Tian and Y. Zhang prepared for sample, performed the experiments and analysed the data. P. C. Mao and M. L. Zheng also analysed the data.

Competing interests

The authors declare no competing interests.

Additional information

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Figures

EeSKOR	MEREIVPEYEINEIVLTIHGSVGSRLSIESEFELNWRGS...SSAISHATNCYGGSFVLENGRIYFMWSNMMFIWSIY	75
TaSKOR	MEREIGPEYEINEILGGTIHGSVGSRLSIEFPEFRWRSSSWHSSSAIRLINNCYGSSFVLENGRWYFMWSNMMFIWSIY	80
TuSKOR	MEREIGPEYEINEILGGTIHGSVGSRLSIEFPEFRWRSSSWHSSSAIRLINNCYGSSFVLENGRWYFMWSNMMFIWSIY	80
AetSKOR	MEREIGPEYEINEILGGTIHGSVGSRLSIEFPEFRWRSS...SSAIRLINNCYGSSFVLENGRWYFMWSNMMFIWSIY	76
TM1		
EeSKOR	SVFYTFFAFCFFFGIFEHILCLECAQIIFIALVAVHFEFLAYRDEHTRHVYDCCRIALFYIKGSFALIMIGCFEFLAVYK	155
TaSKOR	SVFYTFFAFCFFFGIFEHILCLECAQIIFIALVAVHFEFLAYRDEHTRHVYDCCRIALFYIKGSFALIMIGCFEFLAVYK	160
TuSKOR	SVFYTFFAFCFFFGIFEHILCLECAQIIFIALVAVHFEFLAYRDEHTRHVYDCCRIALFYIKGSFALIMIGCFEFLAVYK	160
AetSKOR	SVFYTFFAFCFFFGIFEHILCLECAQIIFIALVAVHFEFLAYRDEHTRHVYDCCRIALFYIKGSFALIMIGCFEFLAVYK	156
TM2		
EeSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYIFTFIRKLTVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	235
TaSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYIFTFIRKLTVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	240
TuSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYIFTFIRKLTVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	240
AetSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYIFTFIRKLTVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	236
TM3		
EeSKOR	TMGDCSYINFRFVCLITRYTTSIYLAIVTMATVGYGLIHAFNIREMVEIVVVSFSLMIGAYLIGNMTAIVKGSPTERF	315
TaSKOR	TMGDCSYINFRFVCLITRYTTSIYLAIVTMATVGYGLIHAFNIREMVEIVVVSFSLMIGAYLIGNMTAIVKGSPTERF	320
TuSKOR	TMGDCSYINFRFVCLITRYTTSIYLAIVTMATVGYGLIHAFNIREMVEIVVVSFSLMIGAYLIGNMTAIVKGSPTERF	320
AetSKOR	TMGDCSYINFRFVCLITRYTTSIYLAIVTMATVGYGLIHAFNIREMVEIVVVSFSLMIGAYLIGNMTAIVKGSPTERF	316
TM4		
EeSKOR	RCKMTEIDRYMNFNRIGSLTSSCVKAHLIIQYESSYKRRIRVCLIEFVAVFSKTIYLETVSKVHLFRGCSEFLSCIVVKI	395
TaSKOR	RCKMTEIDRYMNFNRIGSLTSSCVKAHLIIQYESSYKRRIRVCLIEFVAVFSKTIYLETVSKVHLFRGCSEFLSCIVVKI	400
TuSKOR	RCKMTEIDRYMNFNRIGSLTSSCVKAHLIIQYESSYKRRIRVCLIEFVAVFSKTIYLETVSKVHLFRGCSEFLSCIVVKI	400
AetSKOR	RCKMTEIDRYMNFNRIGSLTSSCVKAHLIIQYESSYKRRIRVCLIEFVAVFSKTIYLETVSKVHLFRGCSEFLSCIVVKI	396
TM5		
EeSKOR	HEEFFIFGEVILECGTVVLCIYIVAHGCLIEEIVAGEGGSEKVISEILFHDVGLVAVICNTRCFYTVRSEICSLIRID	474
TaSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCLIEEIVAGEGGSEKVISEILFHDVGLVAVICNTRCFYTVRSEICSLIRID	479
TuSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCLIEEIVAGEGGSEKVISEILFHDVGLVAVICNTRCFYTVRSEICSLIRID	480
AetSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCLIEEIVAGEGGSEKVISEILFHDVGLVAVICNTRCFYTVRSEICSLIRID	476
EeSKOR	KQSITSLICMYIFLSRQILSNLIKGRFTESRKGKLESITITYLIAKCEALIVLGVNNAAYEGGLFRLKGLICAGALFSKFD	554
TaSKOR	KQSITSLICMYIFLSRQILSNLIKGRFTESRKGKLESITITYLIAKCEALIVLGVNNAAYEGGLFRLKGLICAGALFSKFD	559
TuSKOR	KQSITSLICMYIFLSRQILSNLIKGRFTESRKGKLESITITYLIAKCEALIVLGVNNAAYEGGLFRLKGLICAGALFSKFD	560
AetSKOR	KQSITSLICMYIFLSRQILSNLIKGRFTESRKGKLESITITYLIAKCEALIVLGVNNAAYEGGLFRLKGLICAGALFSKFD	556
EeSKOR	YLGFTSLHVAASFGYELIVRFILRGANVNSILKSGKSEILCAVKSCHFIISILVAGCAAINLELAGGYICRVVACGKI	634
TaSKOR	YLGFTSLHVAASFGYELIVRFILRGANVNSILKSGKSEILCAVKSCHFIISILVAGCAAINLELAGGYICRVVACGKI	639
TuSKOR	YLGFTSLHVAASFGYELIVRFILRGANVNSILKSGKSEILCAVKSCHFIISILVAGCAAINLELAGGYICRVVACGKI	640
AetSKOR	YLGFTSLHVAASFGYELIVRFILRGANVNSILKSGKSEILCAVKSCHFIISILVAGCAAINLELAGGYICRVVACGKI	636
EeSKOR	CLIFRLIRFGLIENCRNYERRTELHVAASEGIEIVAGMIVEEGADLVAFIRWGNTFILEFRRCGSKFEVVRILECTTFAVA	714
TaSKOR	CLIFRLIRFGLIENCRNYERRTELHVAASEGIEIVAGMIVEEGADLVAFIRWGNTFILEFRRCGSKFEVVRILECTTFAVA	719
TuSKOR	CLIFRLIRFGLIENCRNYERRTELHVAASEGIEIVAGMIVEEGADLVAFIRWGNTFILEFRRCGSKFEVVRILECTTFAVA	720
AetSKOR	CLIFRLIRFGLIENCRNYERRTELHVAASEGIEIVAGMIVEEGADLVAFIRWGNTFILEFRRCGSKFEVVRILECTTFAVA	698
EeSKOR	ADQ	717
TaSKOR	EAD	722
TuSKOR	ADH	723
AetSKOR	...	698

Figure 1

Multiple comparison of amino acids among EeSKOR, TaSKOR, TuSKOR, and AetSKOR Note: The five putative transmembrane domains (TM1–TM5) were underlined.

EeSKOR	MEREIVPEYEINEIVLTIHGSGVSRISIFEFELNWRGS...SSAISHETNCYGGSFVNLENGRIYFMWSNMMFIWSIY	75
TaSKOR	MEREICPEYEINEILGGTIHGSGVSRISIFEFELNWRSSSWHSSSAIRLINNCYGGSFVNLENGRIYFMWSNMMFIWSIY	80
TuSKOR	MEREICPEYEINEILGGTIHGSGVSRISIFEFELNWRSSSWHSSSAIRLINNCYGGSFVNLENGRIYFMWSNMMFIWSIY	80
AetSKOR	MEREICPEYEINEILGGTIHGSGVSRISIFEFELNWRSS...SSAIRLINNCYGGSFVNLENGRIYFMWSNMMFIWSIY	76
TM1		
EeSKOR	SVFYTFFAFCFFFGIFEHLICIECAQLIFIALVAVHFFLAYRDSHTRVVYDQCRIALFYIKGSFAIMIGCFEFLAVYK	155
TaSKOR	SVFYTFFAFCFFFGIFEHLICIECAQLIFIALVAVHFFLAYRDSHTRVVYDQCRIALFYIKGSFAIMIGCFEFLAVYK	160
TuSKOR	SVFYTFFAFCFFFGIFEHLICIECAQLIFIALVAVHFFLAYRDSHTRVVYDQCRIALFYIKGSFAIMIGCFEFLAVYK	160
AetSKOR	SVFYTFFAFCFFFGIFEHLICIECAQLIFIALVAVHFFLAYRDSHTRVVYDQCRIALFYIKGSFAIMIGCFEFLAVYK	156
TM2		
EeSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYLFTEFKIATVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	235
TaSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYLFTEFKIATVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	240
TuSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYLFTEFKIATVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	240
AetSKOR	FTGRMEVVRVYLVWIRLYRARKICGFEKKMERLIRISYLFTEFKIATVEIYCTHTAACVFYLLATTIIFAEEGGTWIGSL	236
TM3		
EeSKOR	TMGLDSYINFRFVCLITRYTISIYLAIVTMATVGYGDIHAPNIREMVFIVVVSFDMIGAYLIGNMTAIVKGSRTERF	315
TaSKOR	TMGLDSYINFRFVCLITRYTISIYLAIVTMATVGYGDVHAVNIREMVFIVVVSFDMIGAYLIGNMTAIVKGSRTERF	320
TuSKOR	AMGLDSYINFRFVCLITRYTISIYLAIVTMATVGYGDVHAVNIREMVFIVVVSFDMIGAYLIGNMTAIVKGSRTERF	320
AetSKOR	TMGLDSYINFRFVCLITRYTISIYLAIVTMATVGYGDVHAVNIREMVFIVVVSFDMIGAYLIGNMTAIVKGSRTERF	316
TM4		
TM5		
EeSKOR	RCKMTEIFRYMNFNRIGSLIESCVKAHLIIQYESSYKRRIRIVCLIEFVAVFSKTIYLETVSKVHLFRGCSECFISQIVVKI	395
TaSKOR	RCKMTEIFRYMNFNRIGSLIESCVKAHLIIQYESSYKRRIRIVCLIEFVAVFSKTIYLETVSKVHLFRGCSECFISQIVVKI	400
TuSKOR	RCKMTEIFRYMNFNRIGSLIESCVKAHLIIQYESSYKRRIRIVCLIEFVAVFSKTIYLETVSKVHLFRGCSECFISQIVVKI	400
AetSKOR	RCKMTEIFRYMNFNRIGSLIESCVKAHLIIQYESSYKRRIRIVCLIEFVAVFSKTIYLETVSKVHLFRGCSECFISQIVVKI	396
EeSKOR	HEEFFIFGEVILECGTVVLCIYIVAHGCIIEEIVAGEGGSEKVISEILFHDVVGELVAVICNTRCFYTVRSEICSLIRID	474
TaSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCIIEEIVAGEGGSEKVISEILFHDVVGELVAVICNTRCFYTVRSEICSLIRID	479
TuSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCIIEEIVAGEGGSEKVISEILFHDVVGELVAVICNTRCFYTVRSEICSLIRID	480
AetSKOR	CEEFFIFGEVILECGTVVLCIYIVAHGCIIEEIVAGEGGSEKVISEILFHDVVGELVAVICNTRCFYTVRSEICSLIRID	476
EeSKOR	KQSITSIIQMYIFLSRQIISNIIKGRFTTESKGRKLESNITYIIARCEALIVIGVNNAAYEGGLFRIKGIICAGALFSKFD	554
TaSKOR	KQSITSIIQMYIFLSRQIISNIIKGRFTTESKGRKLESNITYIIARCEALIVIGVNNAAYEGGLFRIKGIICAGALFSKFD	559
TuSKOR	KQSITSIIQMYIFLSRQIISNIIKGRFTTESKGRKLESNITYIIARCEALIVIGVNNAAYEGGLFRIKGIICAGALFSKFD	560
AetSKOR	KQSITSIIQMYIFLSRQIISNIIKGRFTTESKGRKLESNITYIIARCEALIVIGVNNAAYEGGLFRIKGIICAGALFSKFD	556
EeSKOR	YCGFTSIHVAAISFGYELIVREFIIRGANVNSICKSGKSEFLICAVKSGHEFIISLIVAGCAAINIEFAGGYICRVVACGKI	634
TaSKOR	YCGFTSIHVAAISFGYELIVREFIIRGANVNSICKSGKSEFLICAVKSGHEFIISLIVAGCAAINIEFAGGYICRVVACGKI	639
TuSKOR	YCGFTSIHVAAISFGYELIVREFIIRGANVNSICKSGKSEFLICAVKSGHEFIISLIVAGCAAINIEFAGGYICRVVACGKI	640
AetSKOR	YCGFTSIHVAAISFGYELIVREFIIRGANVNSICKSGKSEFLICAVKSGHEFIISLIVAGCAAINIEFAGGYICRVVACGKI	636
EeSKOR	CLIFRIIRFEGIDENCRNYERRTELHVAAEGEILVAGMIVEEGADLVAFIRWGNTFIEFAFRCGSKFEVVRILEQTTPAVA	714
TaSKOR	CLIFRIIRFEGIDENCRNYERRTELHVAAEGEILVAGMIVEEGADLVAFIRWGNTFIEFAFRCGSKFEVVRILEQARAAAA	719
TuSKOR	CLIFRIIRFEGIDENCRNYERRTELHVAAEGEILVAGMIVEEGADLVAFIRWGNTFIEFAFRCGSKFEVVRILEQARAAAA	720
AetSKOR	CLIFRIIRFEGIDENCRNYERRTELHVAAEGEILVAGMIVEEGADLVAFIRWGNTFIEFAFR.....	698
EeSKOR	ALQ	717
TaSKOR	FAD	722
TuSKOR	ALH	723
AetSKOR	...	698

Figure 1

Multiple comparison of amino acids among EeSKOR, TaSKOR, TuSKOR, and AetSKOR Note: The five putative transmembrane domains (TM1–TM5) were underlined.

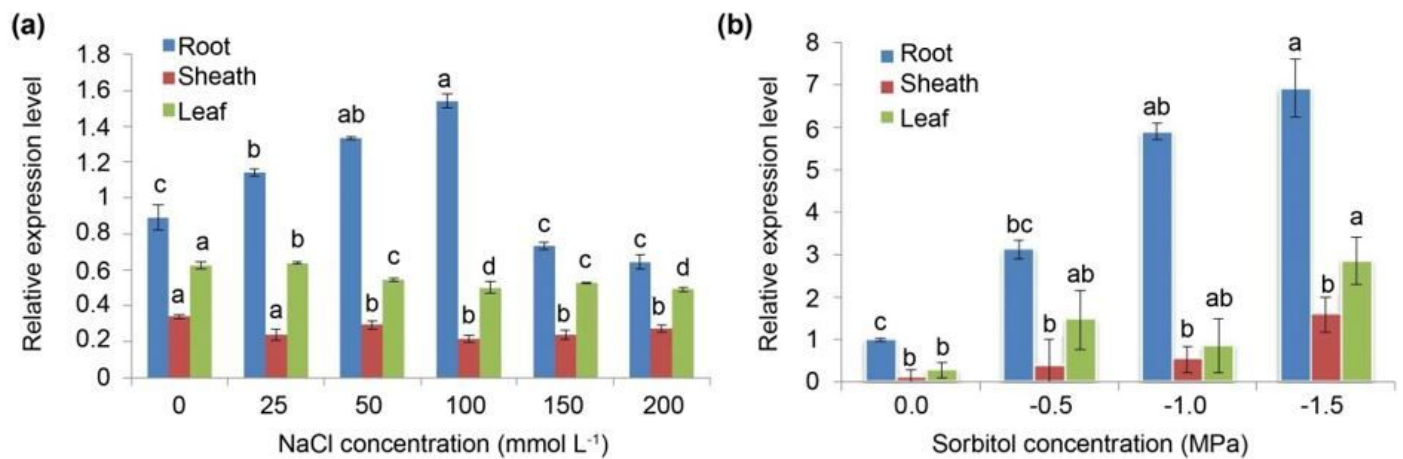


Figure 2

EeSKOR expression patterns in roots, sheaths, and leaves of *E. elongata* under the different salt and drought treatments. Notes: Effects of 24 hr treatments of (a) different NaCl concentration and (b) different sorbitol concentration on EeSKOR expression levels in roots, sheaths, and leaves of *E. elongata*. Means \pm SDs, n = 5, different letters indicate significant differences at P < 0.05 (Duncan's multiple range test).

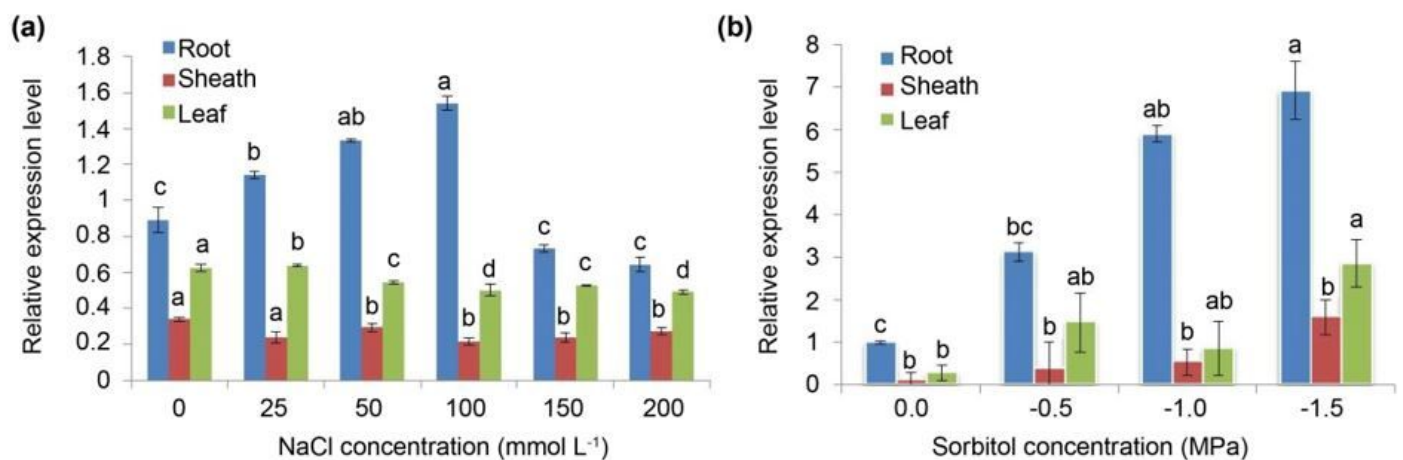


Figure 2

EeSKOR expression patterns in roots, sheaths, and leaves of *E. elongata* under the different salt and drought treatments. Notes: Effects of 24 hr treatments of (a) different NaCl concentration and (b) different sorbitol concentration on EeSKOR expression levels in roots, sheaths, and leaves of *E. elongata*. Means \pm SDs, n = 5, different letters indicate significant differences at P < 0.05 (Duncan's multiple range test).

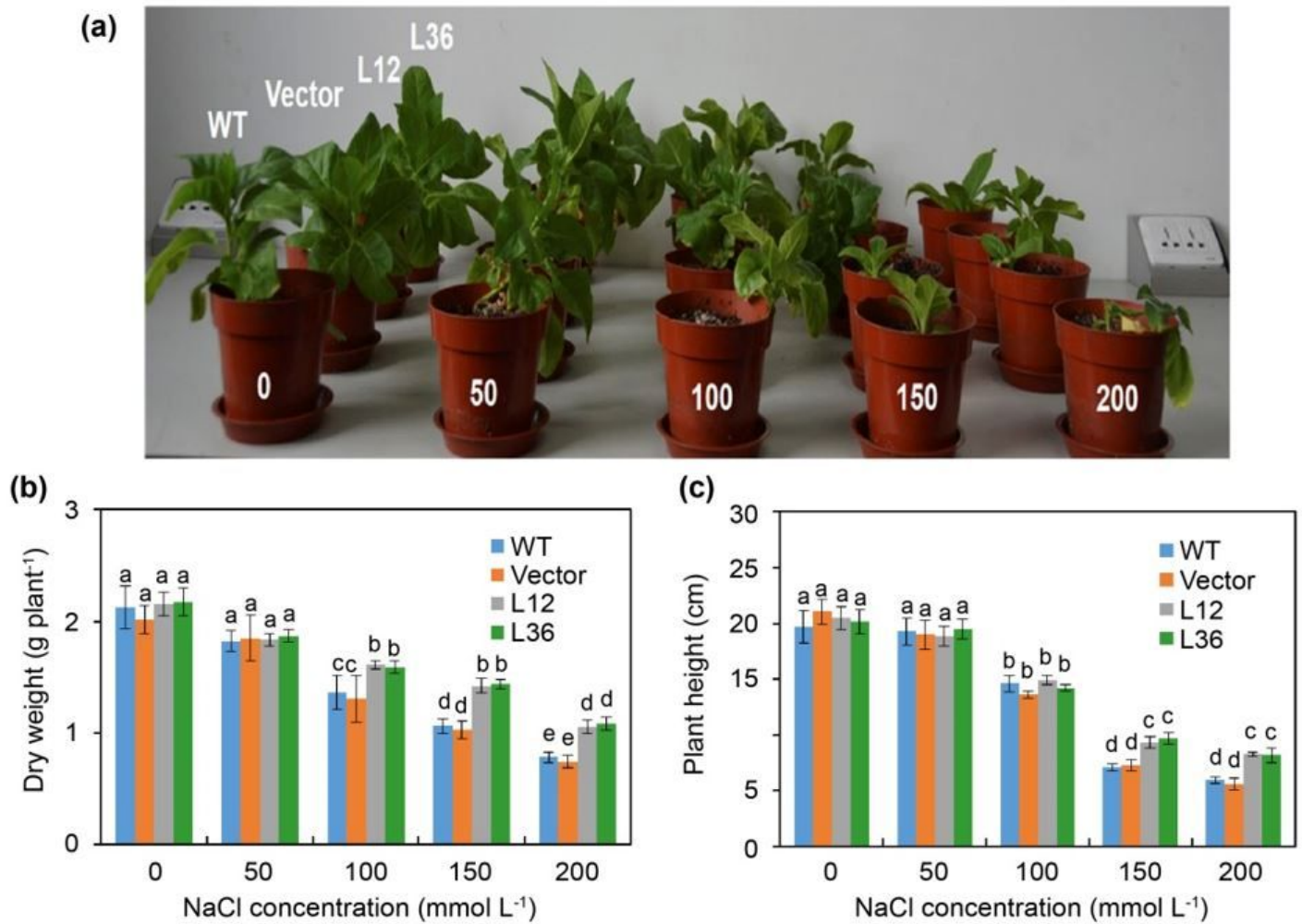


Figure 3

Effects of different concentrations of NaCl (0, 50, 100, 150, 200 mmol·L⁻¹) on the phenotypes (a), dry weight (b) and plant height (c) of WT, Vector and transgenic (L12 and L36) plants after a 21 d treatment. Means ± SDs, n = 5, different letters indicate significant differences at P < 0.05 (the Duncan's multiple range test).

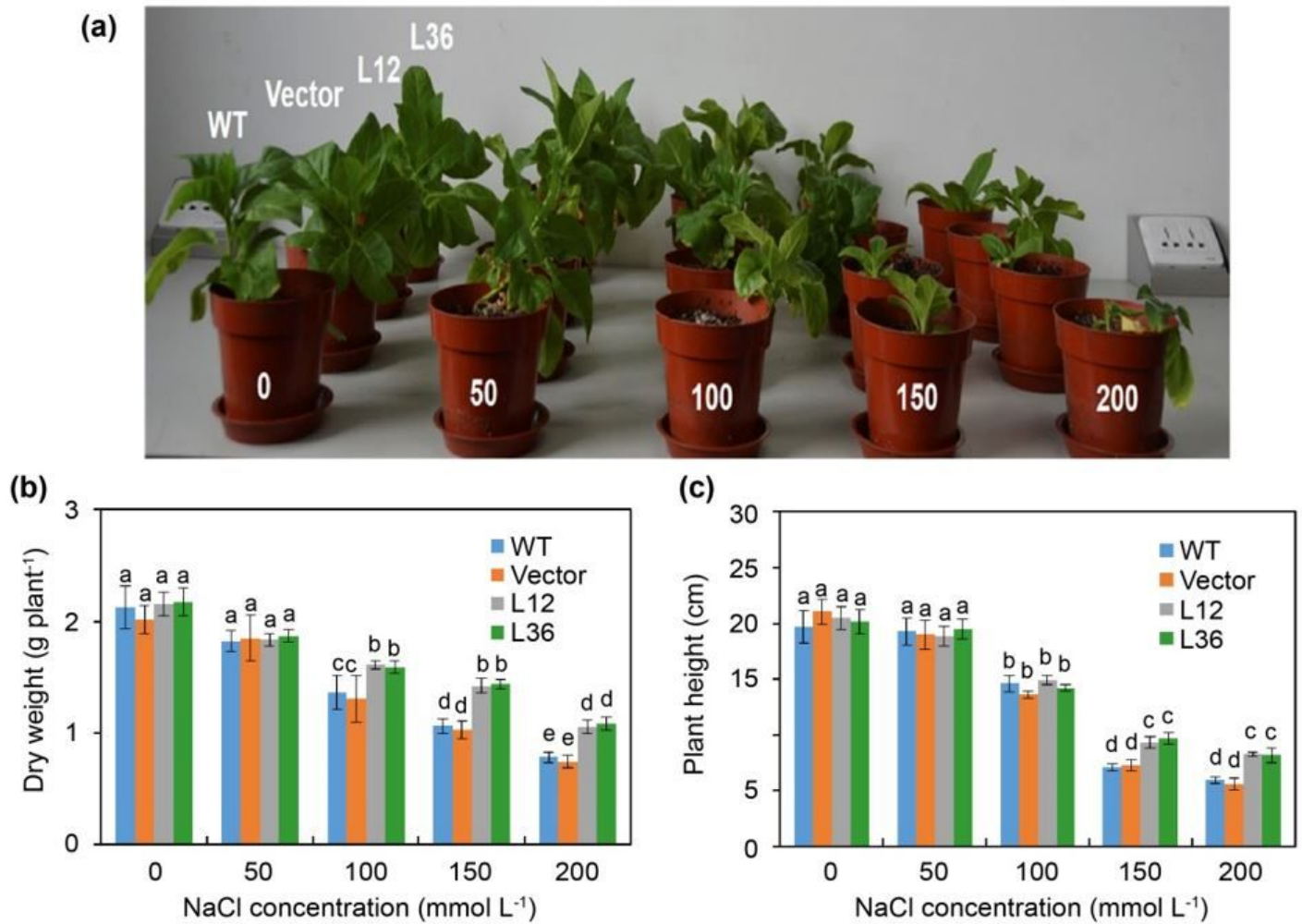


Figure 3

Effects of different concentrations of NaCl (0, 50, 100, 150, 200 mmol·L⁻¹) on the phenotypes (a), dry weight (b) and plant height (c) of WT, Vector and transgenic (L12 and L36) plants after a 21 d treatment. Means ± SDs, n = 5, different letters indicate significant differences at P < 0.05 (the Duncan's multiple range test).

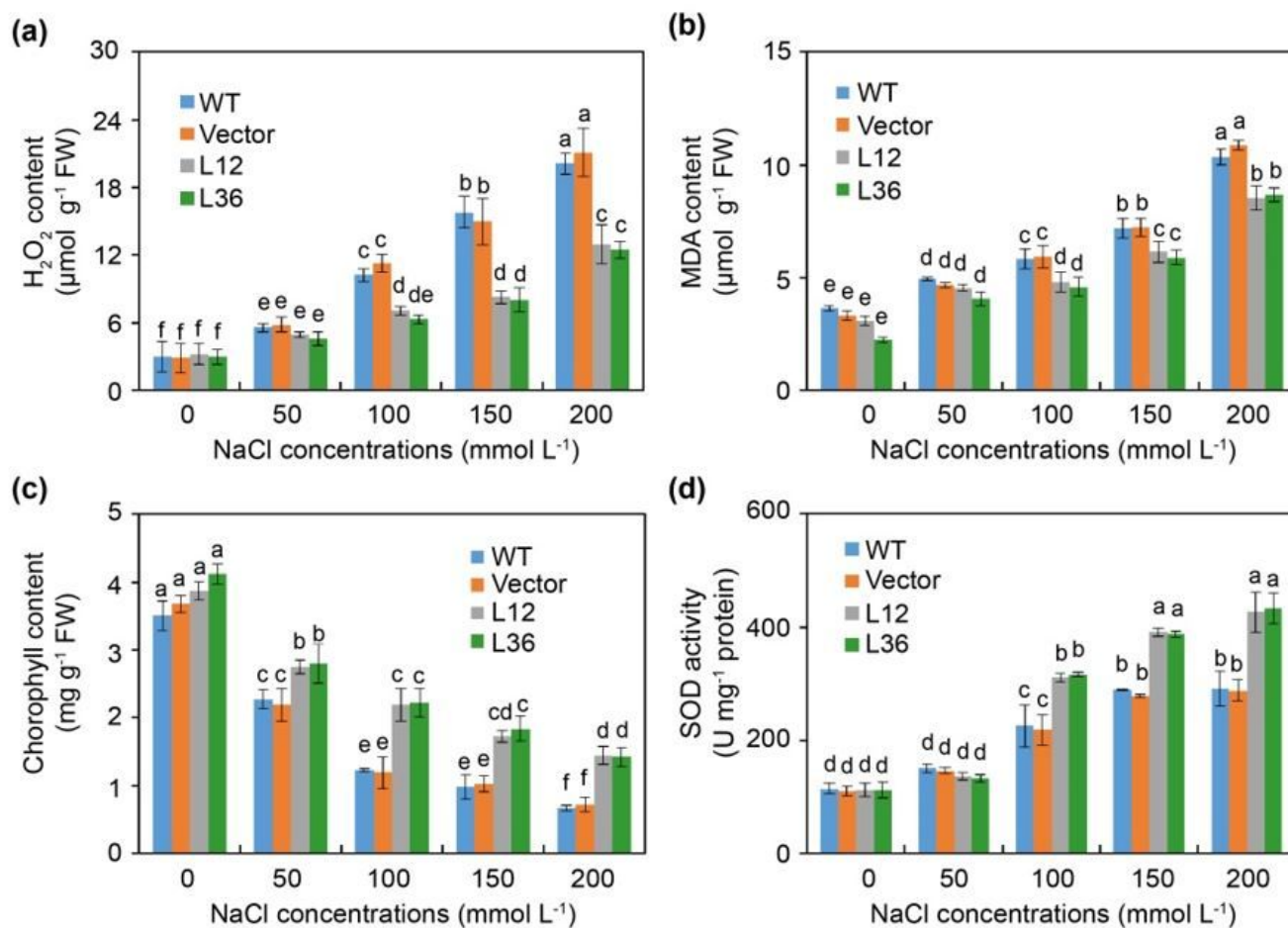


Figure 4

Effects of different concentrations of NaCl (0, 50, 100, 150, 200 mmol·L⁻¹) on H₂O₂ content (a), MDA content (b), chlorophyll content (Chl) (c) and SOD activity (d) of WT, Vector and transgenic (L12 and L36) plants after 21 d of treatment. Means ± SDs, n = 5, different letters indicate significant differences at P < 0.05 (the Duncan's multiple range test).

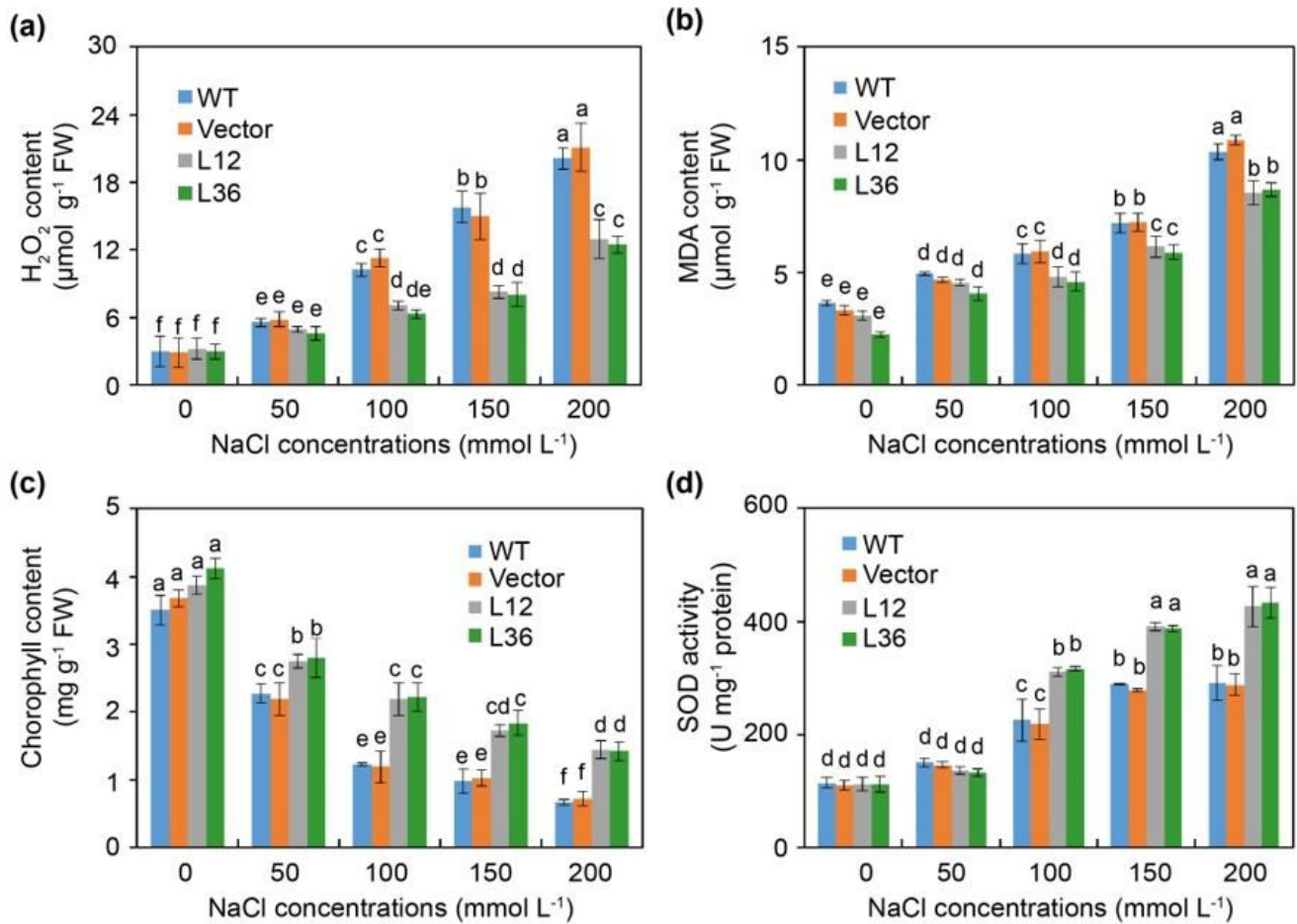


Figure 4

Effects of different concentrations of NaCl (0, 50, 100, 150, 200 mmol·L⁻¹) on H₂O₂ content (a), MDA content (b), chlorophyll content (Chl) (c) and SOD activity (d) of WT, Vector and transgenic (L12 and L36) plants after 21 d of treatment. Means ± SDs, n = 5, different letters indicate significant differences at P < 0.05 (the Duncan's multiple range test).

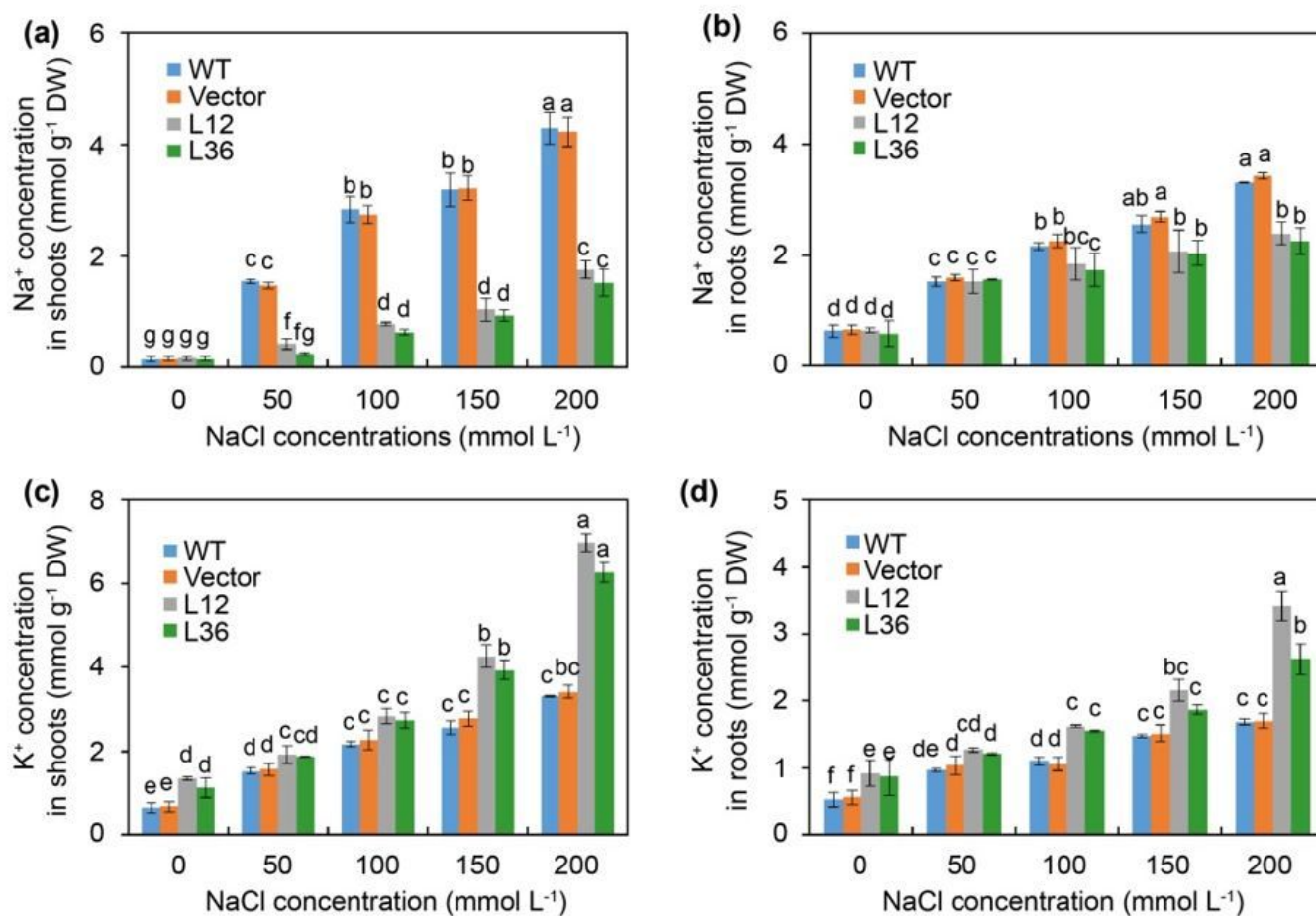


Figure 5

Effects of different concentrations of NaCl (0, 50, 100, 150, 200 mmol·L⁻¹) on Na⁺ and K⁺ concentrations in aboveground and root of WT, Vector, and transgenic plants (L12 and L36) after 21 d of treatment. Means ± SDs, n = 5, different letters indicate significant differences at P < 0.05 (Duncan's multiple range test).

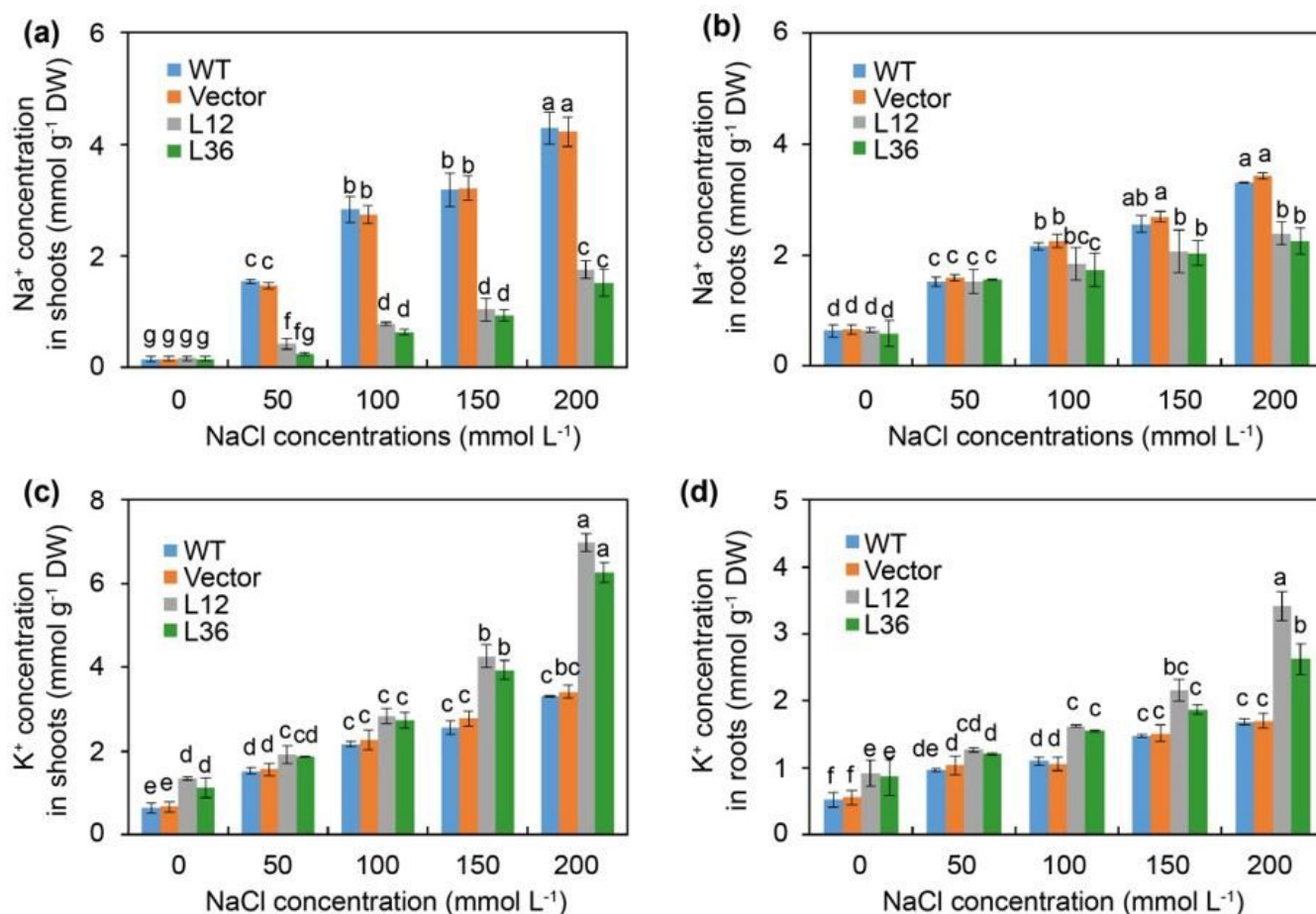


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