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Genome-wide Identification of the SWEET Gene Family in Elymus dahuricus and Functional Characterization of EdSWEET15 in Salt Tolerance

Yuanbo Pan
College of Pratacultural Science, Gansu Agricultural University
Peng zhang
College of Pratacultural Science, Gansu Agricultural University
Miaomiao Huang
Academy of Agriculture and Forestry Sciences of Qinghai University
Zeliang Ju
Academy of Animal Husbandry and Veterinary Sciences, Qinghai University
Kuiju Niu
niukj@gsau.edu.cn
College of Pratacultural Science, Gansu Agricultural University
Jingyan Guo
College of Pratacultural Science, Gansu Agricultural University
Yizhe Wang
College of Pratacultural Science, Gansu Agricultural University
Yiran Li
College of Pratacultural Science, Gansu Agricultural University
Panpan Wang
College of Pratacultural Science, Gansu Agricultural University

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Abstract

The SWEET (Sugars Will Eventually be Exported Transporter) family, a class of sugar transporters identified in recent years, is extensively involved in regulating plant growth and development. Beyond their basic physiological functions, existing studies have suggested that SWEET family members participate in plant stress responses, such as salt tolerance. However, the specific function of SWEET genes from *Elymus dahuricus* Turcz. in salt stress adaptation remains unclear. In this study, A total of 12 *EdSWEET* genes were identified from the whole-genome database of *E. dahuricus* Turcz., which were distributed across 8 chromosomes. The EdSWEET proteins exhibited substantial variations in molecular weight. Phylogenetic analysis classified these genes into four major subfamilies. Abundant regulatory elements were found to be associated with plant hormone signaling and stress responses, indicating that *EdSWEET* genes play crucial roles in coping with abiotic stresses. Expression profiling of *E. dahuricus* Turcz. roots and leaves under salt stress revealed that *EdSWEET15* was significantly up-regulated in both tissues after salt stress treatment, suggesting its involvement in the salt stress response. The *EdSWEET15* gene was cloned from *E. dahuricus* Turcz. Its open reading frame (ORF) is 930 bp in length, Further verification via heterologous overexpression in *Arabidopsis thaliana* demonstrated that, compared with the wild type (WT), *EdSWEET15* overexpressing Arabidopsis lines displayed significantly enhanced salt stress resistance. Specifically, the accumulation of reactive oxygen species (ROS) was remarkably reduced under salt stress, while the scavenging activities of antioxidant enzyme systems, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), were significantly elevated. This study clarifies the novel function of *EdSWEET15* in plant salt stress responses and provides a theoretical basis and candidate gene resource for the genetic improvement of salt tolerance in *E. dahuricus* Turcz.

1 Introduction

Elymus dahuricus Turcz., a perennial herbaceous plant belonging to the genus Elymus (Poaceae), possesses prominent characteristics such as cold tolerance, drought resistance, and poor soil adaptability [1]. Rich in crude protein, minerals, and various essential amino acids, it exhibits excellent palatability and serves as a high-quality forage resource for animal husbandry [2]. Meanwhile, with its well-developed root system and strong tillering capacity, *E. dahuricus* Turcz. plays an irreplaceable role in soil and water conservation, degraded grassland restoration, and saline-alkali land ecological remediation [3]. However, the global problem of soil salinization is increasingly severe, and salt stress has become a key abiotic stress factor limiting the growth, yield formation, and ecological adaptability of *E. dahuricus* Turcz. Salt stress induces a series of physiological and biochemical reactions, including disruption of cellular osmotic balance, accumulation of ionic toxicity, and outbreak of reactive oxygen species (ROS), which significantly inhibit seed germination, seedling growth, root development, and biomass accumulation of *E. dahuricus* Turcz [4]. These adverse effects severely impair its cultivation performance and ecological restoration function in saline-alkali areas. Therefore, systematically deciphering the physiological mechanisms underlying salt tolerance in *E. dahuricus* Turcz. and exploring

key salt-tolerant gene resources not only hold important scientific value for elucidating the molecular regulatory network of stress adaptation in this species but also provide theoretical basis and genetic support for breeding new *E. dahuricus* Turcz. varieties with enhanced salt tolerance and broad adaptability through molecular breeding approaches.

Salt stress is one of the major abiotic stresses affecting plant growth and development worldwide. Under high-salt conditions, the osmotic pressure of soil solution increases significantly, leading to difficulty in water absorption by plant roots, which triggers cellular dehydration and physiological drought, thereby damaging the integrity and stability of cell membranes [5, 6]. Meanwhile, harmful ions such as Na^+ and Cl^- influx into cells in large quantities through roots and accumulate continuously. This not only interferes with the normal absorption and transport of essential ions (e.g., K^+ and Ca^{2+}), causing ionic homeostasis imbalance, but also exerts direct toxic effects on enzyme activity, nucleic acid structure, and metabolic pathways [7–9]. More critically, salt stress disrupts the dynamic balance between the production and scavenging of ROS in plants, inducing the explosive accumulation of ROS such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2). This triggers oxidative stress responses, resulting in protein oxidative damage, lipid peroxidation, and DNA fragmentation, ultimately leading to cell apoptosis [10, 11].

Plant tolerance mechanisms under salt stress involve multiple regulatory processes, including ionic balance regulation, osmotic adjustment, ROS scavenging, and signal transduction. Among these, sugar metabolism and transport play a crucial role in plant responses to abiotic stresses. As the core substrate for plant energy metabolism, sugars also exert key functions in osmotic adjustment, antioxidation, and stress signal transduction [12]. In recent years, the SWEET (Sugars Will Eventually be Exported Transporter) gene family, a novel class of sugar transport-related genes, has attracted extensive attention in plant stress resistance research. Transporters encoded by SWEET genes can mediate the transmembrane transport of various hexoses (e.g., glucose and fructose) and play important roles in plant organ development, pollen maturation, and stress responses [13–16]. Particularly under salt stress, SWEET proteins assist plants in maintaining osmotic balance and metabolic stability by regulating intercellular sugar distribution, thereby providing important support for salt tolerance [17]. Salt stress causes ionic imbalance and oxidative stress in plants, leading to cellular damage. Plants cope with these stresses through a series of mechanisms, among which sugar metabolism and antioxidant mechanisms play pivotal roles. Carbohydrates are not only the main energy source for plant growth and development but also important regulatory factors in stress responses. Under salt stress, plants maintain cellular osmotic balance and alleviate salt-induced damage by regulating the synthesis, transport, and accumulation of sugars [18–19].

To date, the SWEET gene family in *E. dahuricus* Turcz. has not yet been systematically identified, and neither the stress-resistance functions of its members nor the underlying regulatory mechanism of salt tolerance in this species have been reported. In the present study, we comprehensively characterized the SWEET gene family in *E. dahuricus* Turcz. via a genome-wide identification approach, and subsequently

screened out the *EdSWEET15* gene using qRT-PCR. Furthermore, we cloned the *EdSWEET15* gene from the leaves of *E. dahuricus* Turcz. and conducted a series of functional assays to investigate its role in salt tolerance. Collectively, this study lays a solid foundation for elucidating the molecular mechanism through which *EdSWEET15* mediates salt tolerance in *E. dahuricus* Turcz.

2 Results and analysis

2.1 Identification and analysis of physicochemical properties of *EdSWEET* genes

In this study, a total of 12 members of the EdSWEET gene family were identified from the genome of *E. dahuricus* Turcz. via homology alignment analysis, which were distributed across 8 distinct chromosomes (Fig. 1). Following the nomenclature of previously annotated *SWEET* genes in closely related species, these members were sequentially designated as *EdSWEET1a, EdSWEET1b, EdSWEET12a, EdSWEET2b, EdSWEET3a, EdSWEET4, EdSWEET12, EdSWEET13, EdSWEET14, EdSWEET15, EdSWEET16* and *EdSWEET17*.Furthermore, a systematic analysis was performed on the distribution characteristics of sequence splicing sites, physicochemical properties, and secondary structural features of the proteins encoded by the EdSWEET gene family in *E. dahuricus* Turcz. (Tables S2). The results showed that: the molecular weights of the proteins in this family ranged from 16132.4 to 33479.72 Da; the isoelectric point (pI) varied from 6.69 to 9.32; and the length of the amino acid sequences spanned 144 to 310 amino acids (aa). Protein stability prediction indicated that six members (*EdSWEET2a, 2b, 12, 14, 16, 17*) had instability index values greater than 40, classifying them as unstable proteins. Hydrophobicity analysis revealed that all proteins in this family exhibited hydrophobic characteristics.

2.2 Predictions of EdSWEET protein secondary and tertiary structures

Except for EdSWEET3a, which lacks a β-turn structure, the secondary structures of the remaining 11 EdSWEET proteins were predicted to comprise α-helices, random coils, β-turns, and extended strands, albeit with variations in the proportion of each structural element (Table S3, Fig. S1). Among all EdSWEET proteins, α-helices were the most dominant component of their secondary structures. The proportion of random coils ranged from 29.67% to 47.39%. β-turns contributed the least to the secondary structure across all EdSWEET proteins. The proportion of extended strands varied between 15.28% and 20.08%. Prediction of the tertiary structures of EdSWEET proteins (Fig. S2) revealed a high degree of similarity between the tertiary structures of EdSWEET1a and EdSWEET1b. The predicted tertiary structures of the remaining proteins exhibited significant divergence, even though α-helices remained the major structural component in all proteins.

2.3 Conserved motif analysis of EdSWEET protein sequences

In the motif prediction analysis of SWEET proteins in *E. dahuricus* Turcz., a total of 9 distinct motif sequences were identified (Fig. S3). All EdSWEET proteins harbored both motif 2 and motif 5, indicating that these two motifs represent the core structural components underlying the evolution of the *E. dahuricus* Turcz. SWEET gene family (Fig. 2A). Most motifs exhibited fixed positional distributions within the protein sequences. Domain analysis revealed that, except for EdSWEET2a and EdSWEET2b (which only contained two copies of the MtN3-slv superfamily domain), the remaining 10 EdSWEET proteins each possessed 1 to 2 MtN3-slv domains,a conserved core domain characteristic of plant SWEET gene families (Fig. 2B). Gene structure analysis showed that members of the *EdSWEET* gene family contained 4 to 6 exons and 3 to 5 introns. Genes with 6 exons and 5 introns were the most prevalent, accounting for 6 genes (50% of all family members) (Fig. 2C).

Figure 2 Conserved motif diagram of EdSWEET proteins. A: Conserved motif analysis of *EdSWEET* family proteins; B: Conservative structural domain analysis; C: Gene structure analysis.

2.4 Phylogenetic analysis of SWEET protein sequences

To verify the homology relationships between SWEET gene family of *E. dahuricus* Turcz. and those of *Avena sativa* L., *Arabidopsis thaliana*, *Oryza sativa* L., *Zea mays* L. and *Hordeum vulgare* L., a phylogenetic tree was constructed using the maximum likelihood method (Fig. 3). This analysis was based on protein sequences including 12 sequences from *E. dahuricus* Turcz., 13 from *A. sativa*, 16 from *H. vulgare*, 17 from *A. thaliana*, 21 from *O. sativa*, and 24 from *Z. mays*. The *E. dahuricus* Turcz. SWEET proteins were further classified into four subgroups: Group Ⅰ (1 member), Group Ⅱ (4 members), Group Ⅲ (3 members), and Group Ⅳ (4 members). The EdSWEET proteins exhibited high homology with EdSWEETs from monocot *A. sativa*, HvSWEETs from *H. vulgare*, OsSWEETs from *O. sativa*, and ZmSWEETs from *Z. mays*. In contrast, various AtSWEET proteins from dicot *A. thaliana* formed a distinct cluster independent of EdSWEETs, AsSWEETs, HvSWEETs, OsSWEETs, and ZmSWEETs.

2.5 Identification of cis-acting elements in EdSWEETs

A total of 222 cis-acting elements were identified by analyzing the 2000 bp upstream sequences of the SWEET gene promoters in *E. dahuricus* Turcz.. These elements are involved in the regulation of plant growth and development, biotic and abiotic stress responses, hormone responsiveness, and light responsiveness. The results showed that nine *EdSWEET* genes contained 4 to 17 light-responsive elements. Among them, five genes (*EdSWEET1a, 1b, 2a, 3a, 13*) harbored one auxin-responsive element each, while seven genes contained 1 to 4 gibberellin-responsive elements. Several genes were found to contain MeJA-responsive elements, salicylic acid-responsive elements, and abscisic acid (ABA)-responsive elements (Fig. 4). In addition, *EdSWEET3a* and *EdSWEET13* also contained defense and stress-responsive elements. Overall, the promoter regions of these *EdSWEET* genes contain a diverse array of cis-regulatory elements, indicating that they may be involved in multiple biological processes and regulatory pathways.

2.6 Responses of EdSWEET genes to salt stress

To investigate the functions of the EdSWEET gene family in salt stress response, the expression patterns of *EdSWEET* genes under salt stress treatment were analyzed via qRT-PCR in this study. Compared with the control group, salt stress remarkably induced differential expression of several *EdSWEET* genes: specifically, the transcript level of *EdSWEET1b* in root tissues exhibited a continuous upward trend with the extension of stress duration; the expression level of *EdSWEET2a* in roots decreased consistently over the stress period; while the expression of *EdSWEET15* in leaves increased significantly at 24 h post stress, and its transcript abundance in roots was continuously up-regulated throughout the entire stress process. These results indicated that the EdSWEET gene family plays important regulatory roles in plant responses to salt stress. Notably, the transcription level of *EdSWEET15* was significantly up-regulated in both roots and leaves under salt stress, a characteristic suggesting that this gene may serve as a key candidate gene mediating plant salt stress tolerance. To further clarify the biological function of *EdSWEET15*, the full-length sequence of this gene has been cloned in the present study, and subsequent functional verification experiments will be carried out.

Figure 5 Responses of *EdSWEET* Genes to Salt Stress, Significant differences are indicated by asterisks (*$p < 0.05$, **$p < 0.01$).

2.7 Cloning and bioinformatics analysis of the *EdSWEET15* gene

The *EdSWEET15* gene was successfully cloned from *E. dahuricus* Turcz., with a full-length coding sequence (CDS) of 930 bp (Fig. 6A). Phylogenetic analysis using the Neighbor-Joining method indicated that *EdSWEET15* from *Elymus dahuricus* shares the closest evolutionary relationship with *PpSWEET15* (from *Poa pratensis*) and *HvSWEET15* (from *Hordeum vulgare*) (Fig. 6B). This result suggests that the *SWEET15* gene is highly conserved among monocotyledonous plants, implying conserved biological functions during evolution. Protein-protein interaction (PPI) analysis results showed that EdSWEET15 potentially interacts with 10 proteins, namely NYC1 (Non-Yellow Coloring 1), NAC92 (NAC Domain-Containing Protein 92), SAG12 (Senescence-Associated Gene 12 Protein), SAG13 (Senescence-Associated Gene 13 Protein), SGR1 (Stay-Green Protein 1), BHLH13 (Basic Helix-Loop-Helix Transcription Factor 13), BHLH14 (Basic Helix-Loop-Helix Transcription Factor 14), MYC4 (Transcription Factor MYC4), MYC3 (Transcription Factor MYC3), and AIB (Auxin-Induced Protein B) (Fig. 6C). Functional annotation of these interacting proteins revealed their involvement in multiple biological processes, including maintenance of nucleic acid structural stability, transcriptional regulation of gene expression (mediated by transcription factors such as NAC92, BHLH13/14, and MYC3/4), and transmembrane transport. Given that EdSWEET15 belongs to the sugar transporter family, the interaction with these proteins suggests that EdSWEET15 may rely on such PPI networks to regulate or enhance its sugar transport activity, thereby participating in the coordination of sugar metabolism, stress responses, or senescence-related processes in *E dahuricus*.

2.8 Validation of *EdSWEET15* sugar transport function

To investigate the plant sugar transport activity of the *EdSWEET15* gene, transgenic *A. thaliana* harboring *EdSWEET15* and wild-type (WT) plants were separately inoculated onto 1/2 Murashige and Skoog (MS) medium plates supplemented with 0.2% glucose, 0.2% fructose, or 0.2% sucrose. Growth phenotypes were observed after three weeks of cultivation.The results showed that *EdSWEET15*-transgenic *Arabidopsis* grew normally on all three sugar-supplemented media. In contrast, the growth of WT plants was significantly inhibited on the medium containing 0.2% glucose. Compared with WT plants, *EdSWEET15*-transgenic *Arabidopsis* exhibited a distinct growth advantage on the 0.2% glucose medium. To eliminate experimental contingency, four seedlings each from the transgenic line and WT grown on 0.2% glucose medium were transferred to fresh 0.2% glucose medium for a replicate experiment, and consistent results were obtained (Fig. 7A). Additionally, the glucose content in *EdSWEET15* transgenic lines was significantly higher than that in WT plants under the condition of 0.2% glucose supplementation (Fig. 7B).Collectively, these findings demonstrate that *EdSWEET15* possesses glucose transport function.

2.9 Functional validation in transgenic *Arabidopsis*

After overexpressing *EdSWEET15* in *Arabidopsis thaliana*, the growth phenotypes of transgenic plants and wild-type (WT) plants were observed under different concentrations of NaCl treatment. Under 100 mmol/L NaCl treatment, the growth of WT plants was slightly inhibited, while transgenic plants remained unaffected and maintained normal growth. When exposed to 150 mmol/L NaCl, WT plants showed severe growth inhibition, with decreased vitality and slow growth, whereas transgenic plants were barely affected. At 200 mmol/L NaCl, both WT and transgenic plants exhibited chlorosis, indicating that the growth and development of both were severely inhibited (Fig. 8A). However, relatively, transgenic plants maintained higher viability than WT plants: transgenic plants still grew slowly, while WT plants completely ceased growth (Fig. 8E).Three *EdSWEET15* overexpressing *Arabidopsis* lines, namely OE-*EdSWEET15*-1, OE-*EdSWEET15*-2, and OE-*EdSWEET15*-3, were obtained through screening, and the effect of salt stress on their seed germination was detected. The results showed that under normal conditions, there was no significant difference in seed germination rate between *EdSWEET15*-overexpressing *Arabidopsis* and WT (Fig. 8B). Under salt stress, the seed germination rate of *EdSWEET15*-overexpressing *Arabidopsis* was significantly higher than that of WT (Fig. 8C, D).The above results indicate that heterologous overexpression of *EdSWEET15* can enhance the salt tolerance of *Arabidopsis thaliana* and improve the survival rate of *Arabidopsis* under salt stress.

heterologous overexpressing *Arabidopsis* lines

A :Seedling growth phenotypes of *A. thaliana* wild-type (WT) and overexpression (OE) lines under different concentrations of salt stress; B: Germination phenotypes of Arabidopsis WT and OE lines under normal growth conditions; C: Germination phenotypes of *Arabidopsis* WT and OE lines under salt stress conditions; D: Statistical analysis of seed germination rates of Arabidopsis WT and OE lines under salt stress ($P < 0.01$, extremely significant difference); E: Dynamic changes in growth phenotypes of Arabidopsis WT and OE lines after different durations of salt stress treatment

2.10 Analysis of reactive oxygen species (ROS)-related physiological indices

After salt stress treatment, compared with wild-type (WT) *A. thaliana*, the transgenic lines (OE-1, OE-2, OE-3) showed extremely significant reductions in both hydrogen peroxide (H_2O_2) content (Fig. 9A) and superoxide anion ($O_2^{-}\cdot$) production rate (Fig. 9B). Meanwhile, the activities of superoxide dismutase (SOD) (Fig. 9C), peroxidase (POD) (Fig. 9D), and catalase (CAT) (Fig. 9E) in the transgenic lines were significantly or extremely significantly higher than those in WT plants.The above results indicate that under salt stress, the transgenic *Arabidopsis* plants exhibited a significantly decreased level of ROS accumulation, while the scavenging capacity of the antioxidant enzyme system was significantly enhanced. Consequently, the degree of salt stress-induced damage in transgenic plants was significantly lighter than that in WT plants, and their salt tolerance was significantly improved. It can be concluded that overexpression of the *EdSWEET15* gene exerts a significant positive regulatory effect on the salt stress resistance of *A. thaliana*.

A: Hydrogen peroxide (H_2O_2) activity;(B): Superoxide anion production rate; C: Superoxide dismutase (SOD) activity;(D) Peroxidase (POD) activity; (E) Catalase (CAT) activity.

3 Discussion

As an important family of sugar transporters, SWEET genes play a central role in the transmembrane transport of sugars in plants. Particularly under environmental stress conditions, they can maintain cellular osmotic balance and enhance plant tolerance to adverse stresses by regulating the accumulation and distribution of sugars in vivo [20]. In the present study, a total of 12 EdSWEET family proteins were identified, and phylogenetic analysis clustered these proteins into four distinct clades. Previous studies have demonstrated that SWEET proteins from different phylogenetic clades exhibit distinct sugar transport specificities [21]. Our results confirmed that *EdSWEET15* possesses glucose transport activity. Notably, other studies have reported that SWEETs in Clade I (SWEET1, 2, and 3) and Clade II (SWEET4, 5, 6, 7, and 8) are primarily involved in the transport of glucose, fructose, and sucrose, while those in Clade III (SWEET9, 10, 11, 12, 13, 14, and 15) are specialized in the efficient transport of sucrose [22],which is consistent with the findings of the present study. Previous studies have confirmed that sugar transport and metabolism are not only the material basis for plant growth and development but also a key regulatory node in plant stress resistance [23], thereby providing a theoretical foundation for investigating the function of EdSWEET gene family in salt stress responses in this study.

The full-length cDNA sequence of *EdSWEET15* was successfully cloned from *E. dahuricus* Turcz. via gene cloning technology. Phylogenetic tree analysis indicated that *EdSWEET15* from *E. dahuricus* Turcz. has a close evolutionary relationship and high sequence similarity with *HvSWEET15* from *H. vulgare* and *PpSWEET15* from *P. pratensis*, suggesting that they may have similar biological functions. It has been reported that *OsSWEET15* in rice can maintain the osmotic balance and energy metabolism stability of

plants under salt stress by regulating sugar transport and metabolism, thereby improving plant salt tolerance [24]. Combined with the conserved structure of *EdSWEET15* (containing two MtN3_slv functional domains) and its evolutionary relationship in this study, it is speculated that *EdSWEET15* may be involved in the regulation of intracellular osmotic pressure in *E. dahuricus* Turcz. under salt stress through a similar sugar transport mechanism. In addition, multiple stress-responsive cis-acting elements were detected in the promoter region of *EdSWEET15*, including the abscisic acid (ABA)-responsive element ABRE [25] and the MYB transcription factor-binding site MBS [26]. The presence of the ABRE element suggests that the expression of *EdSWEET15* may be regulated by the ABA signaling pathway, which is consistent with the widely existing ABA-dependent regulatory pathway in plant stress responses [27–29]. The MBS element implies that it may interact with MYB transcription factors to further participate in salt stress responses. Previous studies have shown that MYB transcription factors can synergize with the ABA signaling pathway to maintain plant osmotic potential and ion homeostasis under high-salt environments by regulating the expression of cell wall synthesis-related genes and ion transporter genes [30–32]. Based on this, it is hypothesized that *EdSWEET15* may be involved in the salt stress response process of *E. dahuricus* Turcz. through the synergistic regulation of ABA and MYB signaling pathways, thereby enhancing plant salt tolerance.

To clarify the salt stress function of *EdSWEET15*, overexpressing *Arabidopsis* lines of *EdSWEET15* were constructed and salt tolerance verification was carried out in this study. The results showed that compared with WT plants, the salt tolerance of overexpressing lines was significantly improved. On the one hand, the seed germination rate of overexpressing lines under salt stress was significantly higher than that of WT. Previous studies have confirmed that salt stress inhibits plant seed germination [33], and the overexpression of *EdSWEET15* can effectively alleviate this inhibitory effect. On the other hand, under different concentrations of NaCl (100, 150, 200 mmol/L) treatment, the growth status of overexpressing lines was significantly better than that of WT. Specifically, under 100 mmol/L NaCl treatment, the growth of WT was slightly inhibited, while the overexpressing lines grew normally; under 150 mmol/L NaCl treatment, the growth of WT was severely inhibited, but the overexpressing lines still maintained a good growth state; under 200 mmol/L NaCl treatment, both lines showed chlorosis, but the overexpressing lines could still grow slowly, while the WT almost stopped growing (this is consistent with the result reported by Zhang, H. et al. [4] that "NaCl concentrations above 100 mmol/L significantly inhibit the growth of *E. dahuricus* Turcz., and the increase in concentration increases the seedling mortality rate"). In addition, physiological index detection showed that under salt stress, the hydrogen peroxide content and superoxide anion production rate of *EdSWEET15*-overexpressing *Arabidopsis* were significantly lower than those of WT, while the activities of antioxidant enzymes such as SOD, POD, and CAT were significantly higher than those of WT. This result indicates that *EdSWEET15* may accelerate ROS scavenging and reduce oxidative damage by enhancing antioxidant enzyme activity.

This study clarified the function and mechanism of *EdSWEET15* in the salt stress response of *E dahuricus*. It not only enriches the research on the stress resistance function of plant SWEET family genes but also provides a theoretical basis and candidate gene resources for improving the salt

tolerance of *E. dahuricus* Turcz. through genetic engineering and breeding new salt-tolerant forage varieties. It is of great significance for promoting the ecological restoration of saline-alkali grasslands and the sustainable development of animal husbandry.

4 Conclusions

In this study, a total of 12 SWEET sugar transporter genes were identified in *E. dahuricus* Turcz., which are distributed across 8 chromosomes of the *E. dahuricus* Turcz. genome. Phylogenetic analysis revealed that the encoded SWEET proteins are clustered into four distinct subfamilies. Additionally, cis-acting elements associated with light responsiveness, growth and development, and stress responses were identified in the promoter regions of these SWEET genes. Among them, *EdSWEET15* was significantly upregulated following stress treatment. Functional validation assays demonstrated that *EdSWEET15* exhibits glucose transport activity. Heterologous overexpression of *EdSWEET15* in *A. thaliana* showed that, compared with WT plants, *EdSWEET15*-overexpressing lines displayed markedly improved salt tolerance under salt stress conditions. Specifically, the seed germination rate was significantly increased, the accumulation of reactive oxygen species (ROS) was notably reduced, and the scavenging capacity of the antioxidant enzyme system, including SOD, POD, and CAT was significantly enhanced. Collectively, this study preliminarily clarifies the function of *EdSWEET15* from *E. dahuricus* Turcz. in mediating salt stress responses, thereby laying a theoretical foundation for the excavation and utilization of stress-responsive functional genes in salt-tolerant plant species.

5 Materials and methods

5.1 Materials

Seeds of *E. dahuricus* Turcz. cv. ‘Duanmang 1’ (the cultivar used in this study) were provided by the Academy of Animal Husbandry and Veterinary Sciences, Qinghai University. A voucher specimen (PE 00489123) of *E dahuricus* has been deposited at the Chinese Virtual Herbarium (https://www.cvh.ac.cn/spms/detail.php?id=f37b7174), and was identified by Dr. Liu Liang on May 5, 1966. This specimen serves as a reference for taxonomic verification. The seeds were sown in plastic pots filled with a mixed substrate (soil: vermiculite = 2:1, v/v). The plants were cultured in a growth chamber under controlled conditions: 25°C (day) / 20°C (night), and a 14 h light : 10 h dark photoperiod. Both the control group and salt treatment group were set with three biological replicates.At 20 days after sowing, the seedlings were exposed to salt stress (150 mmol/L NaCl solution). Root and leaf tissues of *E. dahuricus* cv. ‘Duanmang 1’ were harvested at 0 h, 3 h, 6 h, 9 h, 12 h, and 24 h post stress treatment. All collected samples were immediately snap-frozen in liquid nitrogen and stored at -80°C for subsequent experimental procedures. The coding sequence of *EdSWEET15* (identified and sequenced in this study) has been deposited in the GenBank database of the National Center for Biotechnology Information (NCBI), with the accession number PX826256.

5.2 Identification of *EdSWEET* genes

The protein sequences of SWEET genes from *A. thaliana*, *O. sativa* L., *Z. mays* L., *H. vulgare* L. and *A. sativa* L. were directly downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/). The genome file of *E. dahuricus* Turcz. was provided by our research group. To identify the *E dahuricus* Turcz. SWEET genes, the BLAST wrapper in TBtools 2.07 was used to align known SWEET genes to the *E. dahuricus* Turcz.genome [34]. Redundant alleles were filtered out from the initially identified gene candidates to obtain the final set of non-redundant gene sequences. The naming of the *EdSWEET* genes was based on the reconstructed evolutionary tree, with gene names assigned according to previously annotated SWEET genes from closely related species.

5.3 Characterization of EdSWEET proteins, sequence structure and phylogenetic analyses

The physicochemical properties of the *EdSWEET* protein sequences, including the number of amino acids, molecular weight, theoretical isoelectric point, instability index, aliphatic index, and total average hydrophilicity, were analyzed using the ExPAsy-ProtParam tool (https://web.expasy.org/protparam/) [35]. The protein's secondary structure was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/), and tertiary structure predictions were generated using SWISS-MODEL (https://swissmodel.expasy.org/) [36–40].

Conserved motif analysis of the SWEET amino acid sequences in *E. dahuricus* Turcz. was conducted using the MEME online tool (http://meme-suite.org/;) [41]. Gene structure and motif distributions were visualized using TBtools 2.07 [42]. A phylogenetic tree of the SWEET family was constructed using the maximum likelihood method in MEGA 11.0, incorporating 12 *E. dahuricus* Turcz. sequences,13 *A. sativa*, sequences, 16 *H. vulgare* sequences, 17 *A. thaliana* sequences, 21 *O. sativa* sequences, and 24 *Z. mays* sequences. The protein sequences used for these analyses can be found in Additional file 2.

5.4 Cis-acting elements and chromosome distributions analysis

The cis-acting elements in the putative promoter regions of the *EdSWEET* genes, defined as the 2,000 bp upstream of the genes, were predicted using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [43], and the predicted cis-acting elements were visualized using TBtools 2.07 [42]. The chromosomal localizations of the SWEET genes were visualized using TBtools 2.07 [42].

5.5 Gene cloning and sequence analysis

Using the cDNA of the *EdSWEET15* gene (930 bp) as the template, specific primers were designed (Table S1). Leaf samples of *E. dahuricus* Turcz.were collected, and total RNA was extracted using the TIANGEN RNA simple Total RNA Kit. Reverse transcription was performed following the manufacturer's instructions (TIANGEN Reverse Transcription Kit). The PCR reaction system (50 μL total volume) consisted of 25μL PrimSTAR Max DNA Polymerase, 2 μL each of forward and reverse primers, 2μL cDNA

template, and 19 μL double-distilled water (ddH_2O). The PCR amplification program was set as follows: initial denaturation at 98°C for 10 s, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 5 s, and extension at 72°C for 15 s, with a final hold at 4°C. After electrophoresis verification, the target PCR product was recovered using the TaKaRa Mini BEST Agarose Gel DNA Extraction Kit Ver.4.0 (Takara Bio Inc.). The recovered product was ligated into the Pcan vector and transformed into Escherichia coli competent cells (DH5α). Positive colonies were identified by colony PCR and sent to Tsingke Biotechnology Co., Ltd. (Xi'an) for Sanger sequencing.

Amino acid sequences with high homology to *EdSWEET15* were retrieved using BLAST on the NCBI website. A phylogenetic tree was constructed by the Neighbor-Joining method using MEGA 7.0 software. Protein-protein interactions among the EdSWEET15 and other proteins were predicted using the online tool STRING (https://cn.string-db.org), and the results were visualized accordingly [44].

5.6 Expression pattern analysis

Total RNA was extracted using the Total RNA Extraction Kit (DP419, Tiangen). Subsequently, complementary DNA (cDNA) was synthesized with the Prime Script™ RT Reagent Kit with gDNA Eraser (R047A, Takara). Specific primers for the EdSWEET gene were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), and their sequences are provided in Table S1. Quantitative real-time PCR (qRT-PCR) was performed using the SuperReal PreMix Plus (SYBR Green) kit. The total reaction volume was 20 μL, comprising 5 μL of diluted template (20 μL of cDNA diluted 20-fold with 180 μL of ddH_2O), 3 μL of ddH_2O, 1 μL of forward primer and 1 μL of reverse primer (each at 10 $\mu mol \cdot L^{-1}$), and 10 μL of 2 × SuperReal PreMix Plus (SYBR Green). qRT-PCR was carried out on a LightCycler®96 Instrument with the following program: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 10 s and annealing at 58°C for 30 s. Each treatment included three biological replicates, and each biological replicate was subjected to three technical replicates. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [45], with the actin gene serving as the reference gene.

5.7 Transgenic functional validation

The *EdSWEET15* gene was cloned into the plant expression vector to construct Pcan-EdSWEET15, which was then transformed into *Agrobacterium tumefaciens*. Transgenic *A. thaliana* plants were generated via the *Agrobacterium*-mediated transformation method.

To validate the function of *EdSWEET15*, sugar transport assays and salt tolerance experiments were performed on the transgenic *Arabidopsis* lines. For the sugar transport assay, the growth status of transgenic plants was observed on culture media supplemented with different sugar substrates. For the salt tolerance experiment, both transgenic and wild-type (WT) *Arabidopsis* plants were first grown in soil using a pot culture method. After reaching the appropriate growth stage, the plants were subjected to salt stress treatment, and subsequent phenotypic changes were monitored to further verify the biological function of *EdSWEET15*.

5.8 Analysis of reactive oxygen species (ROS)-related physiological indicators

Leaf samples were collected from transgenic *A. thaliana* plants and wild-type (WT) controls at 6 days after salt stress treatment for the determination of ROS-related physiological indicators. The hydrogen peroxide (H_2O_2) content was measured using the potassium iodide (KI) spectrophotometric method [46]. The production rate of superoxide anion ($O_2^{-}\cdot$) was assayed according to the protocol described by Ke et al. [47]. The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined via the nitroblue tetrazolium (NBT) reduction method [48], which is based on the principle that SOD inhibits the reduction of NBT to formazan by scavenging $O_2^{-}\cdot$. Peroxidase (POD) activity was measured using the guaiacol method [49], where POD catalyzes the oxidation of guaiacol by H_2O_2 to form a reddish-brown product (tetra methoxyphenol) with a characteristic absorption peak at 470 nm. Catalase (CAT) activity was determined following the method reported by Chakrabarty et al. [46]. All measurements were performed in three biological replicates to ensure statistical reliability.

5.9 Data processing

Experimental data were statistically analyzed and graphically visualized using Microsoft Excel. Statistical significance was determined via one-way analysis of variance (ANOVA) using SPSS 26.0 software.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

YP and PZ : Conceptualization, Validation, Software, Writing – original draft. MH , YW, YL,PW and JG : Visualization, Validation, Formal analysis. ZJ : Supervision, Funding acquisition. KN : Conceptualization, Funding acquisition, Writing – review & editing.

Acknowledgments

Not applicable

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The genome file of E. dahuricus Turcz. was provided by our research group. The protein sequences of SWEET genes in E. dahuricus , A. thaliana , O. sativa , H. vulgare , and Z. mays were directly downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/), and all protein sequences can be found in the Additional file 2. The coding sequence of EdSWEET15 has been deposited in the GenBank database of the NCBI, with the accession number PX826256.

References

1. Miao J, Zhang X, Chen S, Ma X, Chen Z, Zhong J, Bai S. Gliadin analysis of Elymus nutans Griseb. from the Qinghai–Tibetan plateau and Xinjiang, China. Grassland Sci. 2011;57:127–34. https://doi.org/10.1111/j.1744-697X.2011.00219.x.
2. Lu BR. 1993. Meiotic studies of Elymus nutans and E. jacquemontii (Poaceae, Triticeae) and their hybrids with Pseudoroegneria spicata and seventeen Elymus species. Plant Syst. Evol. 186, 193–212. https://doi.org/10.1007/BF00940798
3. Fu J, Chu X, Sun Y, Miao Y, Xu Y, Hu T. Nitric oxide mediates 5-aminolevulinic acid-induced antioxidant defense in leaves of Elymus nutans griseb. exposed to chilling stress. PLoS ONE. 2015;10:e0130367. https://doi.org/10.1371/journal.pone.0130367.
4. Zhang H, Tian Y, Guan B, Zhou D, Sun Z, Baskin CC. The best salt solution parameter to describe seed/seedling responses to saline and sodic salts. Plant Soil. 2018;426:313–25. https://doi.org/10.1007/s11104-018-3623-8.
5. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651–81. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
6. Zhu JK. Abiotic stress signaling and responses in plants. Cell. 2016;167:313–24. https://doi.org/10.1016/j.cell.2016.08.029.
7. Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;9:681. https://doi.org/10.3390/antiox9080681.

8. Shabala S, Cuin TA. Potassium transport and plant salt tolerance. Physiol Plant. 2008;133:651–69. https://doi.org/10.1111/j.1399-3054.2007.01008.x.
9. Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 2012, 217037. https://doi.org/10.1155/2012/217037
10. Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiol Biochem. 2020;156:64–77. https://doi.org/10.1016/j.plaphy.2020.08.042.
11. Jogawat A. Osmolytes and their role in abiotic stress tolerance in plants. Mol plant abiotic stress: biology Biotechnol. 2019;91–104. https://doi.org/10.1002/9781119463665.ch5.
12. Sabagh AE, Mbarki S, Hossain A, Iqbal MA, Islam MS, Raza A, et al. Potential role of plant growth regulators in administering crucial processes against abiotic stresses. Front Agron. 2021;3:648694. https://doi.org/10.3389/fagro.2021.648694.
13. Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, et al. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature. 2010;468:527–32. https://doi.org/10.1038/nature09606.
14. Pan Y, Niu K, Miao P, Zhao G, Zhang Y, Ju Z, et al. Genome-wide analysis of the SWEET gene family and its response to powdery mildew and leaf spot infection in the common oat (Avena sativa L). BMC Genomics. 2024;25:995. https://doi.org/10.1186/s12864-024-10933-8.
15. Gill RA, Ahmar S, Ali B, Saleem MH, Khan MU, Zhou W, Liu S. The role of membrane transporters in plant growth and development, and abiotic stress tolerance. Int J Mol Sci. 2021;22:12792. https://doi.org/10.3390/ijms222312792.
16. Ji J, Yang L, Fang Z, Zhang Y, Zhuang M, Lv H, Wang Y. Plant SWEET family of sugar transporters: structure, evolution and biological functions. Biomolecules. 2022;12:205. https://doi.org/10.3390/biom12020205.
17. Sami F, Yusuf M, Faizan M, Faraz A, Hayat S. Role of sugars under abiotic stress. Plant Physiol Biochem. 2016;109:54–61. https://doi.org/10.1016/j.plaphy.2016.09.005.
18. Gautam T, Dutta M, Jaiswal V, Zinta G, Gahlaut V, Kumar S. Emerging roles of SWEET sugar transporters in plant development and abiotic stress responses. Cells. 2022;11:1303. https://doi.org/10.3390/cells11081303.
19. Saddhe AA, Manuka R, Penna S. Plant sugars: Homeostasis and transport under abiotic stress in plants. Physiol Plant. 2021;171:739–55. https://doi.org/10.1111/ppl.13283.
20. Gong Z, Yang S. Drought meets SWEET. Nat Plants. 2022;8:25–6. https://doi.org/10.1038/s41477-021-01032-7.
21. Mizuno H, Kasuga S, Kawahigashi H. The sorghum SWEET gene family: stem sucrose accumulation as revealed through transcriptome profiling. Biotechnol Biofuels. 2016;9:1–12. https://doi.org/10.1186/s13068-016-0546-6.

22. Baker RF, Leach KA, Braun DM. SWEET as sugar: new sucrose effluxers in plants. Mol Plant. 2012;5:766–8. https://doi.org/10.1093/mp/SSS054.
23. Wu Y, Chen H, Wu M, Zhou Y, Yu C, Yang Q, et al. A vacuolar invertase gene SlVI modulates sugar metabolism and postharvest fruit quality and stress resistance in tomato. Hortic Res. 2025;12:uhae283. https://doi.org/10.1093/hr/uhae283.
24. Mathan J, Singh A, Ranjan A. Sucrose transport in response to drought and salt stress involves ABA-mediated induction of OsSWEET13 and OsSWEET15 in rice. Physiol Plant. 2021;171:620–37. https://doi.org/10.1111/ppl.13210.
25. Sun F, Deng Y, Zhao W, Xiong Y, Zhao L, Zhang L. High-throughput quantitative assessment of ABA-responsive elements at single-nucleotide resolution. Quant Biol. 2025;13:e87. https://doi.org/10.1002/qub2.87.
26. Sun W, Gao Z, Wang J, Huang Y, Chen Y, Li J, et al. Cotton fiber elongation requires the transcription factor GhMYB212 to regulate sucrose transportation into expanding fibers. New Phytol. 2019;222:864–81. https://doi.org/10.1111/nph.15620.
27. Zeng Z, Lyu T, Lyu Y. LoSWEET14, a sugar transporter in lily, is regulated by transcription factor LoABF2 to participate in the ABA signaling pathway and enhance tolerance to multiple abiotic stresses in tobacco. Int J Mol Sci. 2022;23:15093. https://doi.org/10.3390/ijms232315093.
28. Zhu J, Zhou L, Li T, Ruan Y, Zhang A, Dong X, et al. Genome-wide investigation and characterization of SWEET gene family with focus on their evolution and expression during hormone and abiotic stress response in maize. Genes. 2022;13:1682. https://doi.org/10.3390/genes13101682.
29. Yang YZ, Tan BC. A distal ABA responsive element in AtNCED3 promoter is required for positive feedback regulation of ABA biosynthesis in Arabidopsis. PLoS ONE. 2014;9:e87283. https://doi.org/10.1371/journal.pone.0087283.
30. Wang Z, Li X, Gao XR, Dai ZR, Peng K, Jia LC, et al. IbMYB73 targets abscisic acid-responsive IbGER5 to regulate root growth and stress tolerance in sweet potato. Plant Physiol. 2024;194:787–804. https://doi.org/10.1093/plphys/kiad532.
31. Balasubramaniam T, Shen G, Esmaeili N, Zhang H. Plants' response mechanisms to salinity stress. Plants. 2023;12:2253. https://doi.org/10.3390/plants12122253.
32. Geng W, Li Z, Hassan MJ, Peng Y. Chitosan regulates metabolic balance, polyamine accumulation, and Na + transport contributing to salt tolerance in creeping bentgrass. BMC Plant Biol. 2020;20:506. https://doi.org/10.1186/s12870-020-02720-w.
33. Wang Y, Wu F, Zou R, Xu M, Shan H, Cheng B, Li X. The maize sugar transporters ZmSWEET15a and ZmSWEET15b positively regulate salt tolerance in plants. Plant Physiol Biochem. 2024;213:108845. https://doi.org/10.1016/j.plaphy.2024.108845.
34. Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, et al. TBtools-II: A one for all, all for one bioinformatics platform for biological big-data mining. Mol Plant. 2023;16:1733–42. https://doi.org/10.1016/j.molp.2023.09.010.

35. Jayhoon AS, Kumar P. 2024. In-silico characterization and expression analysis of the Waxy gene in rice (Oryza sativa L.). agriRxiv 20240292736. https://doi.org/10.31220/agriRxiv.2024.00260
36. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 46. 2018;W296–303. https://doi.org/10.1093/nar/gky427.
37. Bienert S, Waterhouse A, De Beer TA, Tauriello G, Studer G, Bordoli L, Schwede T. The SWISS-MODEL Repository—new features and functionality. Nucleic Acids Res. 2017;45:D313–9. https://doi.org/10.1093/nar/gkw1132.
38. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis. 2009;30:S162–73. https://doi.org/10.1002/elps.200900140.
39. Studer G, Rempfer C, Waterhouse AM, Gumienny R, Haas J, Schwede T. QMEANDisCo—distance constraints applied on model quality estimation. Bioinformatics. 2020;36:1765–71. https://doi.org/10.1093/bioinformatics/btz828.
40. Bertoni M, Kiefer F, Biasini M, Bordoli L, Schwede T. Modeling protein quaternary structure of homo- and hetero-oligomers beyond binary interactions by homology. Sci Rep. 2017;7:10480. https://doi.org/10.1038/s41598-017-09654-8.
41. Ghorbel M, Zribi I, Chihaoui M, Alghamidi A, Mseddi K, Brini F. Genome-Wide Investigation and Expression Analysis of the Catalase Gene Family in Oat Plants (Avena sativa L). Plants. 2023;12:3694. https://doi.org/10.3390/plants12213694.
42. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13:1194–202. https://doi.org/10.1016/j.molp.2020.06.009.
43. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res. 2002;30:325–7. https://doi.org/10.1093/nar/30.1.325.
44. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2012;41:D808–15. https://doi.org/10.1093/nar/gks1094.
45. Damgaard MV, Treebak JT. Protocol for qPCR analysis that corrects for cDNA amplification efficiency. STAR Protoc. 2022;3:101515. https://doi.org/10.1016/j.xpro.2022.101515.
46. Chakrabarty D, Datta SK. Micropropagation of gerbera: Lipid peroxidation and antioxidant enzyme activities during acclimatization process. Acta Physiol Plant. 2008;30:325–31. https://doi.org/10.1007/s11738-007-0125-3.
47. Ke D, Sun G, Wang Z. Effects of superoxide radicals on ACC synthase activity in chilling-stressed etiolated mungbean seedlings. Plant Growth Regul. 2007;51:83–91. https://doi.org/10.1007/s10725-006-9150-2.

48. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44:276–87. https://doi.org/10.1016/0003-2697(71)90370-8.
49. Muñoz-Muñoz JL, García-Molina F, García-Ruiz PA, et al. Enzymatic and chemical oxidation of trihydroxylated phenols. Food Chem. 2009;113:435–44. https://doi.org/10.1016/j.foodchem.2008.07.076.

Figures

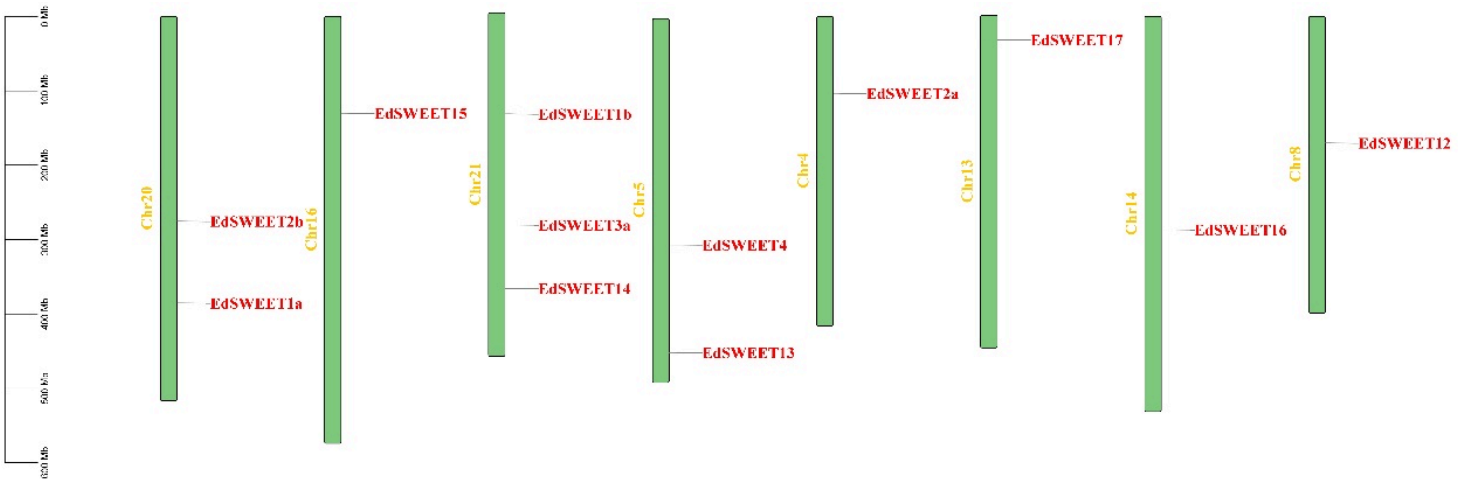


Figure 1

Distribution of *EdSWEETs* genes across *E. dahuricus* Turcz.chromosomes. Green bars represent the eight chromosomes that contain *EdSWEET*genes. The scale indicates the length of each chromosome, and the black lines mark the position of each EdSWEET gene.

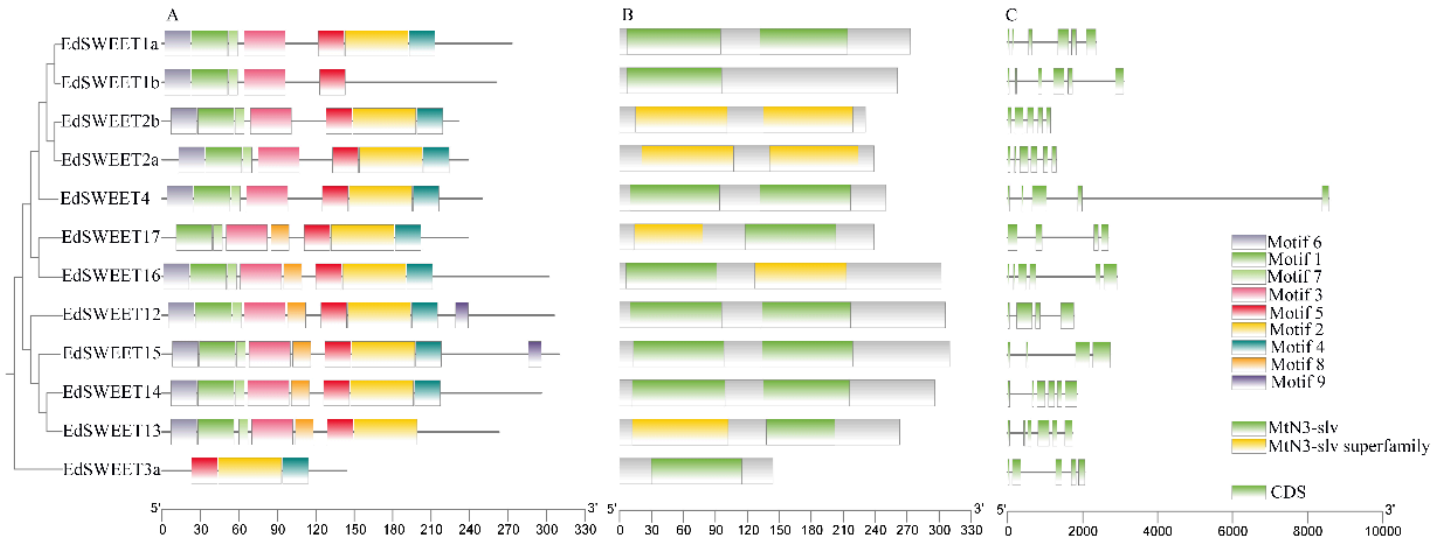


Figure 2

Conserved motif diagram of EdSWEET proteins. A: Conserved motif analysis of *EdSWEET* family proteins; B: Conservative structural domain analysis; C: Gene structure analysis.

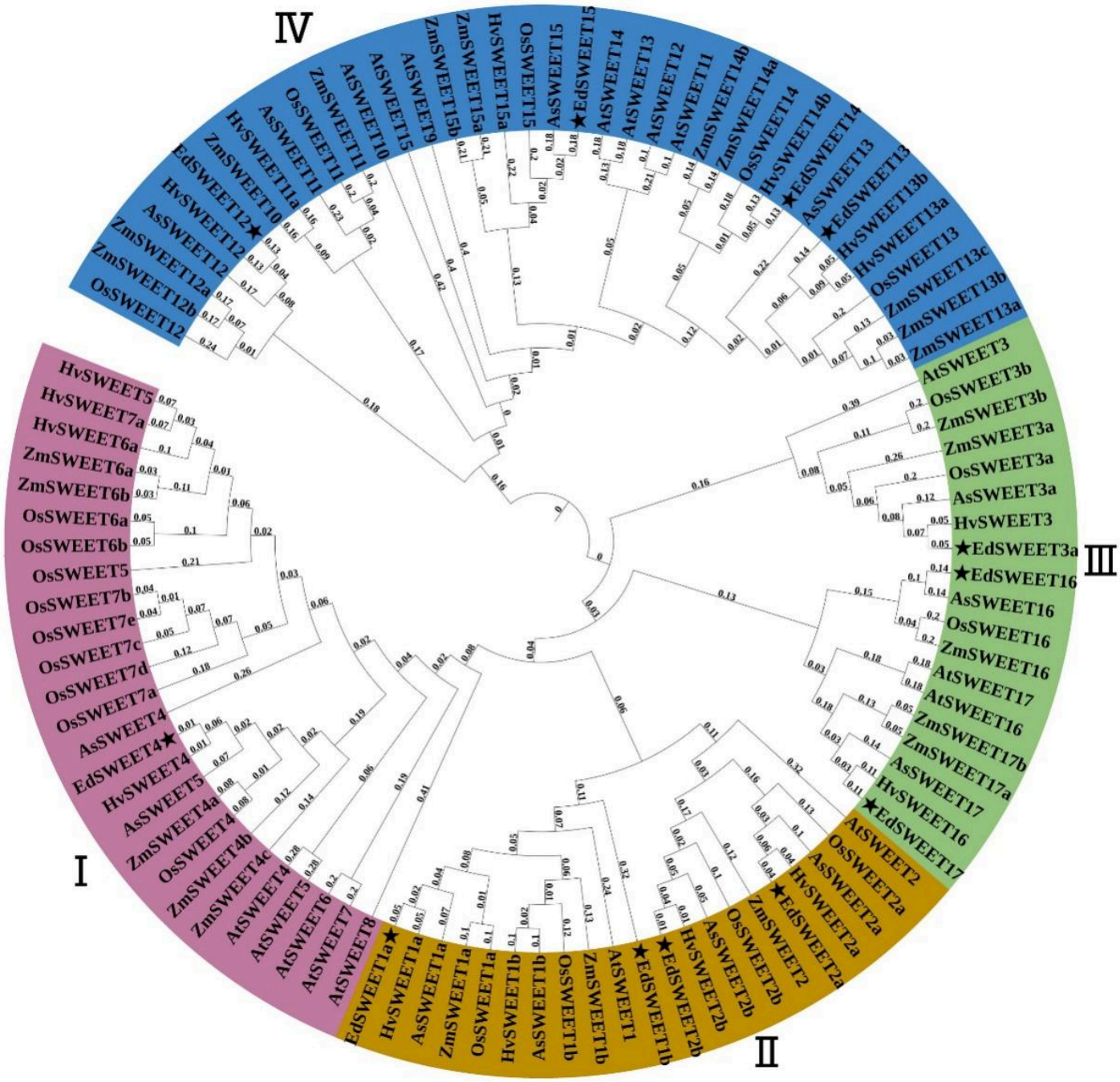


Figure 3

Phylogenetic analysis of SWEET protein sequences in *E. dahuricus*Turcz., *A. thaliana*, *A. sativa*, *O. sativa* and Z. mays. *E. dahuricus*Turcz. *EdSWEET* proteins are marked with stars.

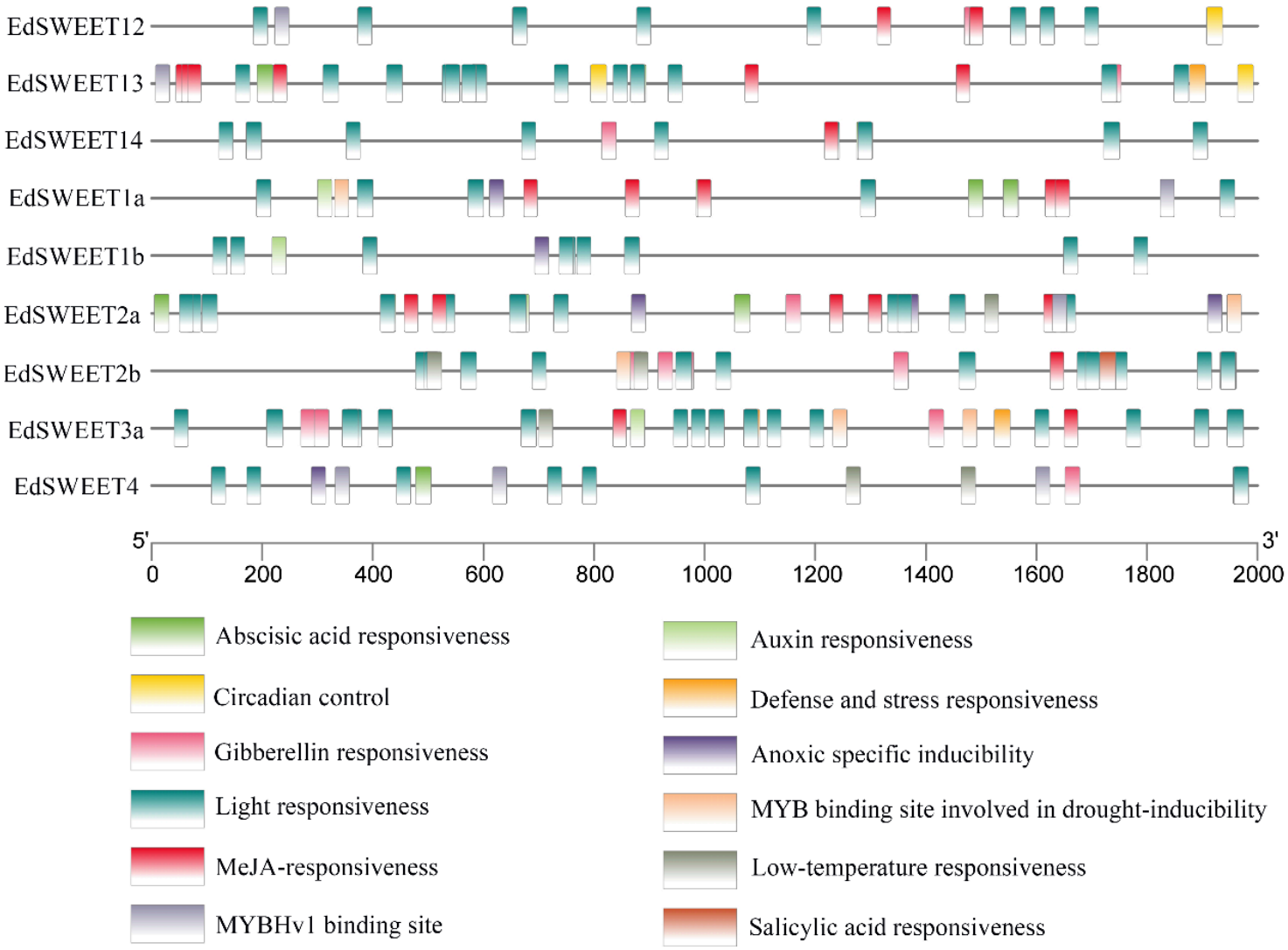


Figure 4

The cis-acting regulatory elements contained in the 2 kb promoter regions of the *EdSWEET* genes.

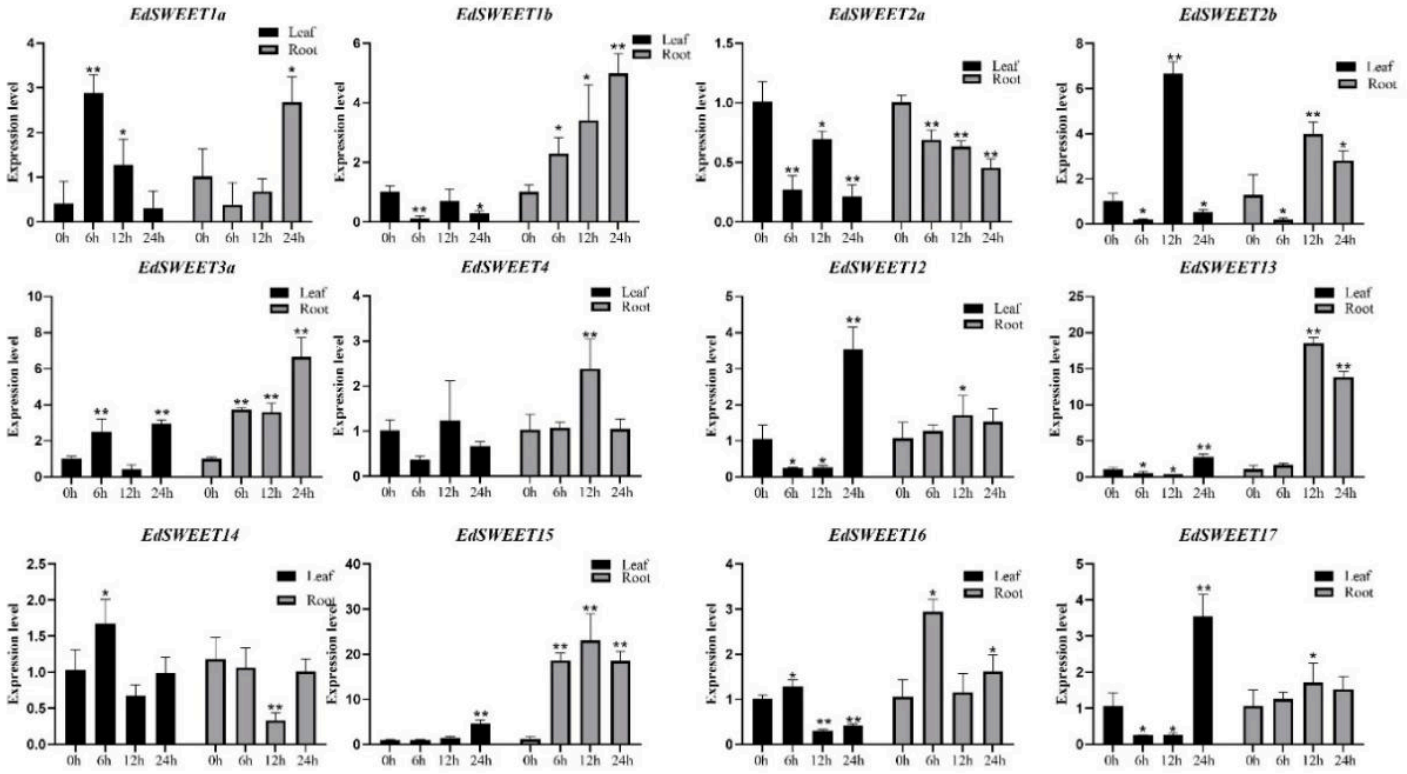


Figure 5

Responses of *EdSWEET* Genes to Salt Stress, Significant differences are indicated by asterisks (*p < 0.05, **p < 0.01).

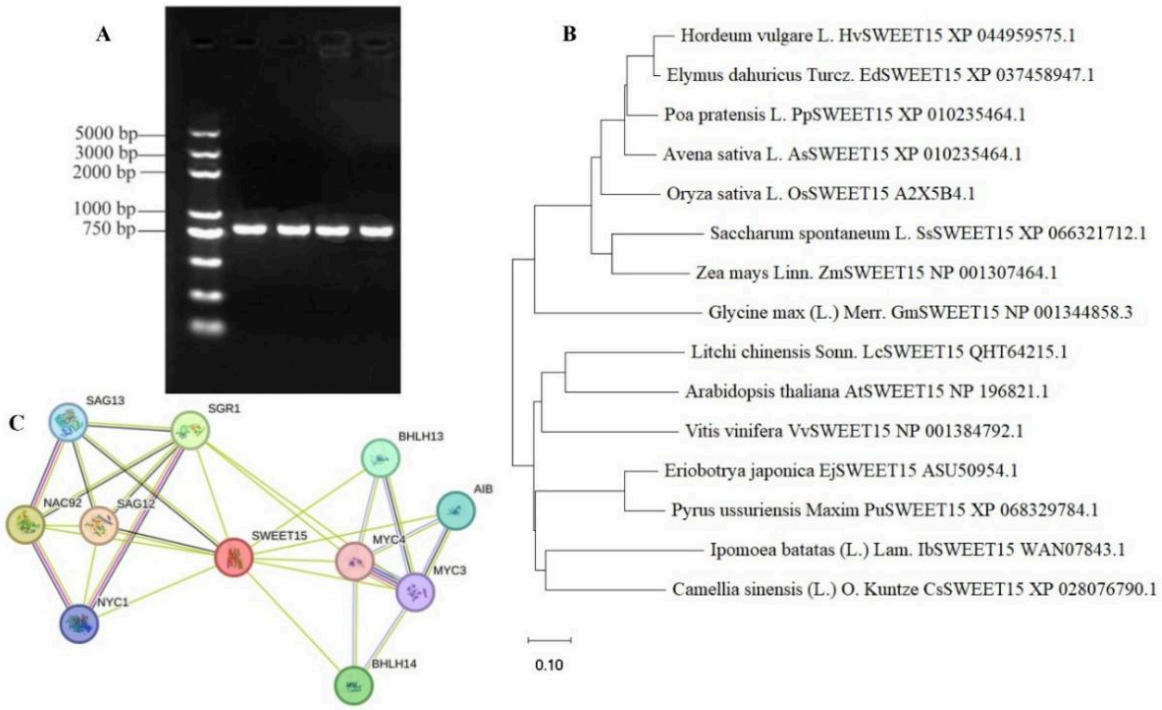


Figure 6

Bioinformatics Analysis of the *EdSWEET15*Gene A: Electrophoretic image of PCR amplification product of *EdSWEET15*gene with lane number (Marker of 5000 bp). B: Phylogenetic tree of EdSWEET15 homologous proteins C: Protein interaction analysis of EdSWEET15

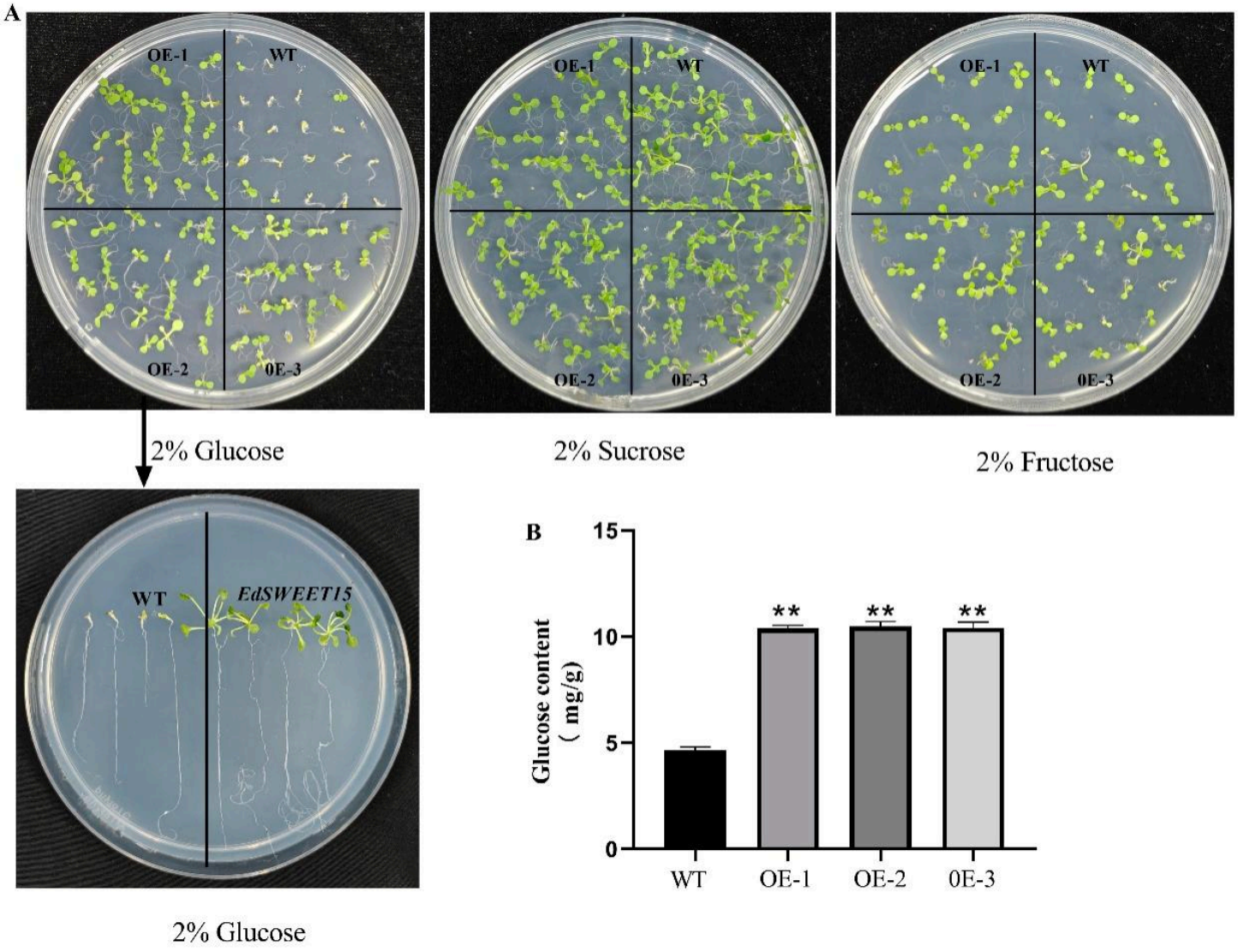


Figure 7

A: Growth performance of *EdSWEET15*-transgenic Arabidopsis on media with different sugars; B: Glucose content analysis

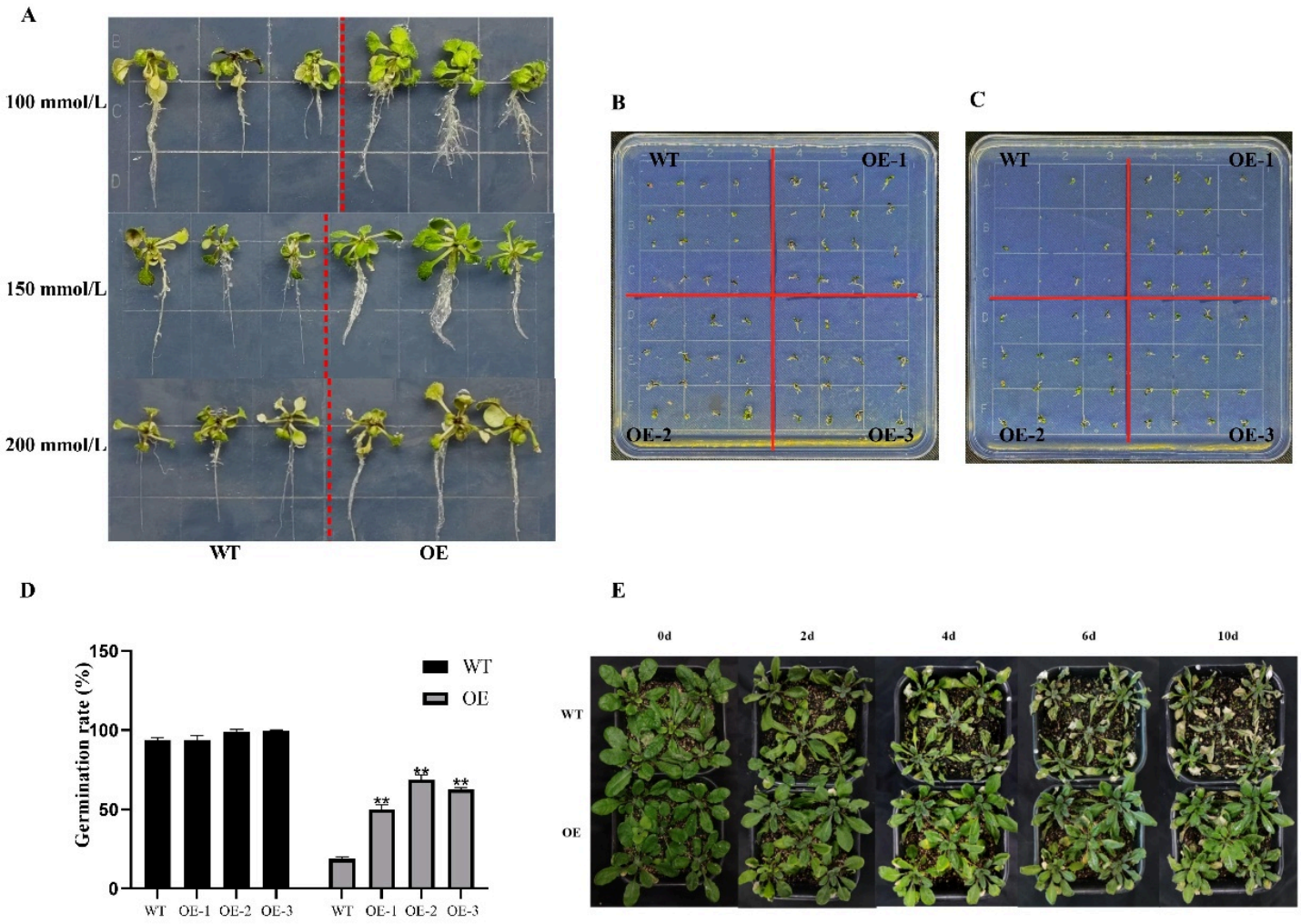


Figure 8

Effects of salt stress on seed germination and seedling growth of *EdSWEET15* heterologous overexpressing *Arabidopsis* lines

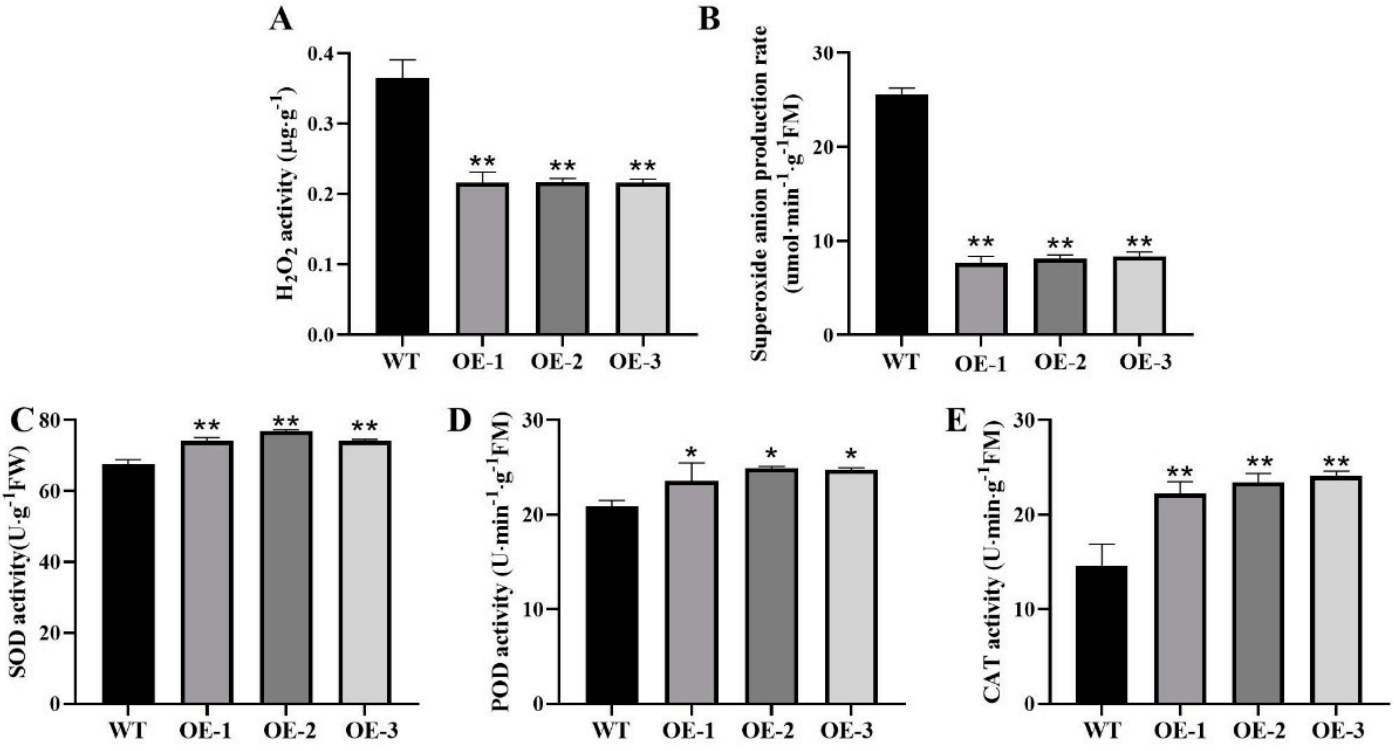


Figure 9

Effects of gene overexpression on reactive oxygen species (ROS)-related physiological indices in plants.

Supplementary Files

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