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Genome-wide identification of HAK gene family in S*partina alterniflora* and functional characterization of *SaHAK13, SaHAK40* and *SaHAK65* in salt stress

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Abstract: KT/HAK/KUP gene family represents the largest class of plant potassium transporters and is central to plant growth and stress adaptation. However, knowledge of this gene family in the halophyte *S. alterniflora* is limited. We identified 70 HAK genes in the *S. alterniflora* genome. These *SaHAK* genes were unevenly distributed across 30 chromosomes and grouped into 11 phylogenetic clusters. Segmental

duplication was identified as the main mechanism underlying *SaHAK* family expansion and may have contributed to functional diversification under saline conditions. Although SaHAK proteins shared conserved motifs and K-trans domains. Their cation-acting elements are still different. Under salt stress, *SaHAK*s showed significant tissue-specific expression with 35.7% *SaHAK*s differently expressed in *S. alterniflora* stems and 25.7% in the roots. GO enrichment result shows that differently expressed *SaHAK*s are mainly enriched in biological processes which related to potassium transport. Furthermore, three salt-inducible *SaHAK*s gene were selected for overexpression in *Nicotiana benthamiana* to explore their functions. After 7d salt stress, transgenic plants had significantly greater root length and root activity than WT. Overexpression of *SaHAK13*,*40* and *65* significantly increased K^+ accumulation and reduced the Na^+/K^+ ratio in *N. benthamiana*. Compare with WT, the K^+ content in transgenic plants increased by 34.51%-49.28% while the Na^+/K^+ ratio significantly decreased by 16.28%-28.96%. Proline, soluble sugar content and SOD, POD and CAT activities were lower in overexpressing lines, whereas chlorophyll and carotenoid contents were higher under stress. Partial least squares path modelling suggested that a lower Na^+/K^+ ratio was associated with reduced accumulation of osmotic regulatory substances, lower activation of the enzymatic antioxidant system and decreased degradation of photosynthetic pigments, thereby contributing to improved plant salt tolerance. Our findings deepen understanding of the biological functions of the plant KT/HAK/KUP gene family and identify potential genetic targets for breeding salt-tolerant crops.

Keywords: *Spartina alterniflora*, KT/HAK/KUP, Gene family, Salt stress,

1. Introduction

Potassium (K) is the most abundant monovalent cation in plants, accounting for nearly 10% of plant dry weight. Most of critical physiological processes are regulate by K^+ including stomatal movement, photosynthesis, enzyme activation, and osmotic adjustment (Li et al., 2022). K^+ deficiency often causes leaf chlorosis and reduced photosynthetic efficiency, thereby limiting plant growth (Luo et al., 2025). As a major osmotic ion, K^+ also helps maintain cellular turgor by regulating intracellular osmotic potential (Marschner et al., 2011). Plant cells typically maintain relatively high cytosolic K^+ concentrations of approximately 100-150 mM (White et al., 2013). Under stress conditions, particularly salinity, the elevated Na^+ impedes K^+ influx across plasma membranes, leading to a critical decline in tissue K^+/Na^+ ratios (Chen et al., 2018). This ionic imbalance disrupts cellular function, causes metabolic disorders and ultimately inhibits plant growth (Nahar et al., 2015).

K^+ uptake and transport in plants are primarily mediated by potassium transporters and ion channels (Feng et al., 2020). The KT/HAK/KUP family represents the largest group of K^+ transporters in plants and play important roles in K^+ absorption and transport (Liu et al., 2025; Feng et al., 2020). The first KT/HAK/KUP genes were cloned by Santa et al. (1997) and Fu et al. (1998) from *Hordeum vulgare* and *Arabidopsis thaliana*. Following yeast complementation assays showed that these genes restored K^+ uptake in K^+ uptake-deficient yeast strains, supporting their function

as potassium transport proteins. Subsequently, KT/HAK/KUP genes have been identified in many plant species, highlighting their broad roles in plant growth and stress defense (Cui et al., 2025).

KT/HAK/KUP proteins belong to the amino acid polyamine organocation (APC) subfamily (Liu et al., 2025). These proteins typically contain 10-15 transmembrane segments, a long cytoplasmic loop between the second and third transmembrane segments, and both N- and C-termini on the cytosolic side of the membrane (Luo et al., 2025). Beyond their transmembrane regions, KT/HAK/KUP proteins contain three recognizable structural features: the N-terminal and C-terminal region, an approximately 70-residue segment between transmembrane domains II and III. These elements share the same membrane orientation (Santa-Maria et al., 2018). Previously reports indicated that the expression of HAK/KUP/KT family genes are affected by Na^+ and K^+ levels in plants (Liu et al., 2025). Some HAK/KUP/KT proteins have shown high affinity for K^+ and to maintaining Na^+/K^+ homeostasis in plants (Feng et al., 2020). For instance, under K^+ starvation, overexpression of *AtHAK5* promotes K^+ uptake of *A. thaliana* (Gierth and Maser, 2007). Similarly, *OsHAK5* and *OsHAK18* also contribute to K^+ acquisition and translocation in rice (Yang et al., 2014; Feng et al., 2019). Some HAK/KUP/KT proteins also mediate Na^+ uptake and transport, highlighting the functional diversity of this transporter family (Liu et al., 2025). A recent study showed that *OsHAK2* participates in Na^+ uptake in rice and increases salt sensitivity (Morita et al., 2023). *ZmHAK4* has also been reported to mediate Na^+ exclusion in maize shoots (Zhang et al., 2019).

Spartina alterniflora is a halophyte and model plant for identifying genes that regulate salt tolerance (Subudhi et al., 2011). Previous studies have demonstrated that *S. alterniflora* can complete its life cycle in the presence of 40 g L^{-1} NaCl and that its seeds remain capable of germination after exposure to 1,000 mM NaCl. (Wei, 2020; Xin et al., 2020). Beyond its value as a stress-tolerance model plant, *S. alterniflora* is also considered a potential resource plant because of its high straw yield and gas production. Its capacity for ion uptake and exclusion is considered central to survival in highly saline conditions (Bradley & Morris, 1991). *S. alterniflora* excludes nearly 97% of Na^+ and Cl^- through salt glands, while its unusually high capacity for K^+ uptake supports robust ion homeostasis (Bradley & Morris, 1991; Hester et al., 2001). However, the molecular mechanisms remain incompletely understood. Recent studies suggest that the expansion of several ion-transport-related gene families, including the *HAK* family, contributes to the remarkable environmental adaptability of *S. alterniflora* (Yan et al., 2025). Although *HAK* genes have been characterized in many plant species, the composition and function of this family in *S. alterniflora* remain unclear.

Given the central roles of HAK transporters in K^+ uptake, ion transport and plant stress tolerance, genome-wide identification and functional characterization of the *HAK* family in *S. alterniflora* are essential for elucidating the molecular mechanisms underlying salt tolerance in this halophyte. Therefore, this study aimed to: (1) identify *HAK* family members in *S. alterniflora*; (2) characterize their expression patterns under salt stress; and (3) investigate the biological functions of *SaHAK* genes in the plant salt-stress response.

2. Material and Methods

2.1 Identification and sequence characterization of *HAK* family members in *S. alterniflora*

The Rice Genome Annotation Project database (https://rice.uga.edu/) and Phytozome (https://phytozome-next.jgi.doe.gov/) were used to downloaded *Oryza sativa* and *Zea mays* HAK protein sequence. These sequences were then used as query sequences in BLASTP searches against the *S. alterniflora* genome (GenBank accession number: JARYIK000000000) (Chen et al., 2024), with an E-value cutoff of 1e-5 and a minimum sequence identity of 70%. Local *S. alterniflora* protein database then was searched by HMMER (v3.0) with the potassium transporter domain profile PF02705, with E-value threshold of 1e-5. Proteins detected by both BLASTP and HMMER were retained as candidate SaHAK proteins. Gene IDs, chromosomal locations, gene lengths, coding sequence lengths and amino acid lengths were extracted from the *S. alterniflora* reference genome (Chen et al., 2024). Physicochemical properties, subcellular localization and transmembrane helices were predicted and identified by web tool (ExPASy: http://web.expasy.orgprotparm/; WoLF PSORT: https://wolfpsort.hgc.jp/; TMHMM: http://www.cbs.dtu.dk/services/TMHMM/). Chromosomal distribution was visualized with MG2C (http://mg2c.iask.in/) according to the physical positions of *SaHAK* genes.

2.2 Synteny and phylogenetic analyses of *SaHAK* family

Genomic position of *SaHAK* genes was extracted from the *S. alterniflora* reference

genome. Synteny analysis and calculation of nonsynonymous (Ka) and synonymous (Ks) substitution rates were performed using TBtools (v2.450), which was also used for visualization. To infer evolutionary relationships, 120 HAK proteins from *S. alterniflora* (70 SaHAKs), *O. sativa* (28 OsHAKs) and *Z. mays* (22 ZmHAKs) were aligned, followed by Neighbor-Joining (NJ) phylogenetic reconstruction using MEGA12 (v12.0.11). Sites with insufficient coverage were excluded by applying partial deletion with a 50% site-coverage threshold, and branch support was evaluated using 1,000 bootstrap replicates. Final phylogenetic tree was visualized using iTOL (https://itol.embl.de/).

2.3 Identification of gene structure, conserved motifs, and cis-acting elements

TBtools (v2.450) was applied to examine and display the coding regions, untranslated regions and intron-exon organization of *SaHAK* genes. Conserved motifs in SaHAK protein sequences were performed with MEME (v5.0.5, http://meme-suite.org/), with the motif number limited to 10 and motif lengths constrained to 6-50 aa. For putative regulatory element analysis, a 2,000 bp fragment preceding the coding region of each *SaHAK* gene was extracted and designated as its promoter. Regulatory cis-elements in the extracted promoter regions were predicted with PlantCARE (http://bioinformatics.psb. ugent.be/webtools/plantcare/html/).

2.4 Plant materials, salt treatment and transcriptome sequencing

Mature *S. alterniflora* plants were collected from public land outside protected areas in Yancheng, Jiangsu Province, China (33.60327619°N, 120.60882581°E), in

compliance with applicable local laws and regulations, and transported to the laboratory for seed harvesting. Harvested seeds were germinated and hydroponically cultivated in Hoagland solution for six weeks inside a growth chamber. The chamber was set to 25°C/24°C day/night temperatures, 70% relative humidity, 15,000 lux light intensity and a 16 h light/8 h dark photoperiod.

Uniformly grown seedlings were selected for salt-stress treatment. Based on the mean seawater salinity in Jiangsu Province, seedlings were stressed at 600 mM NaCl for 24 h. Seedlings grown without NaCl treatment served as controls. After treatment, shoot and root samples were harvested separately for transcriptome sequencing. Each treatment and tissue type included three independent biological replicates.

2.5 The gene expression analysis using transcriptome sequencing and RT-qPCR

The FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing, China) was used to extract total RNA according to the manufacturer's protocol. Subsequent RNA library sequencing was performed by Tsingke Biotechnology using the Illumina HiSeq platform. Low-quality sequences were removed from the raw reads with fastp (v0.23.4). The filtered reads were subsequently aligned using HISAT2 to the *S. alterniflora* reference genome (accession: GWHCBIM00000000) using HISAT2. Because the *S. alterniflora* reference genome reported by Chen et al. (2024) was not yet available when RNA-seq was performed, an alternative reference genome was used for read mapping. To establish correspondence between genes in the two reference genomes, BLASTP was performed with threshold of 1e-10 (E-value) and sequence

identity threshold of 95% (Table S1). The raw RNA-seq reads have been deposited in the China National Center for Bio-information (CNCB) under BioProject accession number PRJCA077579.

Gene expression between the two treatment groups were evaluated in R with DESeq2 (v1.26.0). P values were corrected for multiple testing by the Benjamini-Hochberg procedure to control the false discovery rate (FDR). Genes meeting the criteria of FDR < 0.05 and $|\log_2(\text{fold change})| \geq 0$ were considered significant DEGs (Table S2). DEG functions were annotated and tested for Gene Ontology (GO) enrichment using a hypergeometric test. GO terms with $P_{adj} \leq 0.05$ were regarded as significantly enriched.

The cDNA was reverse transcribed from total RNA employing the TSK302M kit (Tsingke, Beijing). RT-qPCR amplification was conducted on an Applied Biosystems StepOnePlus™ System with ArtiCanCEO SYBR qPCR Mix (Tsingke). Primers targeting specific genes were engineered via SnapGene (v7.2.1; Table S3). Transcript abundance was quantified via the 2-ΔΔCT formula, normalized against the endogenous control gene *SaGAPDH*.

2.6 Overexpression and salt-tolerance evaluation in *N. benthamiana*

Full-length coding regions of *SaHAK13*, *SaHAK40* and *SaHAK65* were cloned and inserted into the pCambia2301-JC vector. The resulting recombinant plasmids were introduced into Escherichia coli DH5α, confirmed by sequencing and subsequently transferred into AGL0. Transformation of *N. benthamiana* was carried out following the leaf-disc protocol reported by Horsch et al. (1985). Overexpressing lines were

selected by hygromycin screening and PCR verification. For salt-tolerance assays, surface-sterilized transgenic seeds were placed on half-strength MS medium containing 30 mg L^{-1} hygromycin, while wild-type seeds were cultured on hygromycin-free medium. Germinated seedlings were transferred to half-strength MS medium supplemented with 150 mM NaCl and stress for 7d under controlled conditions (16h light/8h dark period, 25°C day/24°C night, 70% RH, 15,000 lux). Seedling phenotypes were photographed (Fujifilm, XT-20), and root length was measured using a root analysis instrument (Comecause, IN-GX01). Root activity, soluble sugar, proline, SOD, POD and CAT activities were measured using Solarbio kits (BC5270, BC0290, BC6410, BC0170, BC0090, BC0205., Beijing, China). Chlorophyll and carotenoid contents were determined by acetone extraction method (Jhanani et al., 2024). Dried tissue samples were digested in an HNO_3-H_2O_2 mixture, followed by quantification of Na^+ and K^+ using an AA240 atomic absorption spectrophotometer (Agilent Technologies).

2.7 Statistics

Statistical analyses utilized SPSS 23.0 (Chicago, IL, USA) with graphical outputs generated in GraphPad Prism 9.1.0 (San Diego, CA, USA). Differences among treatments were tested by one-way ANOVA followed by Dunnett's post hoc test (α=0.05). Transcriptomic data processing and DEG visualization employed R (v4.4.3) and TBtools (v2.097). Partial least squares path modeling (PLS-PM) was performed with R (v4.4.3).

3. Results

3.1 Identification of *SaHAK*s

Total of 70 *SaHAK* genes were identified in the *S. alterniflora* genome and named according to their chromosomal positions (Figure 1, Table S4). Chromosomes 13 and 14 contained the largest numbers of *SaHAK* genes (Figure 1). Among the *SaHAKs*, *SaHAK3* encodes the largest protein (1,909 amino acids), while *SaHAK16* encodes the smallest (161 amino acids) (Table S4). The predicted molecular weights of *SaHAK*s range from 18,145.26 Da to 218,906.62 Da, and their isoelectric points range from 5.84 to 10.96 (Table S4). Most SaHAK proteins are predominantly localized to the plasma membrane, whereas SaHAK7 and SaHAK19 are predicted to localize to chloroplasts, SaHAK43 to the nucleus, and SaHAK16 and SaHAK33 to the vacuole according to Subcellular localization predictions (Table S4).

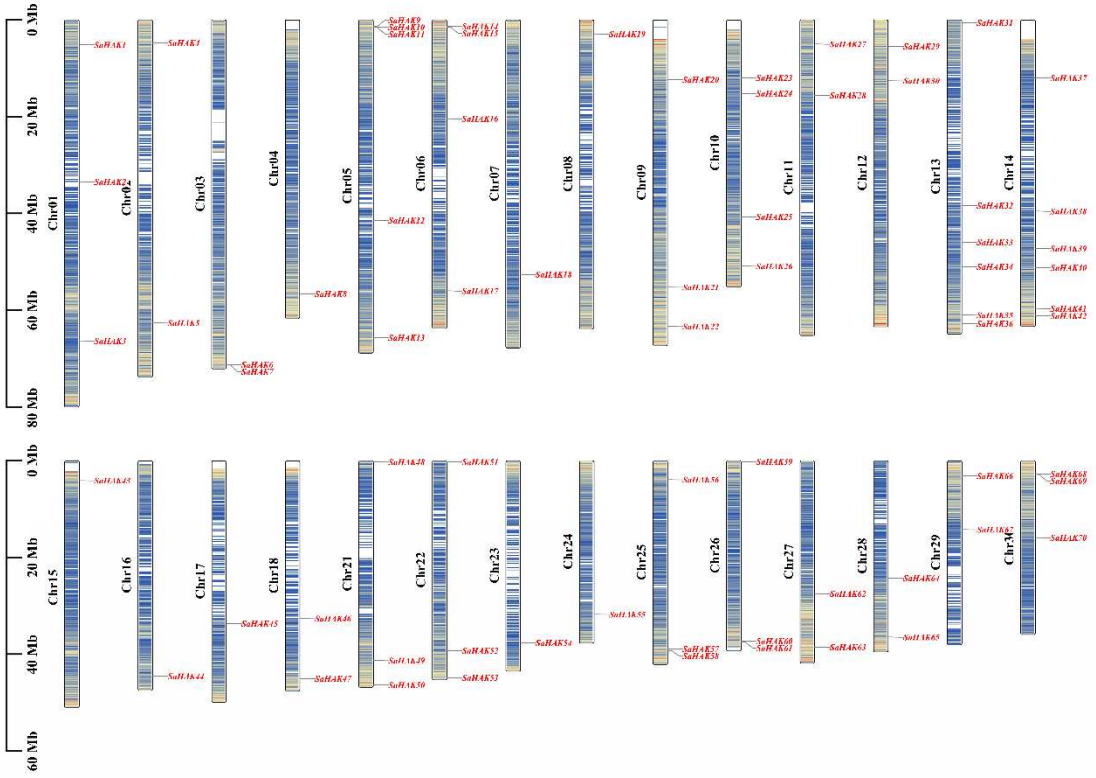


Figure 1. The Chromosomal localization of the *SaHAK* genes. The black line indicated

chromosome length. Different colors within the chromosomes represented the gene density.

3.2 Phylogenetic analysis of *SaHAK*s

To assess the evolutionary relationships among HAK family members, proteins from *O. sativa* (*28* OsHAKs), *Z. mays* (*22* ZmHAKs), and *S. alterniflora* (*70* SaHAKs) were subjected to phylogenetic analysis (Figure 2). The resulting tree grouped SaHAKs into 11 clusters, among which clusters IV, VI, VII, and IX were each further divided into two subclusters, whereas cluster XI was divided into three subclusters. Clusters IV and XI contained the largest number of HAK proteins, with 16 members each (cluster IV: 3 ZmHAKs, 3 OsHAKs, and 10 SaHAKs; cluster XI: 6 ZmHAKs, 4 OsHAKs, and 6 SaHAKs). Clusters VI, VII, and IX were also relatively large, with 12, 15, and 10 members, respectively (Figure 2).

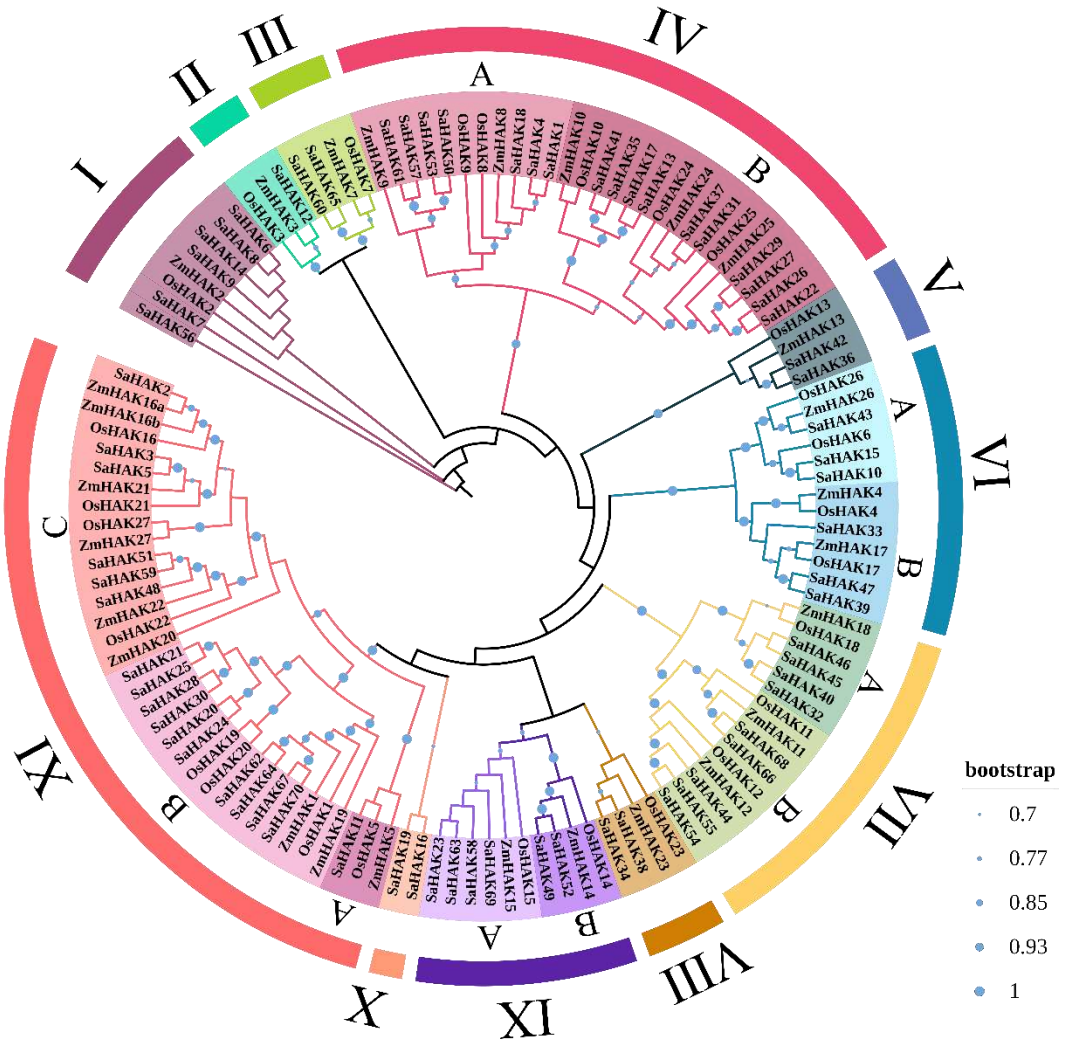


Figure 2. Phylogenetic tree of the HAK proteins from *Oryza sativa*, *Zea mays* and *S. alterniflora*. The blue dots on the branches represent bootstrap values. Higher bootstrap values indicated a relatively higher confidence level for the corresponding branch. The different colors represent clusters which classified by bootstrap. Sa, *Spartina alterniflora*; Os, *Oryza sativa*; Zm, *Zea mays*.

3.3 Synteny analysis of *SaHAK*s

Syntenic relationships among *SaHAK* genes were analyzed to infer the mechanisms underlying expansion of the *HAK* family in *S. alterniflora*. We identified 47 collinear *HAK* genes in *S. alterniflora*, all associated with segmental duplication (Figure 3A), with chromosome 13 containing the largest number of collinear pairs. The Ka/Ks ratios of all homologous *SaHAK* gene pairs were below 1, suggesting that these

genes have predominantly evolved under purifying selection (Table S5). Interspecies collinearity analysis between *O. sativa* and *S. alterniflora* identified 87 orthologous gene pairs, with more pairs located on *S. alterniflora* Chr 13 and 14 than on the other Chromosomes (Figure 3B).

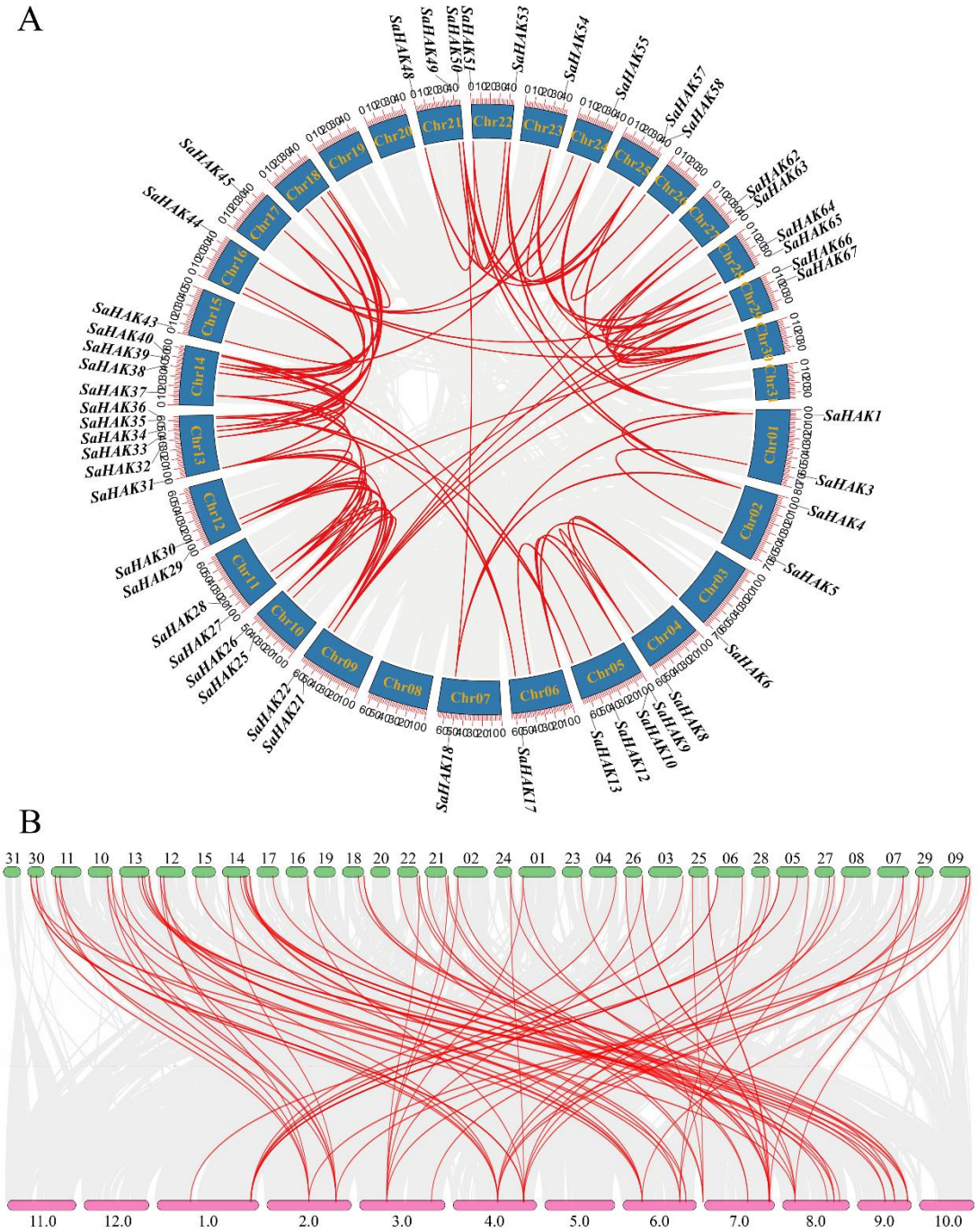


Figure 3. The collinearity analysis of *HAK*s genes. (A) Collinearity analysis in *S. alterniflora*. (B) Collinearity analysis between *S. alterniflora* and *O. sativa*. The grey lines represented all collinear gene pairs and red lines represented *HAK* gene pairs.

3.4 Structure, domain and motif analyses of *SaHAKs*

Most SaHAK proteins contain 10 conserved motifs, except for SaHAK37, SaHAK4, SaHAK48, SaHAK6, SaHAK7, SaHAK8, SaHAK42, SaHAK45, SaHAK16, SaHAK23, SaHAK59, SaHAK62, SaHAK28, SaHAK20, SaHAK19, and SaHAK56, which indicates structural divergence within this subset. All members of cluster VII and cluster VIII contained 9 motifs, pointing to potential functional similarity within these groups (Figure 4A and B). In addition, SaHAK19 and SaHAK23 contained only a single motif each, likely due to their shorter amino acid sequences (Figure 4B). Motif 2 and 7 were present in most SaHAKs, except for SaHAK16, SaHAK19 and SaHAK5, indicating that these motifs may occupy core positions in the family (Figure 4B, E and F). Finally, conserved domain annotation showed that all SaHAK proteins contained the K-trans domain (Figure 4C). Gene structure analysis indicated that *SaHAK* genes contained 1 to 15 introns. Most *SaHAK*s contained 6-8 introns, except for *SaHAK*56 *SaHAK*19, *SaHAK*51, *SaHAK*48, *SaHAK*59, *SaHAK*23, *SaHAK*16, *SaHAK*7 and *SaHAK*6 (Figure 4D).

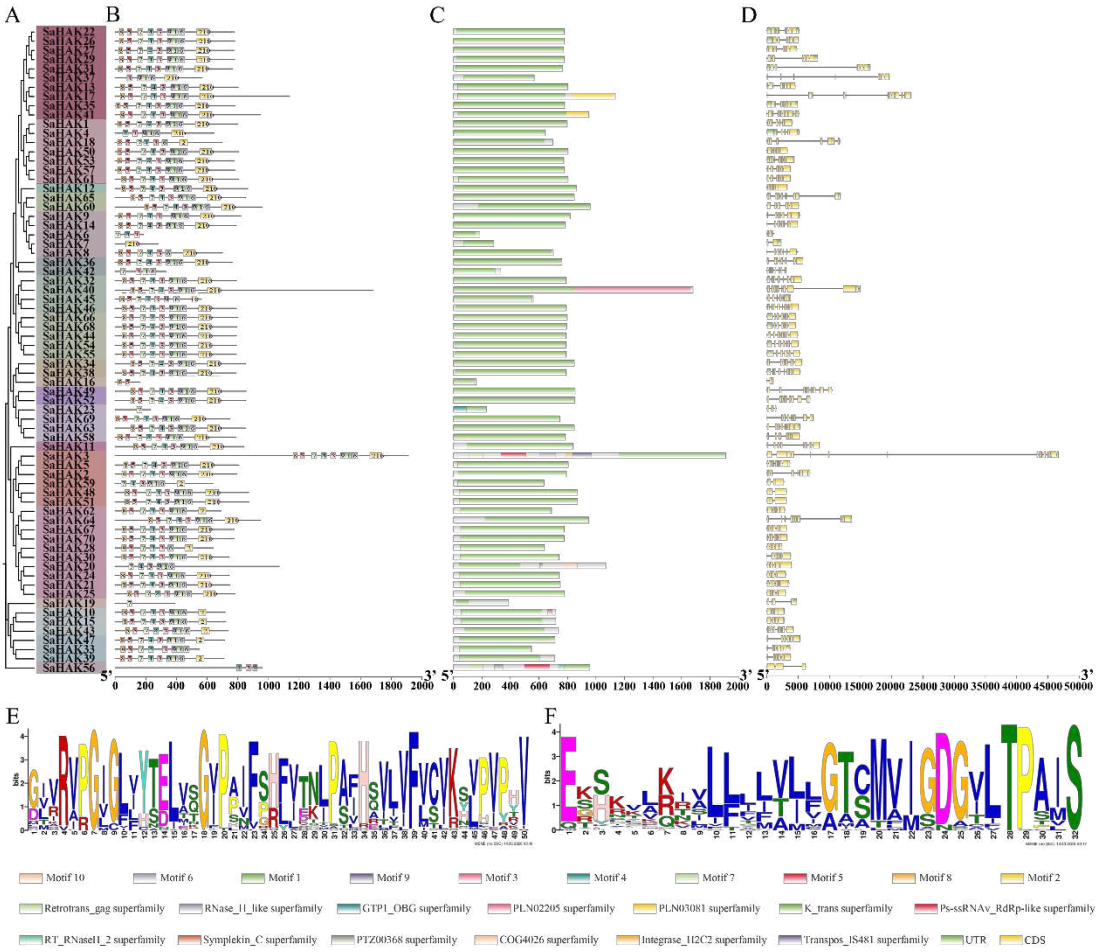


Figure 4. The phylogenetic tree, protein and gene structure of *SaHAK*s. The phylogenetic tree (A), conserved motif analysis (B), conserved domains (C) and gene structure (D) of *SaHAK*s. (E) and (F) The Conserved residues in motif 2 (E) and motif 7 (F). The phylogenetic tree clusters, motifs and conserved domains were represented by different colors. The scale bar represents the amino acid sequence length (aa) and base pair sequence length (bp). CDS, coding sequence; UTR, untranslated regions.

3.5 Cis-acting elements analysis of *SaHAK*s promoter

In total, 2,089 cis-acting elements were detected in the promoter regions of *SaHAK* genes and assigned to six functional categories: light and environmental stress response, hormone response, developmental regulation, stress and defense response, metabolic regulation, and miscellaneous elements. Among these, the light-responsive elements were extensively distributed across *SaHAKs* promoters and constituted the largest

300 category (733, 35.09%). Additionally, numerous stress-responsive elements were
301 detected in the *SaHAK*s promoter regions, including anaerobic induction elements (126,
302 6.03%), defense and stress response elements (22, 1.05%), low-temperature
303 responsiveness elements (33, 1.58%), drought-inducibility elements (51, 2.44%), and
304 anoxic-specific inducibility elements (20, 0.96%). Furthermore, numerous hormone-
305 related regulatory elements were also detected including abscisic acid responsive
306 elements (278, 13.30%), salicylic acid responsive elements (73, 3.49%), auxin-
307 responsive elements (64, 3.06%) and gibberellin responsive elements (46, 2.20%).
308 Development-related elements, including meristem expression regulatory elements (73,
309 3.49%) and endosperm expression element (21, 1.01%) were also identified (Figure 5).



Figure 5. The cis-acting elements analysis of *SaHAK*s promoter. Various colors represented different cis-acting elements. The scale bar represents the base pair sequence length (bp).

3.6 The differential expression and functional enrichment analysis of *SaHAK*s under salt stress

RNA-seq analysis identified 41, 389 and 41, 718 genes with significant differential expression in shoot and root tissues, respectively (Figure 6A and B). Among these differentially expressed genes (DEGs), most *SaHAK*s were induced by salt stress and exhibited tissue-specific expression patterns. Under 24 h salt stress (600 mM NaCl), 25 *SaHAK*s (35.7%) were differentially expressed in *S. alterniflora* shoots, while 45 *SaHAK*s (64.3%) remained unchanged. The *SaHAK31*, *SaHAK37*, *SaHAK13*,

SaHAK29, *SaHAK60*, *SaHAK8*, *SaHAK65*, and *SaHAK46* were significantly upregulated (fold change: 1.67-9.24), whereas *SaHAK5*, *SaHAK4*, *SaHAK51*, *SaHAK48*, *SaHAK2*, *SaHAK34*, *SaHAK40*, and *SaHAK44* were significantly downregulated (fold change: -10.33--3.37). In *S. alterniflora* roots, 18 *SaHAK*s (25.7%) were significantly differentially expressed, whereas 52 *SaHAK*s (74.3%) were not. *SaHAK13*, *SaHAK17*, *SaHAK2*, *SaHAK22*, *SaHAK9*, *SaHAK46*, *SaHAK23*, and *SaHAK63* were significantly upregulated (fold change: 1.44-13.47), whereas *SaHAK18*, *SaHAK1*, *SaHAK3*, *SaHAK61*, *SaHAK69*, *SaHAK21*, *SaHAK57*, and *SaHAK50* were significantly downregulated (fold change: -12.56--2.06). Furthermore, the *SaHAKs* in cluster I，IV, VIIA and IX showed high expression in both shoots and roots, while those in cluster VIA were specifically upregulated in shoots. By contrast, downregulated *SaHAK*s were mainly classified into clusters V and XIC, which exhibited predominant expression in shoots (Figure 6C).

GO enrichment analysis showed that shoot-expressed *SaHAK* genes were predominantly associated with potassium transport-related biological processes including potassium ion transport (GO:0071804), potassium ion transmembrane transport (GO:0010163), and potassium ion import across the plasma membrane (GO:0010107). Cell tip growth (GO:0009932) and cotyledon development (GO:0048825) were also enriched. In addition, several *SaHAK* genes, including *SaHAK2*, *SaHAK18*, *SaHAK54* and *SaHAK55*, were annotated to the cellular response to cesium ion term (GO:0071278) (Figure 6D). In terms of molecular function, the differentially expressed *SaHAK*s in shoots were enriched for potassium ion

transmembrane transporter activity (GO:0022817), symporter activity (GO:0015293), adenylate cyclase activity (GO:0004016), potassium: proton symporter activity (GO:0015387), and potassium: sodium symporter activity (GO:0009674). The roots differentially expressed *SaHAK*s in shoots showed a similar enrichment pattern to those in shoots, with the same major biological process and molecular function categories (Figure 6E).

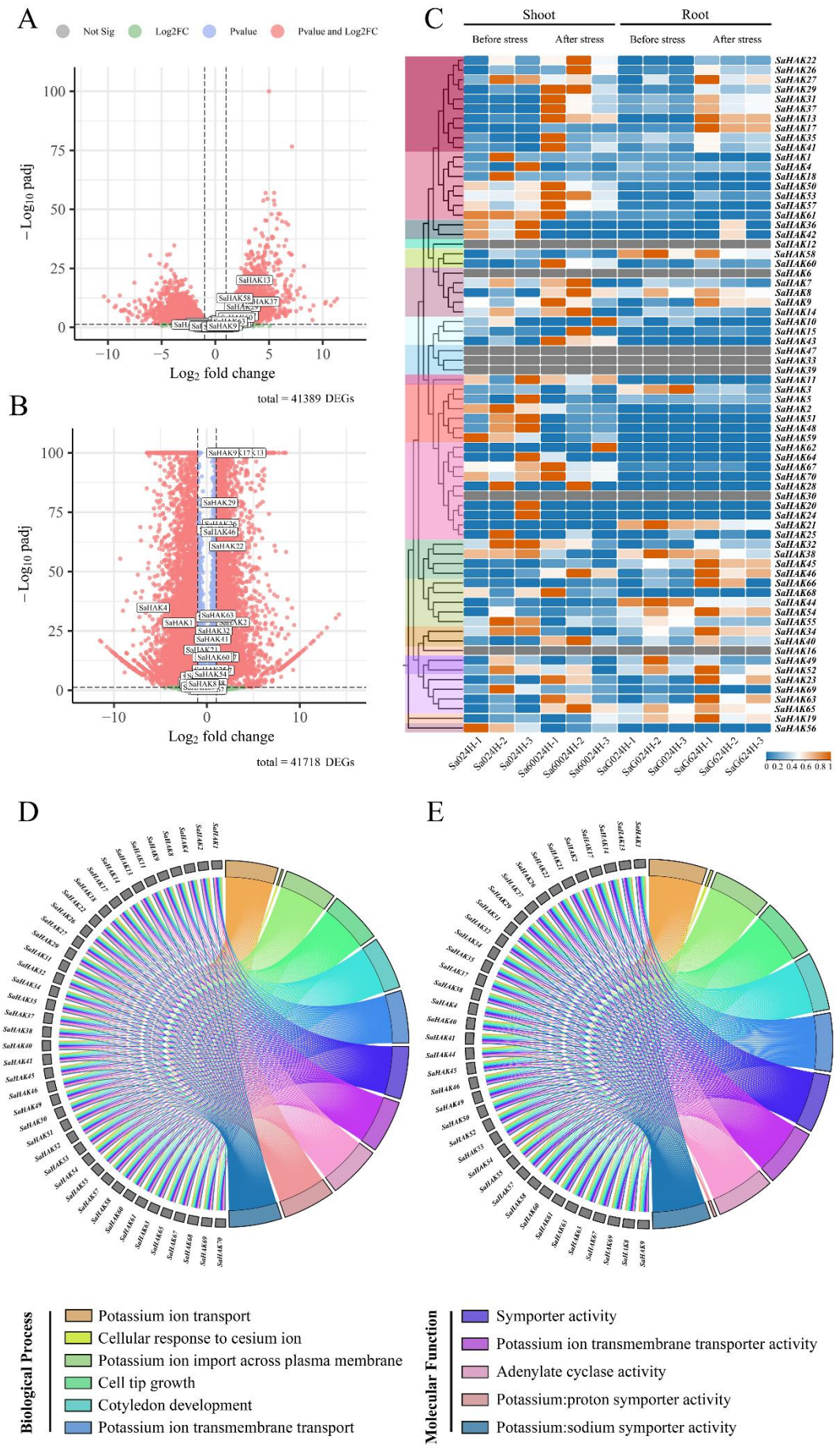

A
Not Sig
Log2FC
Pvalue
Pvalue and Log2FC
−Log10 padj
Log2 fold change
total = 41389 DEGs
B
−Log10 padj
Log2 fold change
total = 41718 DEGs
C
Shoot
Root
Before stress
After stress
Before stress
After stress
SaHAK22
SaHAK26
SaHAK27
SaHAK29
SaHAK31
SaHAK37
SaHAK13
SaHAK17
SaHAK35
SaHAK41
SaHAK1
SaHAK4
SaHAK18
SaHAK50
SaHAK53
SaHAK57
SaHAK61
SaHAK36
SaHAK42
SaHAK12
SaHAK58
SaHAK60
SaHAK6
SaHAK7
SaHAK8
SaHAK9
SaHAK14
SaHAK10
SaHAK15
SaHAK43
SaHAK47
SaHAK33
SaHAK39
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SaHAK23
SaHAK69
SaHAK63
SaHAK65
SaHAK19
SaHAK56
Sa024H1-1
Sa024H1-2
Sa024H1-3
Sa6002H1-1
Sa6002H1-2
Sa6002H1-3
SaG024H1-1
SaG024H1-2
SaG024H1-3
SaG624H1-1
SaG624H1-2
SaG624H1-3
0 0.2 0.4 0.6 0.8 1
D
E
Biological Process
Potassium ion transport
Cellular response to cesium ion
Potassium ion import across plasma membrane
Cell tip growth
Cotyledon development
Potassium ion transmembrane transport
Molecular Function
Symporter activity
Potassium ion transmembrane transporter activity
Adenylate cyclase activity
Potassium:proton symporter activity
Potassium:sodium symporter activity

Figure 6. Transcriptomic analysis of *SaHAK*s under salt stress (600mM NaCl). The DEGs in *S. alterniflora* shoots (A) and roots (B). (C) The expression patterns of *SaHAK*s. The GO enrichment results for *SaHAK*s in the shoots (D) and roots (E) of *S. alterniflora*. The different colors in heatmap represents normalized FPKM values.

To further verify the expression patterns of selected *SaHAK*s, eight DEGs were analyzed by RT-qPCR in different tissues of *S. alterniflora* under salt stress (Figure 7A-H). The results indicated that *SaHAK13*, *SaHAK27*, *SaHAK34*, *SaHAK40* and *SaHAK65* showed significant expression changes following salt treatment, indicating their potential involvement in salinity responses in *S. alterniflora* (Figure 7A, C, D, E and G). *SaHAK13*, *SaHAK40* and *SaHAK65* showed markedly upregulated in both shoot and root tissues. Compared with pre-stress expression levels, *SaHAK13*, *SaHAK40* and *SaHAK65* upregulated by 4.7, 2.57 and 2.79 respectively (Figure 7A, E and G), implying their potential importance in the salt-stress response of *S. alterniflora*.

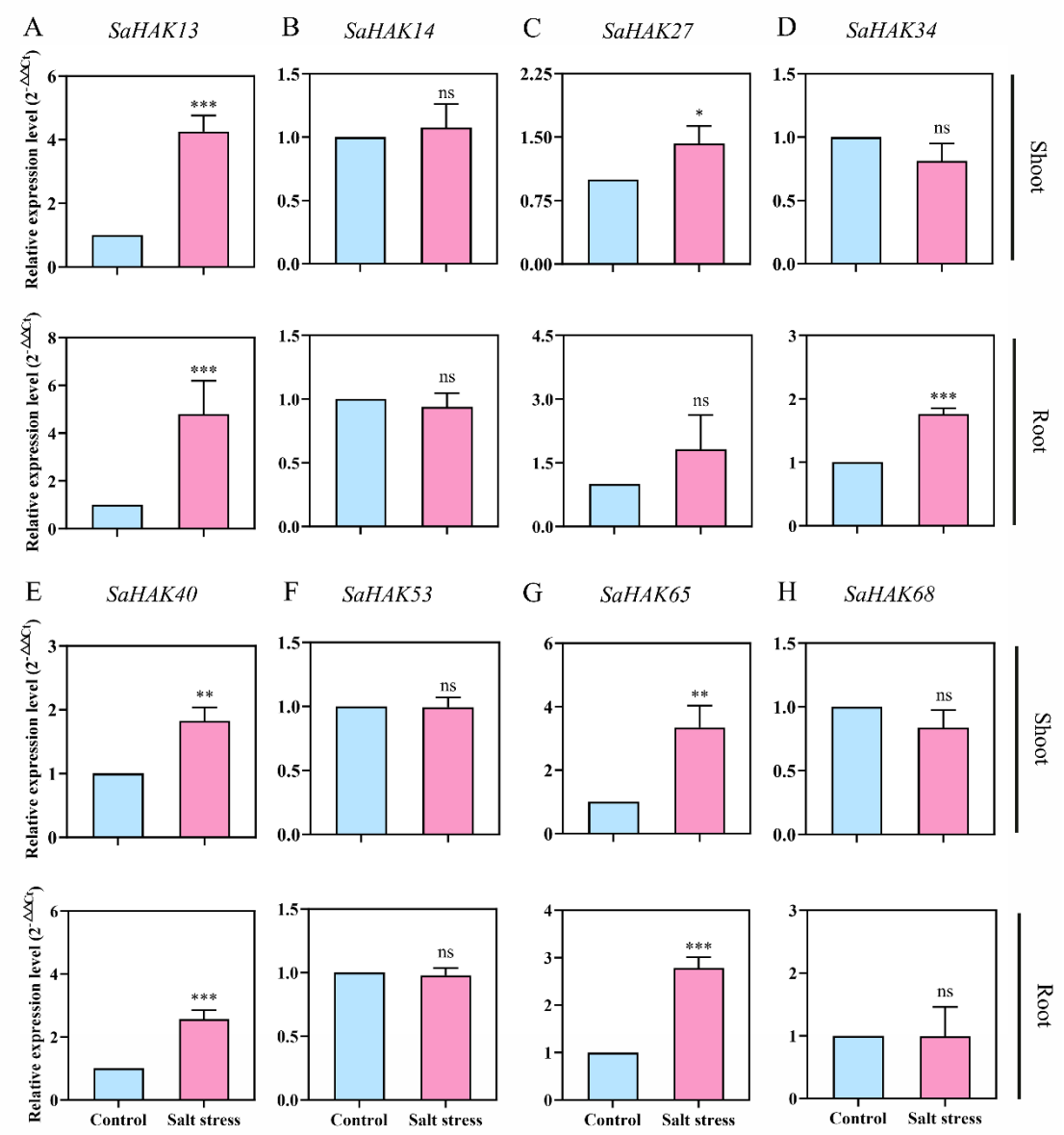


Figure 7. Relative expression levels of 8 *SaHAK*s by RT-qPCR under salt stress. The expression level was calculated using the $2^{-\Delta\Delta Ct}$ method, with *SaGAPDH* as the reference gene. Error bars represent standard deviations (SD) (n=9). Significance: ns ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.005$) and **** ($P < 0.001$).

3.7 Evaluation of salt tolerance in OE-*SaHAK13*, OE-*SaHAK40* and OE-*SaHAK65 N. benthamiana*.

To investigate the functions of *SaHAK13*, *SaHAK40* and *SaHAK65*, these genes were overexpressed in *N. benthamiana*, then they were salt stressed for 7 days. After stress，all overexpressed lines developed stronger root systems than WT plants (Figure

8A). Root length in OE-*SaHAK13,* OE-*SaHAK40* and OE-*SaHAK65* increased by 147.84%,183.18% and 200.61%, respectively, and their root activities increased by 46.24%, 65.59% and 98.11% compared with those of the WT (Figure 8B and C). The Na^+ content of overexpressed plants increased by 17.57%, 17.83% and 10.07%, respectively (Figure 8D). Similarly, the K^+ content of these plants significantly increased by 37.46%, 34.51% and 49.28%, while the Na/K ratio significantly decreased by 18.17%, 16.28% and 28.96% (Figure 8E and F). Additionally, we also found that the overexpressed plants proline content decreased by 77.86%, 81.52% and 91.69%, and soluble sugar contents decreased by 18.48%, 16.41% and 17.65%, respectively (Figure 8G and H). Under salt stress, leaf chlorosis was observed in the WT plants (Figure 8A). The chlorophyll content in OE-*SaHAK13*, OE-*SaHAK40* and OE-*SaHAK65* increased by 59.44%, 100.04% and 96.82%, respectively, and the carotenoid content increased by 62.74%, 69.32% and 105.90%, respectively (Figure 8I and J). Antioxidant enzyme activities were also significantly reduced in the overexpression lines compared with WT plants, with SOD activities decreased by 16.79%, 17.71% and 18.07%, the POD activities decreased by 72.29%, 75.96% and 79.64%, and the CAT activities decreased by 38.25%, 57.47% and 49.49%, respectively (Figure 8K-M).

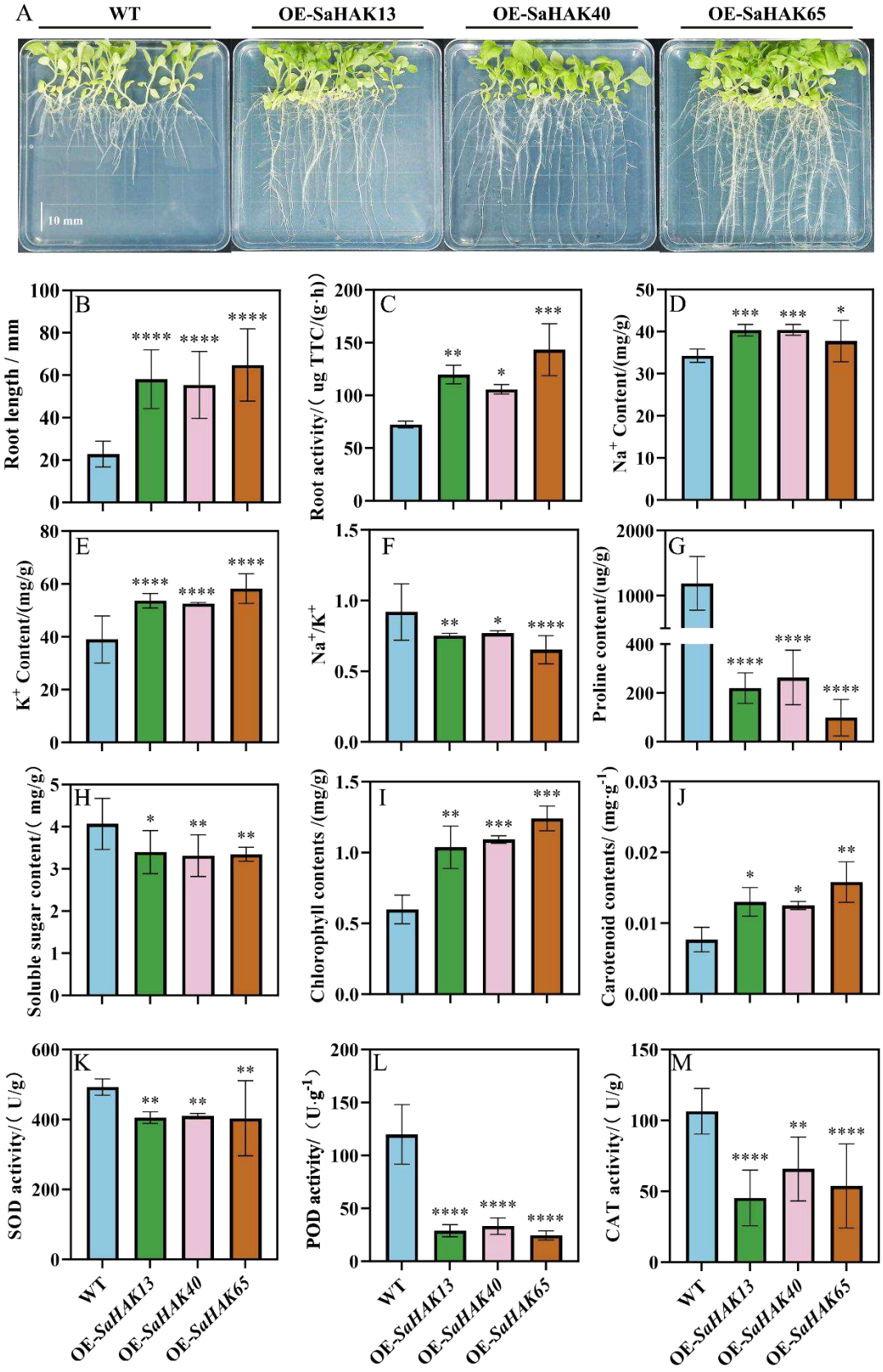


Figure 8. Evaluation of salt stress tolerance in OE-*SaHAK13*, OE-*SaHAK40* and OE-*SaHAK65 Nicotiana benthamiana*. The Phenotype (A), root length (B), root activity (C), Na^+

content (D), K^+ content (E), Na^+/K^+ ratio (F), proline content (G), soluble sugar content (H), chlorophyll content (I), carotenoid content (J), SOD activities (K), POD activities (E), CAT activities (F) of OE-*SaHAK13*, OE-*SaHAK40* and OE-*SaHAK65 Nicotiana benthamiana* after 7d salt stress (150 mM NaCl). Error bars represent standard deviations (SD) (n=9). Significance: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.005$) and **** ($P < 0.001$).

4. Discussion

4.1 Phylogenetic and collinear analyses reveal segmental duplication as the driver of *SaHAK* expansion and evolutionary conservation

Potassium is an essential plant macronutrient required for growth, development and stress responses (Zhang et al., 2019). K^+ accumulation can mitigate Na^+ toxicity and is therefore considered a key strategy for plants salt stress defenses (Wang et al., 2021). *HAK* gene family constitutes a major transport system for K^+ acquisition and long-distance translocation in plants and has been identified in diverse species, including *A. thaliana, O. sativa*, *Z. mays*, *Sorghum bicolor*, *Saccharum officinarum*, *Solanum tuberosum* (Ahn et al., 2004; Amrutha et al., 2007; Zhang et al., 2012; Feng et al., 2020; Liu et al., 2025). In this study, 70 *HAK* family members were identified from the *S. alterniflora* genome (Table S4), representing a substantially larger family than those reported in *O. sativa* (26 *OsHAK*s), *Z. mays* (27 *ZmHAK*s), and *Solanum lycopersicum* (27 *SlHAK*s) (Amrutha et al., 2007; Zhang et al., 2012; Feng et al., 2020). This family is also larger than those reported in several halophytes, including *Salicornia europaea* (24 *SeHAK*s) and *Medicago sativa* (22 *MsHAK*s) (Li et al., 2022; Wei et al., 2022). The expanded *HAK* family in *S. alterniflor*a may reflect gene-family expansion and may have provided genetic material for functional diversification among *SaHAK* members

(Liu et al., 2025). This finding agrees with previous evidence that 8,728 gene families have expanded in *S. alterniflora* compared with other grasses, with many are associated with salt-tolerance-related functions (Hao et al., 2024). Similarly, two whole genome duplication events in *S. alterniflora* have been proposed to drive the expansion of salt-stress-related genes and transcription factors, thereby enhancing adaptation to saline environments (Chen et al., 2024). Our collinearity analysis showed that 47 *SaHAK*s arose from segmental duplication, whereas no tandem duplication events were detected (Figure 3A). Segmental duplication therefore appears to be the main force that drive for the expansion of *SaHAK*s family. Gene-family expansion is important for functional reinforcement, sub-functionalization or neofunctionalization, and may contribute to improving stress adaptation in plants (Banuelos et al., 2002; Zhang et al., 2012). All duplicated *SaHAK* gene pairs had Ka/Ks ratios below 1, consistent with strong purifying selection and evolutionary conservation (Table S5) (Liu et al., 2025). This suggests that the functions of these expanded genes are subject to selective constraint. Rather than promoting extensive functional divergence, *SaHAK* expansion may primarily enhance K^+ transport capacity through functional redundancy, dosage effects, or tissue-specific expression (Zhao et al., 2025). Notably, clusters IV and XI were the largest *SaHAK* clusters and corresponded to the largest HAK clusters in *O. sativa* and *Z. mays* (Figure 2). These clusters have been reported to undergo preferential expansion and retain conserved functions during plant evolution (Gupta et al., 2008). In *S. alterniflora*, the 47 expanded *SaHAK* genes were also mainly concentrated in clusters IV and XI, suggesting that these expanded genes are conserved in K^+ transport and salt

stress adaptation.

4.2 Structural, regulatory and localization features implicate functional diversification of *SaHAK*s

Variation in exon-intron organization may affect transcriptional regulation, splicing efficiency and transcript stability under stress conditions (Sonis, 2015). Genes with a greater number of exons can generate multiple protein isoforms via alternative splicing, thereby increasing the flexibility of plant stress responses (Liu et al., 2025). Introns may also regulate gene expression by influencing alternative splicing and mRNA transcription (Wang et al., 2023). In this study, most *SaHAK*s contained 8-15 introns and 6-16 exons (Figure 4D), which is consistent with *HAK* genes reported in other plants (Cai et al., 2021; Liu et al., 2025). This structural similarity suggests that *SaHAK*s may share conserved expression patterns and biological functions with *HAK* homologs in other species. Consistent with HAK proteins in *O. sativa* and *Z. mays*, all SaHAK proteins contained the conserved K-trans superfamily domain (cl15781), supporting their close association with K^+ transport (Figure 4C) (Banuelos et al., 2002; Zhang et al., 2012). As K^+ transporters, HAK proteins in many plant species are predominantly localized to the plasma membrane, where they mediate active K^+ transport across cellular membranes. Predicted subcellular localization placed most SaHAK proteins at the plasma membrane, further supporting conservation of their K^+ transport function (Table S4).

Cis-acting elements are critical for gene expression regulating. In this study, light-

responsive elements constituted the largest category, consistent with previous reports (Cai et al., 2021; Liu et al., 2025), suggesting that *SaHAK*s may participate in light-regulated processes (Figure 5). Recent studies indicated that K^+ deficiency significantly inhibits plant photosynthesis, and HAK proteins are crucial for plants to maintain photosynthesis under low- K^+ conditions (Cui et al., 2025; Wang et al., 2026). *SaHAK* promoters also contained diverse stress-related regulatory elements, including elements associated with anaerobic induction, defense and stress responses, low-temperature responsiveness, drought inducibility and anoxia-specific induction (Figure 5). These elements suggest that *SaHAK*s may be broadly involved in abiotic stress responses. HAK proteins can contribute to cellular ion homeostasis through K^+ acquisition and translocation, thereby mitigating Na^+ toxicity under salt stress (Zhang et al., 2021). They may also regulate stomatal and cellular osmotic potential through K^+, influencing drought responses (Sun et al., 2024). In addition, HAK proteins have been reported to limit reactive oxygen species (ROS) accumulation by promoting antioxidant enzyme activity, a process associated with tolerance to anaerobic, drought and salt stresses (Chen et al., 2017). Although the mechanisms by which these elements regulate stress-induced expression of *SaHAK*s remain unclear, our results provide further insight into the potential functions of the *SaHAK* gene family. Overall, these findings suggest that although all *SaHAK*s retain the core features of K^+ transporters, differences in subcellular localization and promoter cis-acting elements may contribute to their potential functional diversification.

4.3 Salt-induced tissue-specific expression of *SaHAKs* supports

functional divergence in ion uptake and transport

Profiling *SaHAK* expression is critical for defining the involvement of this gene family in the salinity response of *S. alterniflora*. As K^+ transporter-encoding genes, HAKs are frequently responsive to salt stress and K^+ deprivation, consistent with their central functions in K^+ uptake and transport (Cui et al., 2025). Consistently, most *SaHAK*s were significantly upregulated under salt stress in this study, indicating that the *SaHAK* family is broadly involved in salinity adaptation in this halophyte (Figure 6A-C). Differentially expressed *SaHAK* genes were mainly concentrated in cluster IV, V, VI, VII, IX and XI, consistent with patterns reported by Luo et al. (2025) and Liu et al. (2025) (Figure 6C). Given the structural similarity of genes within the same phylogenetic clusters, *SaHAK*s may have functions like those of other characterized *HAK* homologs in the corresponding clusters. For instance, *OsHAK8* and *OsHAK10* in cluster IV mediate K^+ uptake and translocation, thereby regulating salt tolerance in *O. sativa* (Wang et al., 2021; Banuelos et al., 2002). *OsHAK17* in Cluster VI is significantly upregulated under salt stress and alleviates salt-induced damage by enhancing K^+ uptake (Nampei et al., 2024). *OsHAK1*, *OsHAK5* and *OsHAK16* in Cluster XI facilitate K^+ translocation from roots to shoots and contribute to salt tolerance in *O. sativa* (Chen et al., 2017; Yang et al., 2020; Feng et al., 2019). GO enrichment analysis of salt-responsive *SaHAK*s further supported their potential involvement in ion transport. These genes were predominantly assigned to potassium transport-related GO terms, including potassium ion transport (GO:0071804),

potassium ion transmembrane transport (GO:0010163) and potassium ion import across the plasma membrane (GO:0010107) (Fig. 6D and E). Collectively, these findings indicate that the *SaHAK* family may be involved in K^+ uptake, K^+ translocation and Na^+/K^+ homeostasis under salt stress, although the specific functions of individual members require further experimental validation.

In most plants, K^+ acquired from the external environment is transported into roots and subsequently loaded into the xylem for delivery to aboveground tissues (Peng et al., 2023). Thus, most HAK transporters are localized in root epidermal cells and xylem-associated tissues, where they participate in K^+ acquisition and systemic translocation (Peng et al., 2023). For instance, *TaHAK1* is induced in roots under K^+ deficiency and facilitates K^+ acquisition from the soil in wheat (Huang et al., 2025). In contrast, *TaHAK18* is preferentially expressed in shoots, and its overexpression promotes K^+ transport from roots to shoots and increases leaf K^+ accumulation (Liu et al., 2025). A comparable tissue-specific expression pattern was detected in *S. alterniflora*. *SaHAK31*, *SaHAK37*, *SaHAK29*, *SaHAK60*, *SaHAK8* and *SaHAK65* showed preferential expression in shoots, indicating possible involvement in K^+ translocation from roots to shoots (Figure 6C). By contrast, *SaHAK17, SaHAK2, SaHAK22, SaHAK9, SaHAK23 and SaHAK63* were highly expressed in roots, suggesting that they may mediate K^+ uptake from the soil (Figure 6C). Together, these findings provide a basis for constructing an ion-homeostasis regulatory network in *S. alterniflora* and for understanding the adaptive mechanisms underlying its high salt tolerance.

4.4 *SaHAK13*, *SaHAK40* and *SaHAK65* enhance *N. benthamiana* salt tolerance by maintaining Na^+/K^+ homeostasis

Potassium is indispensable for plant growth. Under high-salinity conditions, excessive Na^+ enters plants from the soil and interferes with K^+ binding and transport, resulting in cellular injury, growth inhibition and, ultimately, plant death (Wang et al., 2013). Therefore, maintaining K^+ uptake and Na^+/K^+ homeostasis is crucial for plant adaptation to salt stress (Liu et al., 2025; Huang et al., 2025). To investigate the functions of *SaHAK*s, three salt responsive genes, *SaHAK13*, *SaHAK40* and *SaHAK65*, were selected and overexpressed in *N. benthamiana*. Results indicate that overexpression of these genes promoted K^+ accumulation and lowered the Na^+/K^+ ratio under salt stress (Figure 8D-F), which is consistent with *HAK* functions reported in several halophytes. For instance, cotton plants overexpressing *AlHAK1* from the halophyte *Aeluropus littoralis* maintained lower Na^+/K^+ ratio and showed enhanced tolerance under high-salinity conditions (Cui et al., 2025). *PtHAK5* from *Puccinellia tenuiflora* is important for maintaining Na^+/K^+ homeostasis (Yang et al., 2018). These findings suggest that HAK genes may support salt tolerance through a conserved mechanism linked to K^+ acquisition and the maintenance of ion balance.

*HAK*s contribute to salt tolerance by regulating K^+-related processes during plant growth and stress adaptation. Previous studies have summarized these roles in organ growth and development, photosynthetic regulation, osmotic adjustment and stomatal regulation (Mostofa et al., 2022). Our results further support these functions. In terms of organ growth, overexpression lines with higher K^+ content possessed significantly

546 greater root length and root activity than WT after salt stress (Figure 8B and C). This
547 may help sustain water acquisition and nutrient absorption under adverse conditions
548 (Dang et al., 2025). The *OsHAK7* and *OsHAK10*, homologues of *SaHAK13* and
549 *SaHAK65*, also mediate K^+ uptake and regulate root development (Mahiwal et al., 2022),
550 suggesting potential functional conservation among these *HAK* members. Root hair
551 development requires the initiation of cell wall outgrowths followed by polarized
552 expansion at the tips of emerging root hairs. Both processes require turgor generation
553 (Sustr et al., 2019). As a major osmotic ion in plants, K^+ is central to these processes
554 (Schoenaers et al., 2017). In this study, differentially expressed *SaHAK*s were enriched
555 in the cell tip growth term (GO:0009932), supporting their potential involvement in
556 root growth regulation (Figure 6D and E). However, the specific roles of these genes in
557 root tip or root hair development require further experimental validation.

558 Osmotic imbalance and associated water loss are major consequences of plants
559 under salt stress (Zhang et al., 2022). As a major inorganic osmolyte, adequate K^+
560 accumulation reduces osmotic potential and supports cellular turgor under osmotic
561 stress (Wang et al., 2013). In this study, adequate K^+ accumulation in the overexpression
562 lines may have alleviated salt-induced osmotic imbalance. Accordingly, the proline and
563 soluble sugar contents in overexpression lines, which are typically induced by osmotic
564 stress, were significantly lower than WT plants (Figure 8G and H). PLS-PM analysis
565 further supported a positive association between Na^+/K^+ homeostasis and reduced
566 osmolyte accumulation (Figure 9). According to Johnson's et al. (2022) report,
567 exogenous K^+ application can maintain cellular turgor, which is important for plant

drought and salt stress defenses. Similarly, *OsHAK18*, a homologue of *SaHAK40*, has been reported mediated this process (Peng et al., 2023). Collectively, these findings indicate that *SaHAK*s may contribute to the regulation of cellular osmotic balance under stress.

The photosynthetic pigments stability and antioxidant response are also influenced by the Na^+/K^+ homeostasis. Salt-induced Na^+ accumulation can restrict K^+ uptake, reduce stomatal conductance, impair CO_2 fixation and promote ROS formation, thereby accelerating pigment degradation (Wang et al., 2013; Havaux, 2014; Wang et al., 2026). Under salt stress, chlorophyll and carotenoid contents declined markedly in WT plants but remained relatively stable in the overexpression lines (Figure 8I and J). The overexpression lines also showed reduced antioxidant enzyme activities compared with WT plants (Figure 8K-M), which differs from some previous reports (Chen et al., 2017). Furthermore, PLS-PM analysis indicated that Na^+/K^+ homeostasis was negatively correlated with photosynthetic pigment degradation and positively correlated with a reduced burden on the enzymatic antioxidant system (Figure 9). One possible explanation is that ionic homeostasis was less perturbed in the transgenic plants, thereby reducing ROS accumulation and restricting the upregulation of antioxidant enzymes (Ahanger et al., 2017). Collectively, these findings indicate that *SaHAK13*, *SaHAK40* and *SaHAK65* enhance salt tolerance primarily by promoting K^+ acquisition and maintaining Na^+/K^+ homeostasis, with ancillary benefits for root growth, osmotic balance and photosynthetic pigment stability.

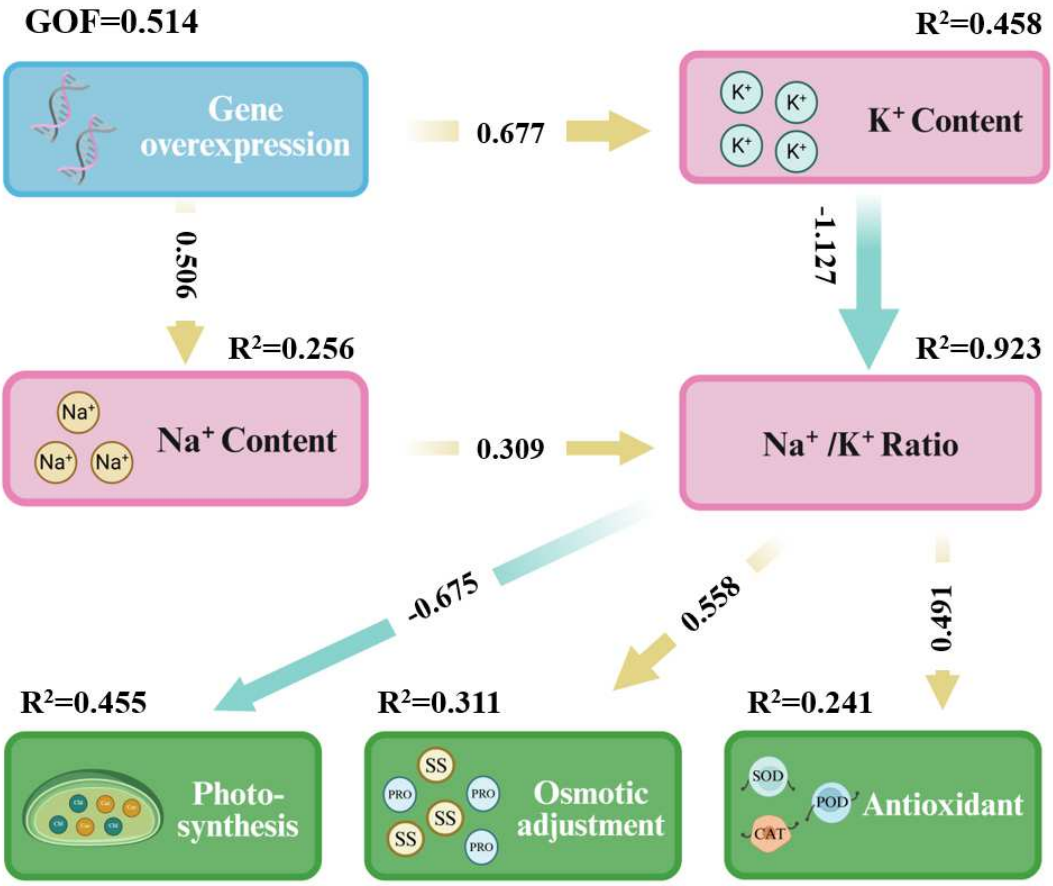


Figure 9. PLS-PM analysis of the SaHAKs's central regulator. Photosynthesis: chlorophyll and carotenoid contents. Osmotic adjustment: proline and soluble sugar contents. Antioxidant: SOD, POD and CAT activities. Solid line: significant relationship ($P < 0.05$); dashed line: non-significant relationship. Yellow line: positive impact; blue line: negative impact. Line thickness indicates causal strength, with a standardized path coefficient. R^2 explains total change in dependent variable; GOF indicates model fit (GOF>0.5).

5. Conclusion

Potassium uptake and transport in plants are governed by the KT/HAK/KUP family. Here, we systematically charaterized 70 *SaHAK* genes in the halophyte *S. alterniflora*, which were phylogenetically resolved into 11 clusters, with clusters IV and XI being the largest. Segmental duplication was identified as the main mechanism underlying *SaHAK* family expansion and may have contributed to functional

diversification under saline conditions. Transcriptional profiling revealed that salt stress dynamically modulated *SaHAK* expression in a tissue-specific manner, suggesting broad participation in the stress response. Notably, heterologous overexpression of three salt-inducible genes，*SaHAK13*, *SaHAK40*, and *SaHAK65*，conferred enhanced salt tolerance in *N. benthamiana*, primarily by promoting K^+ accumulation and reducing the Na^+/K^+ ratio. Further PLS-PM analysis established that a lowered Na^+/K^+ ratio was functionally linked to reduced osmolyte accumulation, attenuated antioxidant enzyme activity, and mitigated photosynthetic pigment degradation, collectively contributing to improved stress resilience. Collectively, these findings not only advance our understanding of *HAK* family function in halophytes but also pinpoint promising candidate genes for engineering salt tolerance in crops.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

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Zhou: Writing-review & editing, Conceptualization, Investigation. **Xiaowen Lin:** Methodology, Investigation. **Jianping Lai:** Formal analysis, Methodology. **Xin Luo:** Methodology, Investigation. **Minnan Sun:** Methodology, Investigation. **Weiwei Lv:** Methodology, Investigation. **Jin-e Liu:** Writing-review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Jinlou Huang:** Writing-review & editing, Methodology. **Bin-Le Lin:** Writing-review & editing, Supervision. **Yanshan Chen**: Conceptualization, Investigation. **Wenzong Zhou:** Conceptualization, Investigation. **Khan Waqas:** Investigation. **Renxi Gu:** Investigation. **Jianguo Lu:** Investigation. **Yunan Zhou:** Investigation. **Qihao Fang：** Investigation. **Yiran Tang:** Investigation.

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Data availability

The transcriptome datasets of *S. alterniflora* seedlings under salt stress were deposited in the China National Center for Bio-information (CNCB, https://www.cncb.ac.cn/) under BioProject accession number PRJCA077579. The corresponding sequencing data accession number is CRA052688. Other data generated or analyzed during this study are included in this article and its supplementary information files.

Declarations

Ethics approval and consent to participate： Not applicable

Consent for publication： Not applicable.

Competing interests： The authors declare no competing interests.

Abbreviations

ANOVA	Analysis of Variance
APC	Amino Acid–Polyamine–Organocation
CAT	Catalase
cDNA	Complementary Deoxyribonucleic Acid
CT / Ct	Cycle Threshold
DEG / DEGs	Differentially Expressed Gene / Genes
DESeq2	DESeq2: Differential Gene Expression Analysis Software
E-value	Expectation Value
FDR	False Discovery Rate
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GO	Gene Ontology
HAK	High-Affinity Potassium Transporter
HISAT2	Hierarchical Indexing for Spliced Alignment of Transcripts 2
Ka	Nonsynonymous Substitutions per Nonsynonymous Site
Ks	Synonymous Substitutions per Synonymous Site
KT	Potassium Transporter
KT/HAK/KUP	Potassium Transporter / High-Affinity Potassium Transporter / Potassium Uptake Permease
K-trans	Potassium Transporter Domain
KUP	Potassium Uptake Permease
mRNA	Messenger Ribonucleic Acid
MS	Murashige and Skoog
NJ	Neighbor-Joining
OE	Overexpression
Padj	Adjusted P-value
PCR	Polymerase Chain Reaction
PlantCARE	Plant Cis-Acting Regulatory Elements
PLS-PM	Partial Least Squares Path Modeling
POD	Peroxidase
qPCR	Quantitative Polymerase Chain Reaction
RH	Relative Humidity
RNA	Ribonucleic Acid

RNA-seq	RNA Sequencing
ROS	Reactive Oxygen Species
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SOD	Superoxide Dismutase
WT	Wild Type

Refernce

Ahanger MA, Tomar NS, Tittal M, Argal S, Agarwal R. Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. Physiology and Molecular Biology of Plants. 2017 Oct;23(4):731-44. Doi: 10.1007/s12298-017-0462-7

Ahn SJ, Shin R, Schachtman DP. Expression of KT/KUP genes in Arabidopsis and the role of root hairs in K+ uptake. Plant Physiology. 2004 Mar 1;134(3):1135-45. Doi: 10.1104/pp.103.034660

Amrutha RN, Sekhar PN, Varshney RK, Kishor PK. Genome-wide analysis and identification of genes related to potassium transporter families in rice (Oryza sativa L.). Plant Science. 2007 Apr 1;172(4):708-21. Doi: 10.1016/j.plantsci.2006.11.019

Banuelos MA, Garciadeblas B, Cubero B, Rodrıguez-Navarro A. Inventory and functional characterization of the HAK potassium transporters of rice. Plant Physiology. 2002 Oct 1;130(2):784-95. Doi: 10.1104/pp.007781

Bradley PM, Morris JT. Relative importance of ion exclusion, secretion and accumulation in Spartina alterniflora Loisel. Journal of Experimental Botany. 1991 Dec 1;42(12):1525-32. Doi: 10.1093/jxb/42.12.1525

Cai K, Zeng F, Wang J, Zhang G. Identification and characterization of HAK/KUP/KT potassium transporter gene family in barley and their expression under abiotic stress. BMC genomics. 2021 May 1;22(1):317. Doi: 10.1186/s12864-021-07633-y

Chen G, Liu C, Gao Z, Zhang Y, Jiang H, Zhu L, Ren D, Yu L, Xu G, Qian Q. OsHAK1, a high-affinity potassium transporter, positively regulates responses to drought stress in rice. Frontiers in Plant Science. 2017 Nov 1;8:1885. Doi: 10.3389/fpls.2017.01885

Chen G, Liu C, Gao Z, Zhang Y, Zhang A, Zhu L, Hu J, Ren D, Yu L, Xu G, Qian Q. Variation in the abundance of OsHAK1 transcript underlies the differential salinity tolerance of an indica and a japonica rice cultivar. Frontiers in Plant Science. 2018 Jan 5;8:2216. Doi: 10.3389/fpls.2017.02216

Chen S, Du T, Huang Z, He K, Yang M, Gao S, Yu T, Zhang H, Li X, Chen S, Liu CM. The Spartina alterniflora genome sequence provides insights into the salt-tolerance mechanisms of exo-recretohalophytes. Plant Biotechnology Journal. 2024 Sep;22(9):2558-74. Doi: 10.1111/pbi.14368

Cui Y, Zhang X, Yang M, Wei X, Guo J. Current understanding of HAK potassium transporters in plant development and stress tolerance. Journal of Plant Physiology. 2025 Sep 23:154617. Doi: 10.1016/j.jplph.2025.154617

Dang TV, Cho HS, Lee S, Hwang I. Salt stress-accelerated proteasomal degradation of LBD11 suppresses ROS-mediated meristem development and root growth in Arabidopsis. Plant Communications. 2025 Apr 14;6(4). Doi: 10.1016/j.xplc.2025.101241

Feng H, Tang Q, Cai J, Xu B, Xu G, Yu L. Rice OsHAK16 functions in potassium uptake and translocation in shoot, maintaining potassium homeostasis and salt tolerance. Planta. 2019 Aug 1;250(2):549-61. Doi: 10.1007/s00425-019-03194-3

Feng X, Wang Y, Zhang N, Wu Z, Zeng Q, Wu J, Wu X, Wang L, Zhang J, Qi Y. Genome-wide systematic characterization of the HAK/KUP/KT gene family and its expression profile during plant growth and in response to low-K+ stress in Saccharum. BMC Plant Biology. 2020 Jan 13;20(1):20. Doi: 10.1186/s12870-019-2227-7

Fu HH, Luan S. AtKUP1: a dual-affinity K+ transporter from Arabidopsis. The Plant Cell. 1998 Jan 1;10(1):63-73. Doi: 10.1105/tpc.10.1.63

Gierth M, Mäser P. Potassium transporters in plants–involvement in K+ acquisition, redistribution and homeostasis. FEBS letters. 2007 May 25;581(12):2348-56. Doi: 10.1016/j.febslet.2007.03.035.

Gupta M, Qiu X, Wang L, Xie W, Zhang C, Xiong L, Lian X, Zhang Q. KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (Oryza sativa). Molecular Genetics and Genomics. 2008 Nov;280(5):437-52.Doi: 10.1007/s00438-008-0377-7

Hao Y, Wang XF, Guo Y, Li TY, Yang J, Ainouche ML, Salmon A, Ju RT, Wu JH, Li LF, Li B. Genomic and phenotypic signatures provide insights into the wide adaptation of a global plant invader. Plant Communications. 2024 Apr 8;5(4). Doi: 10.1016/j.xplc.2024.100820

Havaux M. Carotenoid oxidation products as stress signals in plants. The plant journal. 2014 Aug;79(4):597-606. Doi: 10.1111/tpj.12386

Hester MW, Mendelssohn IA, McKee KL. Species and population variation to salinity stress in Panicum hemitomon, Spartina patens, and Spartina alterniflora: morphological and

physiological constraints. Environmental and Experimental Botany. 2001 Dec 1;46(3):277-97. Doi：10.1016/S0098-8472(01)00100-9

Horsch RB, Fry JE, Hoffmann NL, Wallroth M, Eichholtz D, Rogers SG, Fraley RT. A simple and general method for transferring genes into plants. science. 1985 Mar 8;227(4691):1229-31. Doi: 10.1126/science.227.4691.1229

Huang Y, Hu N, Yang X, Zhou S, Song M, Zhang J, Chen X, Du X, He D. Root response to K+-deprivation in wheat (Triticum aestivum L.): coordinated roles of HAK transporters, AKT2 and SKOR K+-channels, and phytohormone regulation. Agriculture. 2025 May 3;15(9):993. Doi：10.3390/agriculture15090993

Jhanani GK, Govindasamy C, Raghavendra T. Germination and biological adaptation approaches as salt-stress tolerance process in selected paddy cultivars under salinity stress. Environmental Research. 2024 Oct 15;259:119566. Doi：10.1016/j.envres.2024.119566

Johnson R, Vishwakarma K, Hossen MS, Kumar V, Shackira AM, Puthur JT, Abdi G, Sarraf M, Hasanuzzaman M. Potassium in plants: Growth regulation, signaling, and environmental stress tolerance. Plant Physiology and Biochemistry. 2022 Feb 1;172:56-69.Doi：10.1016/j.plaphy.2022.01.001

Li Q, Du W, Tian X, Jiang W, Zhang B, Wang Y, Pang Y. Genome-wide characterization and expression analysis of the HAK gene family in response to abiotic stresses in Medicago. BMC genomics. 2022 Dec 1;23(1):791. Doi：10.1186/s12864-022-09009-2

Liu K, Qin Y, Wu L, Yi R, Shi X, Yu J, Shi X, Dong W, Jia L, Fan M. Genome-wide identification, characterization and expression pattern analysis of HAK/KUP/KT potassium transporter gene family in potato. Frontiers in Plant Science. 2025 Jan 16;15:1487794. Doi：10.3389/fpls.2024.1487794

Liu T, Zhang Y, Xie Y, Yang R, Yuan M, Li Y, Xu H, Zhu X, Song T, Cheng X. Impact of the potassium transporter TaHAK18 on wheat growth and potassium uptake under stressful K+ conditions. Journal of Plant Physiology. 2025 Apr 1;307:154459. Doi：10.1016/j.jplph.2025.154459

Liu X, Shi L, Chen Y, Wang J, Liu A, Li P. Identification, classification, and stress-responsive regulation of HAK family genes in poplar. Frontiers in Plant Science. 2025 Nov 14;16:1690537. Doi：10.3389/fpls.2025.1690537

Luo HB, Cao HQ, Huang CM, Wu XJ, Ye LP, Wei YW. Genome wide identification and functional analyses of HAK family potassium transporter genes in passion fruit (Passiflora edulis Sims) in response to potassium deficiency and stress responses. Plant Physiology and

747 Biochemistry. 2025 Aug 1;225:109995. Doi：10.1016/j.plaphy.2025.109995

748 Mahiwal S, Pandey GK. Potassium: a vital nutrient mediating stress tolerance in plants.
749 Journal of Plant Biochemistry and Biotechnology. 2022 Dec;31(4):705-19. Doi：
750 10.1007/s13562-022-00775-4.

751 Marschner H, editor. Marschner's mineral nutrition of higher plants. Academic press; 2011
752 Aug 8. Doi：10.1016/C2009-0-63043-9

753 Mekawy AM, Assaha DV, Li J, Yusuf A, Mostafa D, Shoulkamy MA, Ueda A. Differential
754 physiological and molecular processes in the root may underlie contrasting salt tolerance in
755 two egyptian rice cultivars at the seedling stage. Journal of Soil Science and Plant Nutrition.
756 2024 Jun;24(2):3100-14. Doi：10.1007/s42729-024-01736-7

757 Morita S, Tamba N, Shibasaka M, Sasano S, Kadoike T, Urase Y, Maruyama M, Fukuoka A,
758 Yanai J, Masumura T, Ogihara Y. In planta evidence that the HAK transporter OsHAK2 is
759 involved in Na+ transport in rice. Bioscience, Biotechnology, and Biochemistry. 2023
760 May;87(5):482-90. Doi: 10.1093/bbb/zbad020

761 Mostofa MG, Rahman MM, Ghosh TK, Kabir AH, Abdelrahman M, Khan MA, Mochida K,
762 Tran LS. Potassium in plant physiological adaptation to abiotic stresses. Plant Physiology and
763 Biochemistry. 2022 Sep 1;186:279-89. Doi：10.1016/j.plaphy.2022.07.011

764 Nampei M, Ogi H, Sreewongchai T, Nishida S, Ueda A. Potassium transporter OsHAK17
765 may contribute to saline-alkaline tolerant mechanisms in rice (Oryza sativa). Journal of Plant
766 Research. 2024 May;137(3):505-20. Doi：10.1007/s10265-024-01529-0

767 Peng L, Xiao H, Li R, Zeng Y, Gu M, Moran N, Yu L, Xu G. Potassium transporter
768 OsHAK18 mediates potassium and sodium circulation and sugar translocation in rice. Plant
769 Physiology. 2023 Nov;193(3):2003-20. Doi：10.1093/plphys/kiad435

770 Schoenaers S, Balcerowicz D, Vissenberg K. Molecular mechanisms regulating root hair tip
771 growth: a comparison with pollen tubes. InPollen tip growth: From biophysical aspects to
772 systems biology 2017 Jun 15 (pp. 167-243). Cham: Springer International Publishing. Doi：
773 10.1007/978-3-319-56645-0_9

774 Sonis ST. Fundamentals of Genetics and Genomics. InGenomics, Personalized Medicine and
775 Oral Disease 2015 Sep 18 (pp. 1-10). Cham: Springer International Publishing. Doi：
776 10.1007/978-3-319-17942-1_1

777 Subudhi PK, Baisakh N. Spartina alterniflora Loisel., a halophyte grass model to dissect salt
778 stress tolerance. In Vitro Cellular & Developmental Biology-Plant. 2011 Aug;47(4):441-57.

779 Doi：10.1007/s11627-011-9361-8

780 Sun W, Xia L, Deng J, Sun S, Yue D, You J, Wang M, Jin S, Zhu L, Lindsey K, Zhang X.
781 Evolution and subfunctionalization of CIPK6 homologous genes in regulating cotton drought
782 resistance. Nature communications. 2024 Jul 9;15(1):5733. Doi：10.1038/s41467-024-
783 50097-3

784 Sustr M, Soukup A, Tylova E. Potassium in root growth and development. Plants. 2019 Oct
785 22;8(10):435. Doi：10.3390/plants8100435

786 Wang M, Zheng Q, Shen Q, Guo S. The critical role of potassium in plant stress response.
787 International journal of molecular sciences. 2013 Apr 2;14(4):7370-90. Doi：
788 10.3390/ijms14047370

789 Wang T, Duan S, Xu C, Wang Y, Zhang X, Xu X, Chen L, Han Z, Wu T. Pan-genome analysis
790 of 13 Malus accessions reveals structural and sequence variations associated with fruit traits.
791 Nature Communications. 2023 Nov 15;14(1):7377. Doi：10.1038/s41467-023-43270-7

792 Wang X, Li J, Li F, Pan Y, Cai D, Mao D, Chen L, Luan S. Rice potassium transporter
793 OsHAK8 mediates K+ uptake and translocation in response to low K+ stress. Frontiers in
794 Plant Science. 2021 Aug 3;12:730002. Doi：10.3389/fpls.2021.730002

795 Wang Y, Chen YF, Wu WH. Potassium and phosphorus transport and signaling in plants.
796 Journal of Integrative Plant Biology. 2021 Jan;63(1):34-52. Doi：10.1111/jipb.13053

797 Wang Z, Yao C, Yang Y, Segbo S, Xu X, Lin X, Zhou P, Gao F, Ni Z, Shi T, Gao Z. Effects of
798 Foliar Potassium Fertilizer on Photosynthetic Capacity and Expression of Potassium and
799 Sugar Transporters in Peach (Prunus persica). Horticulturae. 2026 Mar 21;12(3):388. Doi：
800 10.3390/horticulturae12030388

801 Wei J, Tiika RJ, Cui G, Ma Y, Yang H, Duan H. Transcriptome-wide identification and
802 expression analysis of the KT/HAK/KUP family in Salicornia europaea L. under varied NaCl
803 and KCl treatments. PeerJ. 2022 Mar 3;10:e12989. Doi：10.7717/peerj.12989

804 Wei W. Functional analysis of Spartina alterniflora SabZIP19 and SaTRA2 genes in rice salt
805 tolerance. Fujian Agriculture and Forestry University, Fuzhou. 2020. Doi：
806 10.27018/d.cnki.gfjnu.2020.000714

807 White PJ. Improving potassium acquisition and utilisation by crop plants. Journal of Plant
808 Nutrition and Soil Science. 2013 Jun;176(3):305-16. Doi：10.1002/jpln.201200121

809 Xin Y，Zou CY, Shi Y, Ran SS, Liu JE, Chen H, Ren LJ. Response of chlorophyll
810 fluorescence parameters of Spartina alterniflora to salt stress [J]. Environmental Ecology.

2020 Feb;2(07): 8-12. Doi：10.3969/j.issn.1000-3142.2012.01.020

Yang H, Zhang W, Chai W, Wang W, Gao L, Zhang J, Wang Y, Wang SM. PtHAK5, a candidate for mediating high-affinity K+ uptake in the halophytic grass, Puccinellia tenuiflora. Engineering Agriculture. 2018;5(1):108. Doi：10.15302/J-FASE-2018200

Yang T, Feng H, Zhang S, Xiao H, Hu Q, Chen G, Xuan W, Moran N, Murphy A, Yu L, Xu G. The potassium transporter OsHAK5 alters rice architecture via ATP-dependent transmembrane auxin fluxes. Plant Communications. 2020 Sep 14;1(5). Doi：10.1016/j.xplc.2020.100052

Yang T, Zhang S, Hu Y, Wu F, Hu Q, Chen G, Cai J, Wu T, Moran N, Yu L, Xu G. The role of a potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. Plant Physiology. 2014 Oct 1;166(2):945-59. Doi：10.1104/pp.114.246520

Zhang H, Zhu J, Gong Z, Zhu JK. Abiotic stress responses in plants. Nature reviews genetics. 2022 Feb;23(2):104-19. Doi：10.1038/s41576-021-00413-0

Zhang L, Sun X, Li Y, Luo X, Song S, Chen Y, Wang X, Mao D, Chen L, Luan S. Rice Na+-permeable transporter OsHAK12 mediates shoots Na+ exclusion in response to salt stress. Frontiers in Plant Science. 2021 Dec 7;12:771746. Doi：10.3389/fpls.2021.771746

Zhang M, Liang X, Wang L, Cao Y, Song W, Shi J, Lai J, Jiang C. A HAK family Na+ transporter confers natural variation of salt tolerance in maize. Nature Plants. 2019 Dec;5(12):1297-308. Doi：10.1038/s41477-019-0565-y

Zhang Z, Zhang J, Chen Y, Li R, Wang H, Wei J. Genome-wide analysis and identification of HAK potassium transporter gene family in maize (Zea mays L.). Molecular Biology Reports. 2012 Aug;39(8):8465-73.Doi：10.1007/s11033-012-1700-2

Zhao K, Tian H, Zhou L, Liu C, Wang H, Yang D, Guan J, Zhang S, Huang X. Genome-wide identification of calcium-dependent protein kinase (CDPK) family genes in pear (Pyrus bretschneideri) and functional characterization of PbrCDPK8 in black spot tolerance. Horticultural Plant Journal. 2025 Dec 29. Doi：10.1016/j.hpj.2025.07.025

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