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ORIGINAL ARTICLE

Uncovering expression and functional analysis of newly discovered high-affinity K^+ transporter family members from sugarcane

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Abstract

Potassium ions are widely involved in a series of physiological and biochemical processes of plants, which is of great significance to plant growth. High-affinity K^+ (*HAK*) transporter mainly absorbed and transported potassium in plants, but there are few studies on *HAK* gene in sugarcane. In this study, the coding region of high-affinity K^+ transporter genes were cloned from sugarcane and designated as *ScHAK9, ScHAK10, ScHAK11* (GenBank accession number: MG564720, MG564721, MG564722). Phylogenetic analysis results confirmed that *ScHAK9* and *ScHAK11* had the closest relationship with *ZmHAK9* and *ZmHAK11*(*Zea mays*), *ScHAK10* had the closest relationship with *SbHAK10* (*Sorghum bicolor*). In subcellular localization experiments, the fusion protein of ScHAK9 and ScHAK11 with green fluorescent protein was specifically localized in the cell membrane, but ScHAK10 green fluorescent protein was not detected, it was speculated to be expressed in Golgi apparatus. The gene expression level of *ScHAK* in different tissues of sugarcane at the growth periods was different, and the gene expression level of *ScHAK* genes were up-regulated by the low-potassium and salt stress. Through the functional characterization experiments of *ScHAK* genes in K^+ uptake-deficient yeasts, it was founded that *ScHAK* genes possessed K^+ transporter activity. The study indicated that *ScHAK* genes might mediate K^+ absorption through the cell membrane and might be participate in maintaining Na^+/K^+ homeostasis in sugarcane under the adversity stress, and the development of plant organs is regulated by the potassium ions transport of *ScHAK* genes.

Keywords Sugarcane · High-affinity K^+ transporter genes(*HAK*) · Gene cloning · Gene function · Gene expression

Abbreviations

HAK	High-affinity K^+ transporter genes
GFP	Green fluorescence protein
qRT-PCR	Quantitative real-time PCR
CaMV	Cauliflower mosaic virus
URA3	Orotidine-5'-phosphate decarboxylase
GAL1	Recombinant galectin 1
CYC1	Cytochrome c-1
AP	Arginine and phosphoric acid

Introduction

Potassium is an essential element in plants and its activity is related to enzyme activity regulation, protein synthesis, osmotic adjustment, and photosynthesis (Ma et al. 2012; Amtmann et al. 2005) as well as stress response of plants (Anschutz et al. 2014). The absorption, transport and distribution of K^+ are primarily realized via potassium ion channels and transport carriers, which can be classified as the KUP/HAK/KT family, HKT/TPK family, CHX family, and KEA family (Maser et al. 2001). High-affinity K^+ (*HAK*) transporter gene belongs to KUP/HAK/KT family and is important in the absorption of plant potassium (Gierth et al. 2005). Identification of *HAK* genes encoding

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were first identified from barley (Santa-Maria et al. 1997), several *HAK* genes were cloned from various plants including maize, rice, mesembryanthemum crystallinum and poplar (Zhang et al. 2012; Yang et al. 2009; Su et al. 2002; He et al. 2012). *HAK* genes were founded to exist widely in plant tissues. *NtHAK1* gene was expressed in various organs of tobacco, particularly in roots (Lu and Yang 2011). After the gene was knocked out, root hair elongation was damaged, indicating that the root growth was affected by *HAK* genes (Zou et al. 2012). Arabidopsis mutants (*AtHAK5*) germinated slowly and their root growth was limited under the low-potassium stress, indicating that *HAK* genes were involved in the physiological processes of seed germination and root elongation (Nieves-Cordones et al. 2010).The KUP/HAK/KT family can be divided into three types such as high-affinity, low-affinity and mixed-affinity potassium transporters according to the affinity of potassium absorption (Rubio et al. 2000). High-affinity potassium transporters participate in potassium absorption under low-potassium conditions (Banuelos et al. 2002). Low-affinity potassium transporters are widely distributed in plant tissues and work together with potassium channels to absorb potassium under a high potassium concentration (Song et al. 2013). Mixed-affinity potassium transporters function as K^+ and Na^+ bidirectional transporters (Maathuis 2006; Takahashi et al. 2007).

In summary, it is reported that many plants contain *HAK* family genes, the research on the physiological function of *HAK* family genes is still in the initial stage, the known functional of *HAK* genes had been identified in a few plants, such as *Arabidopsis*, rice and maize, but their physiological functions and regulatory mechanisms in sugarcane remain unknown. Sugarcane is allopolyploid, and it is speculated that the *HAK* family in sugarcane will also be a large family with complex functions. The study on the phylogenetic evolution, structure and function of the *HAK* genes would lay a foundation for the mechanism of potassium absorption and transport in sugarcane. In this study, three members of sugarcane high-affinity K^+ transporter (*ScHAK*) gene family *c*oding sequence were cloned, and the primary features and structures of *ScHAK* genes along with its relationship with HAK from other genera were characterized. The sugarcane HAK gene expression characteristics were analyzed in the roots, stems and leaves at the physiological maturity stage, and under low-potassium stress treatment (0.1 mmol/L) and salt stress treatment (1% NaCl) in sugarcane leaves, subcellular localization analyses revealed that ScHAK9 and ScHAK11 protein were localized to the plasma membrane in tobacco epidermal cells. When expressed in K^+ uptake-deficient yeast cells, *ScHAK* genes were clearly improve K^+ acquisition of the yeast and tolerance to low K^+ environment. The functional and expression analysis of *ScHAK* genes in sugarcane may provide the way to improve the potassium absorption efficiency in sugarcane.

Materials and methods

Plant material

Sugarcane variety "ROC22" in China was used for gene cloning and gene expression studies. Sugarcane (*Saccharum officinarum*) was planted with hoagland nutrient solution added in water in the greenhouse (average temperature, 25 °C, relative humidity, 80 ± 5%). One group was set as the control and the other two treatments included low-potassium stress treatment (0.1 mmol/L) and salt stress treatment (1% NaCl). Leaf samples were harvested at different intervals (24 h, 48 h, 72 h and 96 h) at the seedling stage, stored at − 80 °C for RNA isolation. Choose consistent and healthy cane stems for planting in a greenhouse (average temperature, 25 °C, soil water content at 25% ± 5%). The complete plants (roots, stems and leaves) were also collected at the age of seedling stage, elongation stage and maturation stage.

Gene cloning and sequence bioinformatics analysis of ScHAK genes

RNA was extracted from leaves with RNA extraction kit (Omega Bio-Tek, Guangzhou, China). First-strand cDNA template for RT-PCR amplification was obtained by using One-Step gDNA Removal and cDNA Synthesis Super-Mix kit (Transgen, Beijing, China). According to the data of sugarcane cDNA library, primer pair were designed from the conserved sequence region. Then PCR was performed with the template of first-strand cDNA. The used primer pair was designed as:

ScHAK9-F: 5'-ATGGATCCCGAGTTCGGCGT-3'.
ScHAK9-R: 5'-TCACAGCACGTACACCATGC-3'.
ScHAK10-F: 5'-ATGAAGAGCCCCCCTGTCAT-3'.
ScHAK10-R: 5'-TCAGATATAGTACATCATGCCG-3'.
ScHAK11-F: 5'-ATGGCATCGCTGTCAGAAAGT-3'.
ScHAK11-R: 5'-TCAGATGTAGTATATCTGGCCG-3'.

The PCR reaction system contained 12.5 μL of PCR Buffer, 5 μL of dNTP mixture (2 mmol/L), 0.75 μL of upstream primer(10 μM/L), 0.75 μL of downstream primer(10 μM/L), 0.5 μL of KOD FX DNA polymerase, and 3.0 μL of first-strand cDNA template and was diluted to a volume of 25.0 μL with double-distilled water. PCR program was composed of initial denaturation at 94 °C for 2 min, 35 cycles of 98 °C for 10 s, 61 °C for 30 s, and

72 °C for 3 min, and final extension at 72 °C for 7 min. Then, PCR products(2 μL) were electrophoresed on 1.2% agarose gel. Remaining PCR products were purified with the gel extraction kit (Biomed Technology, Beijing, China), then cloned into pEASY-Blunt Simple Cloning vector, transformed into E. coli T1, and sequenced.

The sequence of *ScHAK* genes were analyzed with Prot Param (https://web.expasy.org/protparam/) and Protter software (http://wlab.ethz.ch/protter/start/) to predict the amino acid sequence, hydropathy, protein structure, molecular weight, and isoelectric point of putative protein. An alignment of ScHAK protein and other plant HAK proteins retrieved from NCBI was carried out in Clustal W. Then with the alignment results, a phylogenetic tree was constructed in MEGA 6.0 according to the neighbor-joining method.

Construction of GFP fusion carrier and transfection

To construct *ScHAK*-GFP fusion fragment for the expression in tobacco epidermal cells, PCR products were digested with *Nco*I and *Spe*I, and then ligated to pCAMBICA1302 vector. The recombinant plasmids (pCAMBICA1302- ScHAK) were confirmed by double digestion with *Nco*I and *Spe*I and sequencing and then transferred to TOP10 clone strain.

According to the previous method (Xu et al. 2014), the empty plasmid pCAMBIA1302 was used as the control. A positive monoclonal antibody containing recombinant plasmid pCAMBIA-ScHAK was amplified in YEP liquid culture (containing 50 mg/mL rifampicin and 25 mg/mL Kanamycin) and cultivated to $OD_{600\ nm}$ = 1.5–2.0 at 28 °C. After centrifugation, Agrobacterium bacteria were collected. Then, 50 mL of Agrobacterium suspension was added, mixed, injected into tobacco epidermis, and then cultured for 72 h under normal illumination conditions. Tobacco epidermis was observed with a confocal laser scanning microscope (excitation wavelength, 488 nm; emission wavelength, 625–725 nm).

Quantitative real-time PCR analysis

Total RNA from roots, stems and leaves were extracted with Spin Column Plant total RNA Purification Kit (Omega Bio-Tek, Guangzhou, China). Then the first-strand cDNA was synthesized with AMV Reverse Transcriptase First Strand cDNA Synthesis Kit (TransGen Biotech, Beijing, China). Specific primers for qRT-PCR of *ScHAK* and 25S rRNA (as house-keeping gene) genes are shown in Table 1.

Real-time quantitative PCR was performed on ABI 7500 with the 20-μL reaction system composed of 7.0 μL of 2 × SYBR Green qPCR Master Mix, 0.5 μL of 10 μM upstream primer, 0.5 μL of 10 μM downstream primer, 3 μL of first-strand cDNA template, and 9 μL of double-distilled water. The amplification program was set as follows: initial denaturation at 95 °C for 10 min, 40 cycles of denaturation and annealing extension at 95 °C for 10 s and 60 °C for 34 s, dissolution curve collection at 95 °C for 30 s and 60 °C for 15 s. Triplicate technical replicates were analyzed for each biological replicate.

Complementation test of yeast mutants

Complementation test of yeast mutants was performed according to the method of Li et al. (2014). According to the ORF sequence of *ScHAK*, primers containing restriction sites were designed (Table 2). After double digestion, *ScHAK11* was connected to the expression vector pYES2 with the same digestion site by T4 DNA ligase. The fused plasmid was composed of the GAL1 promoter, the CYC1 terminator and a selective marker URA3. The vector pYES2 was used as a negative control. The transformants were selected on Glc-containing SC-agar plates with 100 mm K^+ and zero uracil. AP (arginine and phosphoric acid) plates containing 0.5, 5, 10, 20, and 50 mM K^+ were used for subsequent growth assays according to the previous method (Horie et al. 2011). There clones were used in growth tests with solid plates under 28 °C for 48–72 h.

Results

Genetic relationship analysis of ScHAK

In order to study the evolutionary relationship of *ScHAK9, ScHAK10* and *ScHAK11* proteins, there related HAK proteins from NCBI protein database were downloader for constructing phylogenetic tree by MEGA 6.0. The phylogenetic relationship suggested that they might have the similar functions.

The phylogenetic results revealed that *ScHAK* genes can be divided into 3 groups, the sequence of *ScHAK9* and *ScHAK11* had the highest identity with *Zea mays HAK*, but the *ScHAK10* had the highest identity with *Sorghum bicolor HAK* genes (Fig. 1). The research on the phylogenetic tree of genes can speculate its functional characteristics.

Protein domain analysis of ScHAK

A coding sequence (CDs) of *ScHAK9, ScHAK10* and *ScHAK11* were obtained from sugarcane leaves and then submitted in NCBI (GenBank accession number: MG564720, MG564721,MG564722).There amino acid sequences encoded by the cloned *ScHAK* genes were

Table 1 Primer sequences used in this study

Genes	Primers	Primer sequences (5'–3')	Size (bp)
25S	25S-F	ATAACCGCATCAGGTCTCCAAG	22
25S	25S-R	CCTCAGAGCCAATCCTTTTCC	21
ScHAK9	ScHAK9-F	CCTCGCCTTCTTCCTCTTCT	20
ScHAK9	ScHAK9-R	GCATGTCGTACTCGTACTTCTTG	23
ScHAK10	ScHAK10-F	AGAGTCTCGGCGTGGTGTA	19
ScHAK10	ScHAK10-R	GAGCGTGAGCGTCCAGAAG	19
ScHAK11	ScHAK11-F	GCAAGTCAAGCCACCATATCT	21
ScHAK11	ScHAK11-R	ATCCAGCAGTCACAGCAATG	20

Table 2 Primer sequences caontain restriction enzyme digestion sites

Genes	Restriction sites	Primer sequences (5'–3')	Size (bp)
ScHAK9-F	*Hind* III	ATCAAGCTTATGGATCCCGAGTTCGGCGT	29
ScHAK9-R	*Xba*I	TACTCTAGATCACAGCACGTACACCATGC	29
ScHAK10-F	*Hind*III	ATCAAGCTTATGAAGAGCCCCCCTGTCAT	29
ScHAK10-R	*EcoR*I	CGCGAATTCTCAGATATAGTACATCATGCCG	31
ScHAK11-F	*Hind*III	ATCAAGCTTATGGCATCGCTGTCAGAAAGT	31
ScHAK11-R	*Xho*I	TACCTCGAGTCAGATGTAGTATATCTGGCCG	31

analyzed by BLASTP of NCBI, and it was found that *ScHAK9, ScHAK10* and *ScHAK11* sequences had a conserved domain of potassium ion transport subfamily, which belonged to the HAK/ KUP /KT protein family, The basic physicochemical properties and function prediction of the coding proteins were analyzed, and the results were shown in Table 3.

The prediction analysis of the transmembrane structure of ScHAK proteins showed that the transmembrane structure of proteins can be divided into two models, the first model has a long ring structure between the second and third transmembrane regions, and the *ScHAK9* gene belongs to this model, the second model has a long ring structure between the first and second transmembrane regions, and the *ScHAK10, ScHAK11* genes belongs to this model. The transmembrane times of different proteins were different, but their structures were similar, and no signal peptide structure was found, then indicating that it was a non-secretory protein as a transport vector and could not be transported after it was synthesized in the cytoplasm, but might exist as a transport carrier (Fig. 2).

Subcellular localization analysis of ScHAK

In order to confirm the subcellular localization of ScHAK protein, the vector containing ScHAK-GFP fusion genes were constructed. The fusion protein expression vector pCAMBIA1302 was constructed by fusing the C-terminus of ScHAK protein to the green fluorescence protein (GFP) with the 35S promoter of Cauliflower Mosaic Virus (CaMV). Then, the DNA plasmids of pCAMBIA1302-ScHAK and the GFP control were transform into tobacco epidermal cells by *Agrobacterium*-mediated transformation. The cellular location of ScHAK protein was confirmed through the fluorescence and confocal analysis of laser microscopy under ultraviolet light. The result showed that the GFP protein was distributed throughout the cell membrane and nucleus with the pCAMBIA1302 vector. However, green fluorescence was specifically detected in the cell membrane transiently transfected with the pCAMBIA-ScHAK9 and pCAMBIA-ScHAK11, and green fluorescence with the pCAMBIA-ScHAK9 was aslo detected in the cell nucleus (Fig. 3b and d), the ScHAK10-GFP fusion protein was not detected which speculated that the protein is expressed in Golgi apparatus (Fig. 3c). These results further proved that ScHAK9 and ScHAK11 were membrane protein.

Expression patterns of ScHAK in sugarcane tissues and abiotic stresses

The tissue-specific expression of genes was related to the specific biological function of genes, so the tissue expression specificity of three genes were analyzed by qRT-PCR. The gene expression profile of *ScHAK* in sugarcane was confirmed, by further investigation in its expression patterns in leaves, roots and stems at different growth stages (seedling, elongating, and mature stages) by qRT-PCR (Fig. 4a, b and c). In the seedling stage, all *ScHAK* genes were expressed in leaves, roots and stems, *ScHAK9* gene was highly expressed in root at elongation stage, *ScHAK10* gene was highly expressed in leaf and stem at maturation

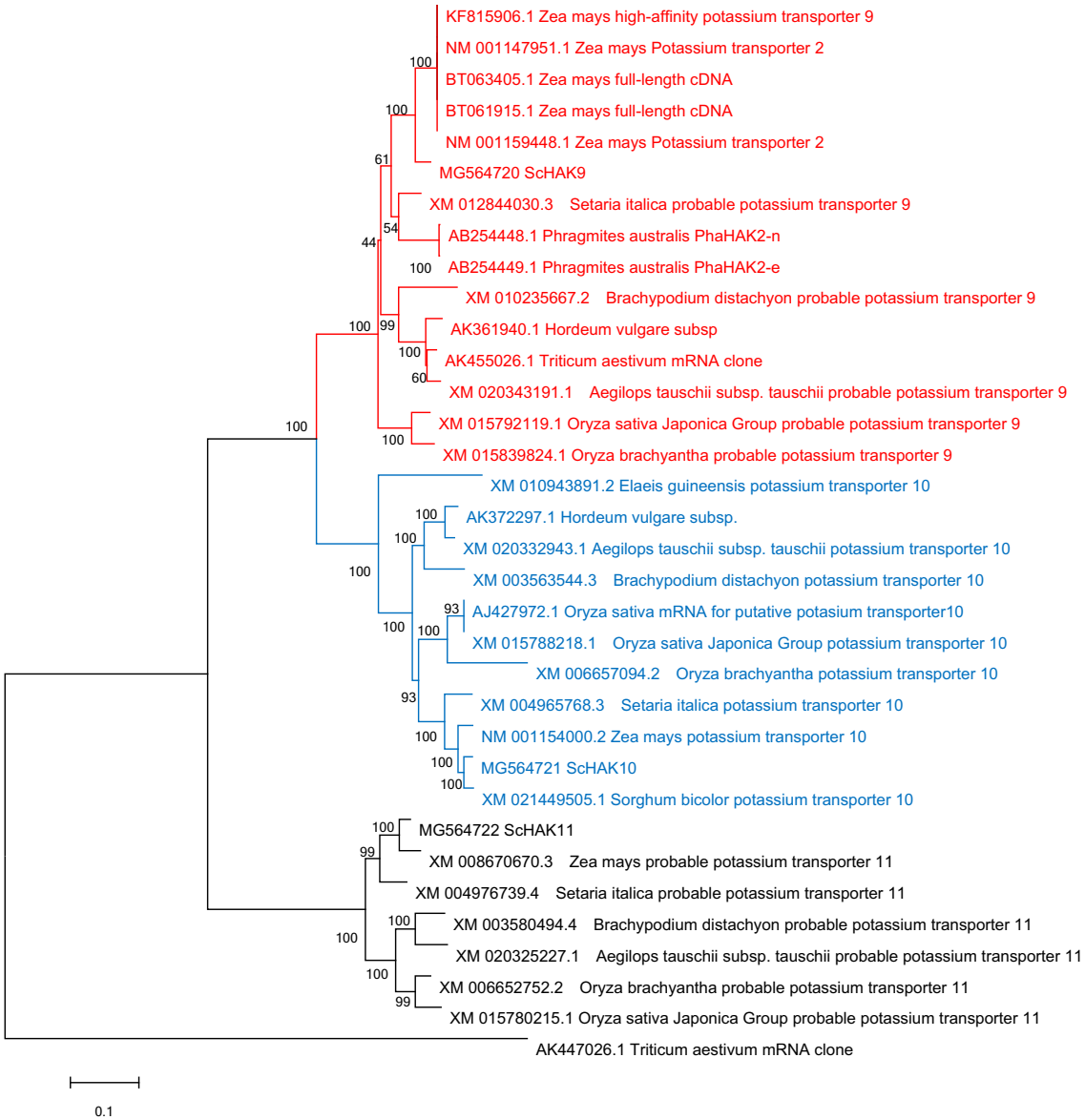


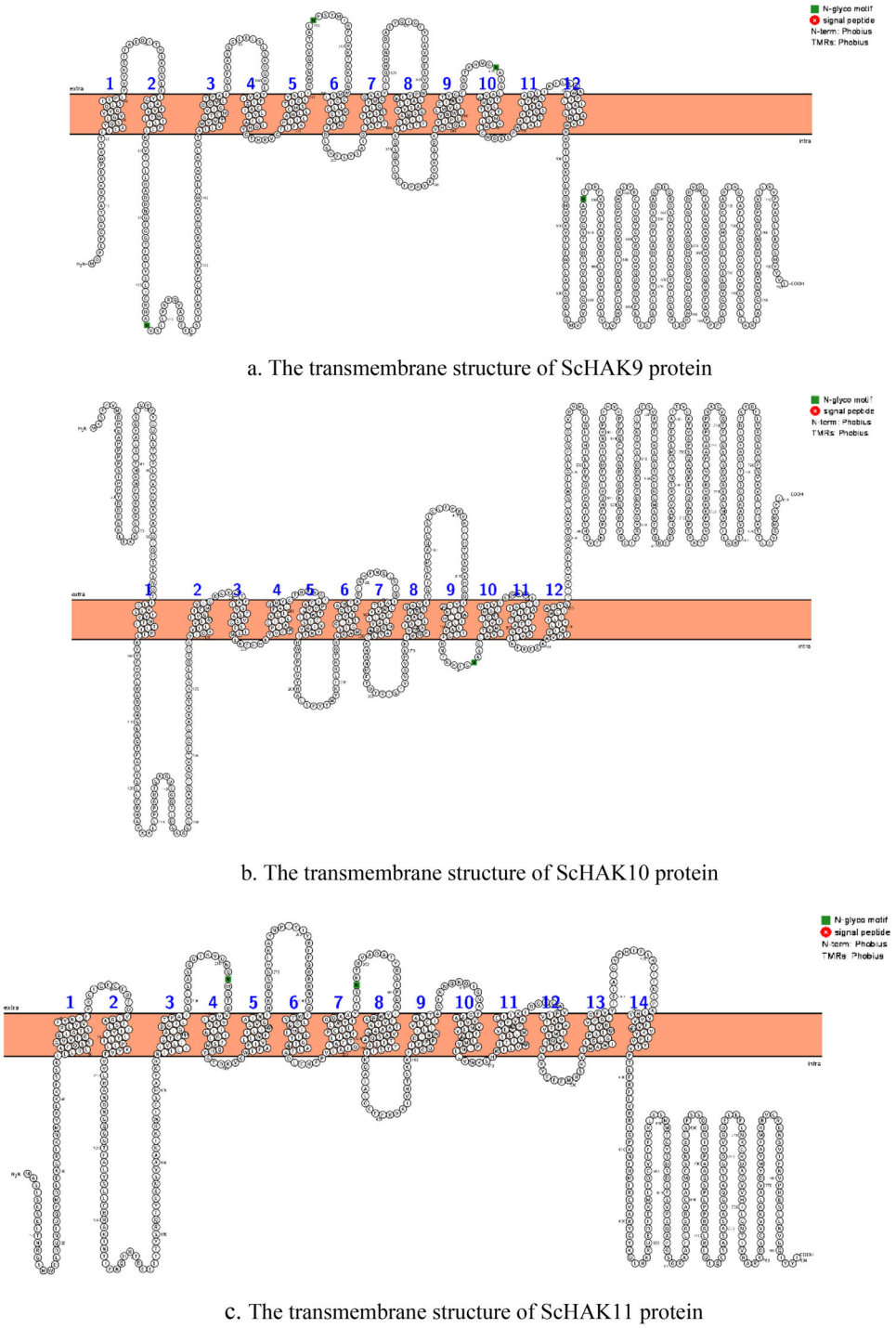
Fig. 1 Phylogenetic tree of ScHAK and other plant HAK proteins. Numbers at branch points show bootstrap support

Table 3 Physical and chemical properties and function prediction of *ScHAK* genes

Gene name	Number of amino acids	Molecular weight (k Da)	Isoelectric point(PI)	Signal Peptide	Transmembr ane domain
ScHAK9	783	86.58	7.87	None	12
ScHAK10	810	89.83	8.46	None	12
ScHAK11	804	89.58	8.2	None	14

stage, *ScHAK11* gene was highly expressed in roots and leaves at elongation stage. These results indicated that expression of *ScHAK* genes were spatiotemporal specific in growth process of sugarcane.

Moreover, the expression profiles of *ScHAK* were also detected under low-potassium and salt stress treatments. In our observations, the expression of *ScHAK* genes were induced under multiple stresses (Fig. 4d, e). The expression of *ScHAK* genes reached the maximum level in 48 h



a. The transmembrane structure of ScHAK9 protein

b. The transmembrane structure of ScHAK10 protein

c. The transmembrane structure of ScHAK11 protein

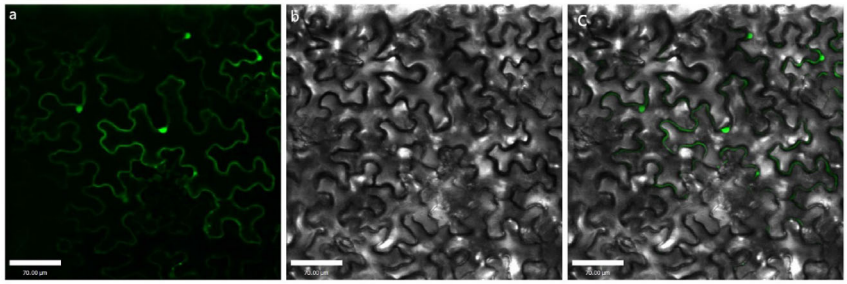
Fig. 2 Transmembrane model of ScHAK proteins

after low potassium treatment, and rapidly induced after 24 h under salt stress and its expression reached the highest level after 96 h. The experimental results suggested that *ScHAK* genes might play an important role in the regulation under salt and low-potassium stress.

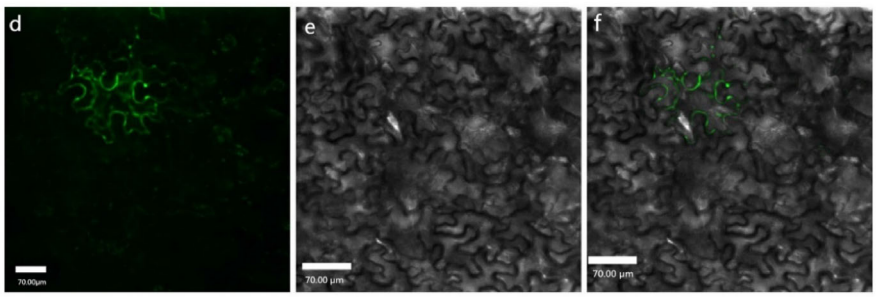
Functional analysis of *ScHAK* genes in yeast cells

In order to explore the transporter functional of ScHAK protein, the transformants were inoculated on AP medium respectively containing 0.5, 5, 10, 20 and 50 mM KCl. On AP medium containing 0.5 mM KCl, the empty pYES2 vector could not grow, but the strain CY162 transformed with *ScHAK* genes rescued its growth defect on AP

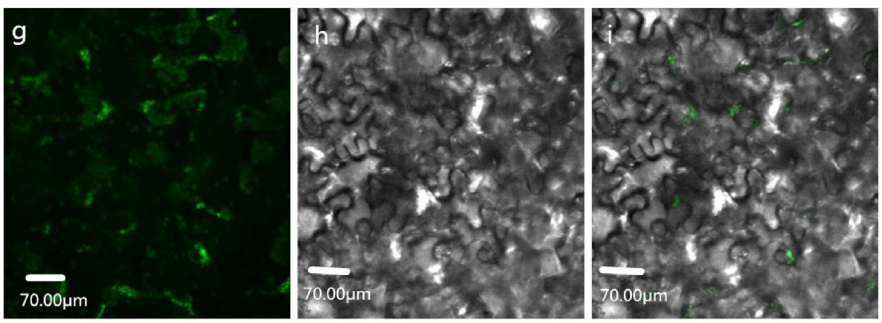
Fig. 3 Subcellular localization of pCAMBIA13021302-ScHAK



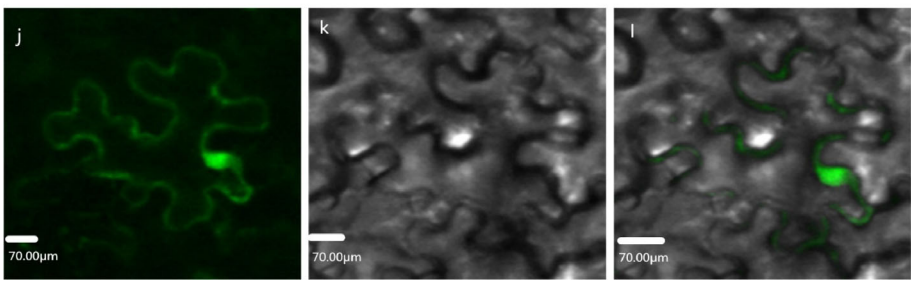
a. Histochemical analysis of transactivation activity of pCAMBIA1302 plasmids in N. *benthamiana* leaves. excitation: 488nm, emission:625 nm. (a) GFP images, (b) dark-field images, (c) Overlap of GFP fluorescence and dark-field images.



b. Histochemical analysis of transactivation activity of pCAMBIA1302-ScHAK9 plasmids in leaves of *N. benthamiana*. excitation: 488 nm, emission:625 nm. (d) GFP images, (e) dark-field images, (f) Overlap of GFP fluorescence and dark-field images.

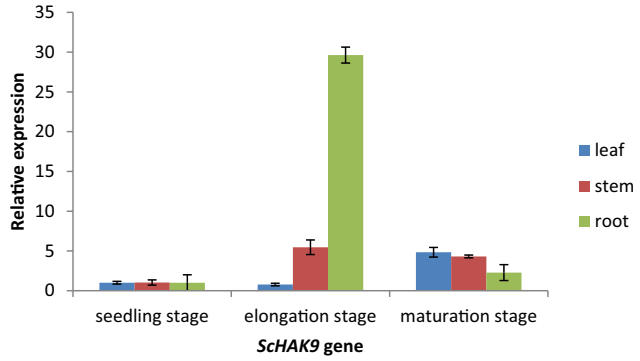


c. Histochemical analysis of transactivation activity of pCAMBIA1302-ScHAK10 plasmids in leaves of *N. benthamiana*. excitation: 488 nm, emission:625 nm. (g) GFP images, (h) dark-field images, (i) Overlap of GFP fluorescence and dark-field images.

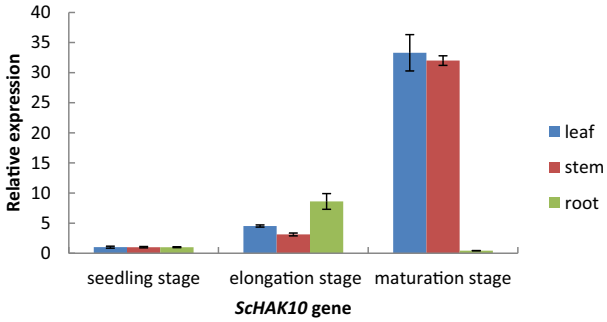


d. Histochemical analysis of transactivation activity of pCAMBIA1302-ScHAK11 plasmids in leaves of *N. benthamiana*. excitation: 488 nm, emission:625 nm. (j) GFP images, (k) dark-field images, (l) Overlap of GFP fluorescence and dark-field images.

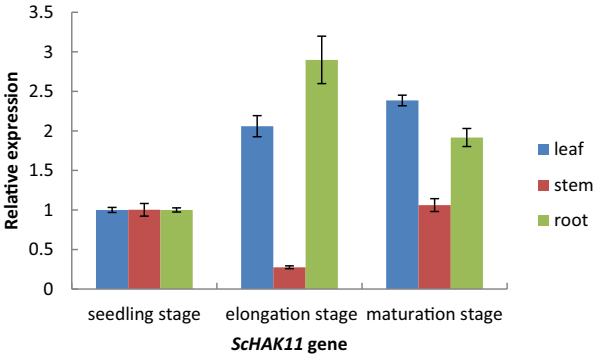
medium containing 5–50 mM KCl. All yeast cells transformed with *ScHAK* genes grew better than the empty vector-transformed cells, and yeast cells transformed with *ScHAK10* grew better than other Transferred *ScHAK* genes yeast strain (Fig. 5).The studies indicate that *ScHAK* genes improves the potassium absorption activity of yeasts



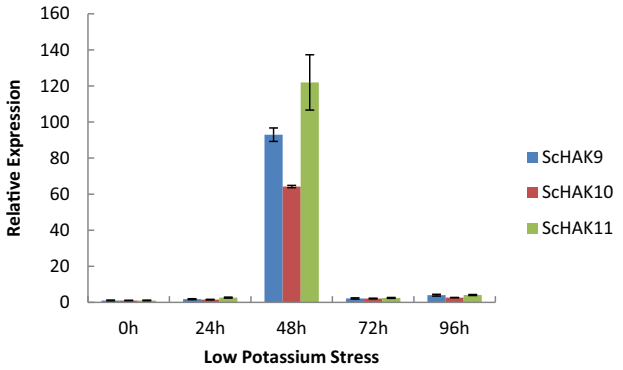
a. Gene expression levels of *ScHAK9* gene in different tissues in growth stages.



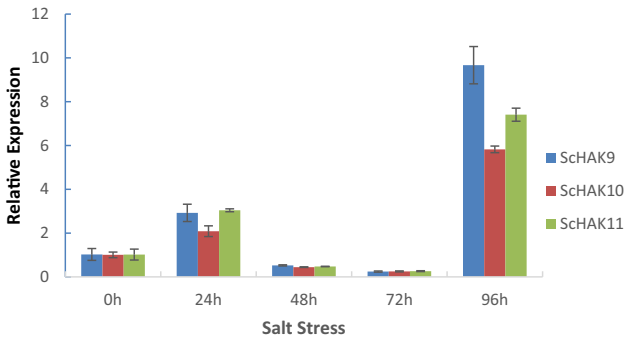
b. Gene expression levels of *ScHAK10* gene in different tissues in growth stages.



c. Gene expression levels of *ScHAK11* gene in different tissues in growth stages.



d. Gene expression levels of *ScHAK* genes under low-potassium stress.



e. Gene expression levels of *ScHAK* genes under salt stress.

Fig. 4 Expression Patterns of *ScHAK* genes in Sugarcane

mutant under low K^+ concentrations, it was speculated that *ScHAK* has the function of transporting K^+.

Discussion

As a C4 plant, sugarcane has a long growth cycle and requires a lot of fertilizers, especially potassium (Huang et al. 2013). *HAK* genes exist in many crops and involved in the absorption and transport of potassium ions in plants. But the *HAK* genes has not been cloned and characterized in sugarcane. Researches discovered that *HAK* gene family contains multiple transmembrane regions with a long cytoplasmic ring structure between second and third transmembrane regions (Sato et al. 2014). In our study, *ScHAK9*, *ScHAK10* and *ScHAK11* gene was first isolated from sugarcane. By comparing the deduced protein sequence of ScHAK proteins in the Protter software, we determined that ScHAK proteins were highly similar and all contained a long cytoplasmic ring structure, but the transmembrane structure model of proteins were different. Sequence phylogenetic analysis results suggested that

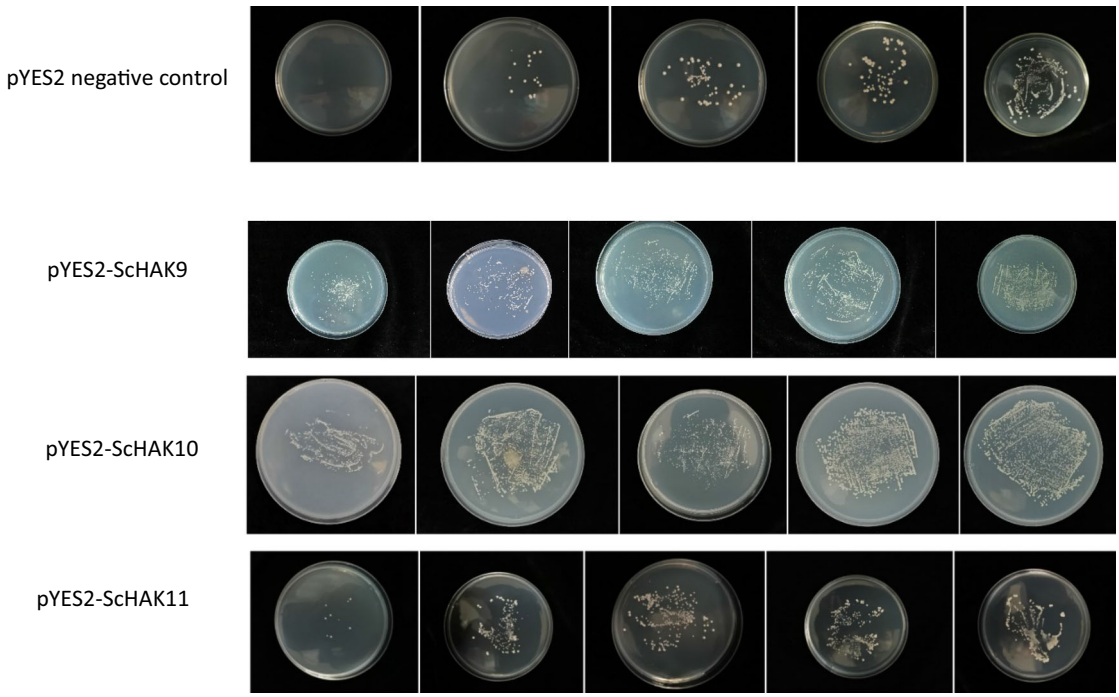


Fig. 5 Effect of *ScHAK* on the potassium absorption-deficient yeast mutant R5421

ScHAK9 and *ScHAK11* was highly homologous to *Zea mays* potassium transporter, and *ScHAK10* was highly homologous to sorghum bicolor *HAK10*, this result indicates that *ScHAK* genes belongs to the *HAK* superfamily, and this result also may explain why *ScHAK* has similar functions to *HAK*, which plays an important role in potassium transport.

Previous research found that 25 HAK genes of maize were located in the plasma membrane, and two HAK genes of maize were located in the vesicle membrane and endoplasmic reticulum. In this study, the localization of ScHAK9 and ScHAK11 protein was obeserved in epidermal cells of tobacco. A transient expression of the ScHAK9 and ScHAK11 protein in tobacco was located in the plasma membrane, what is more, the green fluorescence signal of gene sufficiently proofed that the protein of ScHAK9 aslo existed in nucleus, subcellular localization indicated that ScHAK9 and ScHAK11 protein was transmembrane protein and have similar transmembrane transporting (Song et al. 2014). The localization of ScHAK10 protein was not observed in epidermal cells of tobacco, it was speculated that the protein was expressed in Golgi apparatus, and the transcriptional regulation mechanism is different from that of other crop homologous proteins.

In many plants, the expression patterns of *HAK* genes are obviously different in various tissues. In *Arabidopsis*, the hight expression level of *AtHAK5* gene was detected in roots and its expression level in other tissues was low (Gierth et al. 2005). In soybean, the expression level of *GmHAK30* gene in roots was 500 times higher than that in other tissues, but the expression levels of *GmHAK10* and *GmHAK25* were the highest in pods and seeds (Chao et al. 2017). However, there is no evidence to show the expression pattern of HAK genes in plant growth cycle. In this study, the expression pattern of ScHAK genes in various stages and tissues was analyzed by qRT-PCR, expression patterns of *ScHAK* genes in sugarcane were different from those of other crop *HAK* genes, *ScHAK* genes expression were related to growth period, *ScHAK9, ScHAK10* and *ScHAK11* gene was expressed throughout the plants, but gene expression levels were different in various growth stages, the relative expression of *ScHAK9* in root was higher than that in leaf and stems at the elongating stage, the relative expression of *ScHAK10* in leaf and stems were higher than that in root at the maturity stage, and the relative expression of *ScHAK11* in leaf and root was higher than that in stems at the elongating and maturity stage, but the gene expression is lower than the other two genes. These results indicated that based on the demand characteristics of sugarcane growth, the members of sugarcane potassium transporter family play different roles, *ScHAK* genes might be involved in regulatory and developmental processes of K^+ absorption and distribution in sugarcane, and the hight expression of genes in different tissues is conducive to promote the development of sugarcane (Feng et al. 2020).

The expression levels of *HAK* genes were affected by abiotic stresses. *AlHAK1* was up-regulated remarkably in

the roots of *Aeluropus littoralis* under the low-potassium stress and the gene expression in leaves lagged behind. It was presumed that the plant injury was reduced by the redistribution of *AlHAK1* gene (Liu et al. 2015). Su et al. (2002) reported that the expression level of *McHAK1* gene in *Mesembryanthemum crystallinum* was up-regulated to maintain stable K^+/Na^+ ratio by enhancing K^+ uptake efficiency under the high-salt stress. In Oryza, OsHAK10 was expressed in root and other tissue, but its expression was not induced by low potassium stress and salt stress (Banuelos et al. 2002). In this study, the expression level of *ScHAK* genes was increased after 24 h under the salt stress and the relative expression of *ScHAK* genes reached the maximum value after 48 h under the low-potassium conditions. The results indicated that ScHAK9, ScHAK10 and ScHAK11 displayed upregulated expression, suggesting that they may play important roles in maintaining normal growth and mediating potassium acquisition under K^+ deficiency. In addition, the expression level of *ScHAK* genes could improve the potassium uptake efficiency and tolerance of sugarcane under salt stress.

Most of the plant HAK proteins belongs to high-affinity potassium ion transporter which play an important role in potassium acquisition under potassium deficiency conditions (Santa-María et al. 1997; Banuelos et al. 2002; Gierth et al. 2005; Nieves-Cordones et al. 2010; Boscari et al. 2009; Yang et al. 2014). For example, the growth of *OsHAK1* invert was detected in the mutant strain of yeasts under different potassium concentration gradients and the functions of three rice potassium transporters in yeasts were identified. (Banuelos et al. 2002) showed that *OsHAK1* had the ability to transport K^+ in *Saccharomyces cerevisiae*. After knocking out *OsHAK1* in rice, Chen et al. (2015) found that the total K^+ uptake was significantly decreased under low-K^+ conditions. In this study, we found that the expression level of *ScHAK* proteins could rescue the growth defect of yeast cells (R5421) in both low-K^+ (0.05 and 0.1 mM) media. In the media with high concentration of K^+ (20 mM, 50 mM), yeast cells transformed with *ScHAK* or empty vector yeast strain showed that no obvious growth difference (Fig. 5), and in the medium with low-K^+ concentration (0.05 mM), transgenic yeast cells grew better than empty carrier yeasts. The results showed that *ScHAK* genes could improve the K^+ uptake activity of K^+-deficient yeast mutant. It is suggested that *ScHAK* genes might have the similar function in sugarcane.

Conclusions

In the present study, three high-affinity K^+ transporter genes cDNA, *ScHAK9, ScHAK10* and *ScHAK11* from sugarcane have been identified. The transmembrane structure of ScHAK proteins can be divided into two models. The qRT-PCR study showed that *ScHAK* genes induced by low-potassium and salt stress, and the expression level of genes in sugarcane tissues were different in growth process. Subcellular localizations and K^+ uptake-deficient yeast revealed that ScHAK proteins may mediate K^+ absorption by the plasma membrane and other organelle, they might be play crucial role in the maintenance of the K^+ homeostasis in sugarcane under low-potassium situations.

Authors contribution HL, CH, and YW designed the research. HL, HZ, HC, SJ, and LX performed the experiments and data analysis. HL wrote the manuscript. ZD, KW and YW contributed with valuable discussions. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication Not applicable.

Ethics approval Not applicable.

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