

Molecular cloning and functional analysis of *SchAK10* gene promoter from sugarcane (*Saccharum officinarum* L.)

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Abstract

Transcriptional regulation of the high-affinity K⁺ (*HAK*) transporter gene is an important mechanism of plant response to low potassium stress. Cloning and analysis of the promoter of potassium transporter gene is not only helpful to its expression pattern and regulation mechanism but also important to improve the potassium absorption efficiency in sugarcane. The potassium transporter gene *ScHAK10* is highly expressed and induced by low potassium stress in sugarcane but the functions of its promoter is still unclear. In the present article, the 1918 bp promoter region of the *ScHAK10* gene (*pScHAK10*) was cloned by genomic walking technique. Computational analysis affirmed the existence of abiotic stress-responsive *cis* and core *cis*-elements, such as TATA box, CAAT box, phytohormone responsive, stress response and light response motifs. GUS histochemical staining of transgenic *Arabidopsis thaliana* seedlings showed that the leaf, corolla, and root tip were deeply stained, and paraffin sections of root tip tissue showed GUS staining in ductal tissue of *A. thaliana* shallowly stained. The 5'-terminal deletion of the promoter was cloned, and the lengths of 1918 (full), 1623 (Q1), 1332 (Q2), 957 (Q3), 576 (Q4), and 357 bp (Q5) were cloned into the GUS reporter vector for *A. thaliana* transient transformation. The transgenic plants generated through a single event exhibited a promising expression of the GUS reporter protein, which was treated with salt, low potassium, IAA and cold stress conditions. The results showed that the promoter activity correlates with the promoter fragment's length, and the long promoter fragment exhibits higher training. The Q5 was the least active and could not drive GUS expression. Under abiotic stress, the expression of GUS enzyme activity varies among different promoter fragments. Under low potassium and high salt stress, Q3 and Q4 showed the highest promoter activity. The Q1 and Q4 led the highest promoter activity during IAA and cold stress. These findings help to understand the molecular mechanism of *ScHAK10* expression regulation and could be an excellent tool for future crop improvement.

Introduction

Sugarcane is a high-efficiency C₄ crop with higher nutrient requirements. Therefore, the key to achieving high sugarcane yield maintaining sufficient nutrient supply. An adequate nutrient supply is crucial for high sugarcane yield [1-3]. The *HAK* potassium transporter gene family is an important K⁺ transporter that plays a major role in the absorption and transport of potassium in sugarcane. The identification and functional analysis of the promoter region of the *HAK* potassium transporter gene family has emerged as a novel approach to enhancing potassium uptake efficiency and developing potassium-efficient sugarcane varieties. Currently, there are few studies on the promoter of *HAK* potassium transporter. It was found that the *AP2/ERF* (ethylene response factor) transcription factor *RAP2.11* binds to the GCC-box region of the *AtHAK5* promoter under growing conditions. Under low potassium conditions, *ARF2* phosphorylated and reduced its binding ability to the promoter region of the *AtHAK5* gene, resulting in the high expression of the *AtHAK5* [4]. In addition, it has also been found that some transcription factors such as *DDF2* (dwarf and delayed flowering 2), *JLO* (jagged lateral organs), and *bHLH121* (basic helix-loop-helix 121) may bind to the promoter of *HAK5* gene [5-7], which can respond and activate the

expression of high-affinity potassium transporter (*HAK5*). Overexpression of these transcription factors can enhance the growth of *Arabidopsis thaliana* roots under low potassium stress[7]. These studies demonstrated that the enhancing expression regulation of potassium transporter genes can increase the growth tolerance of plants during low potassium conditions [3].

Promoters are crucial for the regulation of gene expression, types and number of *cis*-acting elements affect the expression pattern and intensity of genes[8]. Therefore, it is necessary to isolate and assess its function as the promoter of genes and clarify the molecular mechanism to improving the efficiency of gene expression in sugarcane. Currently, research on the expression regulation of *HAK* genes mainly focuses on *Arabidopsis* and rice, and there have not yet to be any report on sugarcane. In our previous study, the CD sequence of the *SchHAK10* was cloned, and preliminary studies on its sequence characteristics and expression properties were performed [9]. The transcript levels of *SchHAK10* were highly expressed in leaf and stem at the maturation stage. Moreover, the expression of *SchHAK10* induced by salinity and low potassium conditions. Transgenic *A. thaliana* that overexpression of *SchHAK10* under *CaMV35S* promoter were more resistant to cold and drought conditions. Studies on the mechanism of regulating the expression of this gene have performed. This study aimed to identify the structure and function of the *SchHAK10* promoter to understand the regulatory mechanism of the *HAK* gene and provide a theoretical basis for manipulating the expression and function of potassium transport-related genes in sugarcane.

Materials And Methods

Plant materials and growth conditions

The sugarcane variety GuiTang 42 (GT 42) was used for cloning the *SchHAK10* promoter. This variety was produced from the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China. Healthy sugarcane budsetts were planted in the greenhouse of Sugarcane Research Institute (average temperature, 28°C, soil water content 25±5%) during plant growth establishment. Leaf samples were collected at seedling stage, frozen in liquid nitrogen and stored at -80°C for DNA isolation. *Arabidopsis* seeds were disinfected with 75% ethanol for 1 min, then disinfected with sterile water once again, and then disinfected with 20% sodium hypochlorite disinfectant for 10 min. The seeds were placed on a clean bench to dry after thoroughly cleaning with disinfectant water six times. The disinfected seeds were vernalized for three days (4 °C) and then grown on MS medium (18-22°C). The photoperiod in the artificial climatic chamber was 16 h light and 8 h of darkness. *Arabidopsis* seedlings were transplanted into pots at the four-leaf stage and grown in the atmospheric environmental conditions. After four weeks, plants were used for genetic transformation for *SchHAK10* promoter analysis.

DNA extraction and *SchHAK10* promoter

Genomic DNA was extracted from sugarcane leaves by DNA extraction kit (TIANGEN Biotech, Beijing, China). Following the instructions of the TaKaRa Genome Walking Kit (Takara, Biotech, Tokyo, Japan). Three pairs of downstream primers were designed for the 5'end of the *SchHAK10* gene CDs sequence

(NCBI No: MG564721), and multiplex PCR amplification was based on the sugarcane genomic DNA as a template. Based on the sequence of *SchAK10*, Primer5.0 designed downstream primers, and the primer sequence is:

pSchAK10-SP1:5'-TGAGCGTGAGCGTCCAGAAGACGAA-3';

pSchAK10-SP2:5'-ACTCTGGTACGCCAGGCTCAGCGTCAT-3'

pSchAK10-SP3:5'-GACATGGGCATTCCGAGATTCAAG-3'.

Perform a multiplex PCR reaction using P1, P2, P3, and P4 primers from the TaKaRa Genome Walking Kit as forward primers and *pSchAK10*-SP1, *pSchAK10*-SP2, and *pSchAK10*-SP3 as reverse primers. Analyze by using 1% agarose gel electrophoresis. Extract the third phase PCR product from the gel and send it to TaKaRa for sequencing.

Analysis of the *SchAK10* promoter sequences

The promoter sequence (1918 bp) of the *SchAK10* gene was analyzed for cis-acting elements and their position using PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) and PlantCE (<https://www.dna.affrc.go.jp/PLACE/?action=newplace>)

Vector construction and genetic transformation

Based on the analysis of the *cis*-acting elements of the promoter, different lengths of forward and reverse sequences were designed for amplification (Table 1). Gene-specific primers were used to amplify the promoter sequence of *pSchAK10*, which consisted of 1918 bp (Full), 1623 bp (Q1), 1332 bp (Q2), 957 bp (Q3), 576 bp (Q4), and 357 bp (Q5) fragments. The PCR procedure was as follows: 95°C for 5 min; 35 Cycle of 95°C for 30 sec, 55°C for 30 sec, and 72 °C for 30 sec; 72 °C for 5 min. Follow the instructions in the Seamless Clone kit (Tsingke Biotechnology, Beijing, China) and transform into competent cells of *Escherichia coli* DH5α. Follow the instructions of the seamless cloning kit to ligate and transform into *E. coli* DH5α capable cells. Observed single clones for sequencing identification. Digest the pEASY-T plasmid containing the full, Q1, Q2, Q3, Q4, and Q5 promoter regions with *HindIII*/ *SmaI*, and then ligate it to the pBI121 expression vector containing the β-glucuronidase (GUS) gene, replacing the 35S promoter upstream of GUS. Construct the recombinant vector *pSchAK10*-pBI121.

Table 1: Forward and reverse primers for different lengths of pSchAK10 promoter

Primer name	Primer sequence (5'-3')
HAK10P-F0	gaccatgattacgcccaagcttGGACTCTATAGGGCGATTGAG
HAK10P-F1	gaccatgattacgcccaagcttCGGAAATTTACACATTCTTGTTTT
HAK10P-F2	gaccatgattacgcccaagcttAAGGGTATTTTTTTTATTAAAATCT
HAK10P-F3	gaccatgattacgcccaagcttCAGATTAGTGTCCATGCTCGGT
HAK10P-F4	gaccatgattacgcccaagcttTTAATCTATTTTTTCTTTGGATCCT
HAK10P-F5	gaccatgattacgcccaagcttATATTCTCTCTCCGCTCTGCAC
HAK10P-R	taagggactgaccacccgggCGCTGCCTCCACTGCCCCGGT

Identification of transgenic *Arabidopsis thaliana*

The recombinant plasmid with the expression vector was introduced into *Agrobacterium* strain *EHA104* by cryogenic method and transformed into *Arabidopsis* plants using the floral dip transformation approach [10]. Transgenic *Arabidopsis* was identified through baster resistance selection and PCR detection. The primer sequence for PCR detection is: F:

5'- gaccatgattacgcccaagcttATATTCTCTCTCCGCTCTGCAC-3'; R: 5'-CTGATCAATTCCACAGTTTTTC-3'

The T_0 transgenic seeds were planted on MS medium containing hygromycin resistance for screening. Screening were designated as T_1 transgenic plants. After the T_1 plants had grown up to two leaves, transplanted into vermiculite and placed in a growth chamber (temperature 22°C and 16 h photoperiod per day). Leaf samples was taken from the T_1 transgenic *Arabidopsis* plants during the seedling stage for PCR analysis, and the plants with positive PCR results were harvested for T_1 seeds at maturity.

Transgenic *Arabidopsis* seedlings grown under abiotic stress

The two-week-old transgenic *A. thaliana* seedlings were grown on MS medium and transferred to adverse environmental condition of MS medium supplemented with 50 μ M KCl, 200 mM NaCl, low temperature (4°C), and 150 μ M IAA. The seedlings were exposed to cold chamber for 3 h for low-temperature (4°C) treatment. After stress condition, qualitative and quantitative GUS analyses were performed.

Histochemical GUS staining and fluorometric assay

Histochemical GUS staining was performed according to Chao et al [10]. Collect plant organs, such as stems and leaves from *A. thaliana*. Added GUS staining solution, incubated at 37°C for 24 hr, and then

used 70% ethanol to decolorize until the background color completely disappeared. Finally, we observed and photographed the staining results under the dissecting microscope (AXIO Zoom.V16, Zeiss).

Determination of GUS enzyme activity in transgenic plants

Following the directions of the GUS enzyme activity kit manual, select transgenic *A. thaliana*, frozen, ground and added GUS extraction solution (1 ml). The mixture was centrifuged (15,000 rpm), and the supernatant was used for the measurement of protein content. Combined 40 µL of protein extract with 160 µL of protein reaction solution (containing P-nitrophenyl-β-D-glucuronide, PNPG) and incubated the mixture at 37°C in the dark. Once incubation was complete, added reaction stop solution (200 µL). The GUS fluorometric assay was measured with the emission at 405 nm using an enzyme-linked immunosorbent assay. GUS activity was expressed as 4-methylumbelliferone (4-MU) produced per min per g of protein.

Paraffin section of transgenic *Arabidopsis thaliana* roots

Place the stained tissue in a container filled with fixative. A vacuum pump removes air from the container, allowing the fixative to saturate the material fully. Add alcohol (70%) for preservation. Gradually dehydrate the material using ethanol solutions of increasing concentrations, such as 85, 95, and 100%, followed by another round of 100% ethanol. The dehydrated material should be submerged in a mixture of xylene and ethanol to achieve clarity. The xylene to 100% ethanol ratio is either 1:2, 1:1, or 2:1. Shift material in pure xylene. Then, load the transparent material into wax cups for impregnation and encapsulation. Cut sections at a thickness of 50µm for microscopic observation.

Results

Clone of *pSchAK10* promoter

The genomic DNA of sugarcane variety GuiTang 42 (GT 42) was used as a template in multiplex PCR reactions, with AP1, AP2, AP3, and AP4 from TaKaRa Genome Walking Kit as forward primers and *pSchAK10*-SP1, *pSchAK10*-SP2, and *pSchAK10*-SP3 as reverse primers. Sequencing of the PCR products revealed a 2.1 kb fragment amplified by the AP3 forward primer. This fragment contains approximately 1918bp of the promoter sequence upstream of the *SchAK10* gene in sugarcane, as assessed by the sequence alignment analysis.

Bioinformatics analysis of the *pSchAK10* promoter

The promoter region of the *SchAK10* contains a diverse array of regulatory elements. It includes 28 TATA-boxes and 41 CAAT-boxes, crucial for gene transcription initiation. In addition to these core elements, the promoter also harbors four plant hormone-responsive elements, i.e., ABRE, CGTCA-motif, TGA-element, and TGACG-motif. Identified six light-responsive factors (Box 4, G-box, GA-motif, GT1-motif, I-box, TCT-motif), five stress-responsive elements (LTR, TC-rich repeats, WUN-motif, GT1GMSCAM4), and one

transcription factor binding site (CCAAT-box). These results suggested that the expression of *ScHAK10* influenced by different environmental factors such as light, phytohormones, etc.

Table 2: Analysis of *cis*-acting elements in the *pScHAK10*

Element type	Name	Copy number	Motif sequence	Function
Basal element	TATA-box	28	TATA/ATATAT/TTTTA	Core promoter element
	CAAT-box	43	CAATT/CAAT/CCAAT	Common cis-acting elements in promoter and enhancer regions
Phytohormone	ABRE	2	ACGTG AACCCGG	cis-acting regulatory element involved in the abscisic acid responsiveness
	CGTCA-motif	2	CGTCA	cis-acting regulatory element involved in the Me JA-responsiveness
	TGA-element	1	AACGAC	Auxin-responsive element
	TGACG-motif	2	TGACG	cis-acting regulatory element involved in the Me JA-responsiveness
Light	Box 4	3	ATTAAT	Part of a light-responsive module
	G-box	1	CACGTC	cis-acting regulatory element involved in light responsiveness
	GA-motif	1	ATAGATAA	Part of a light-responsive element
	GT1-motif	1	GGTTAA	Light responsive element
	I-box	1	GGATAAGGTG	Part of a light-responsive element
	TCT-motif	1	TCTTAC	Part of a light-responsive element
Stress	LTR	1	CCGAAA	cis-acting element involved in low-temperature stress responsiveness
	TC-rich repeats	1	ATTCTCTAAC	cis-acting element involved in defense and stress responsiveness
	WUN-motif	3	AAATTTTCCT AAATTACT	Mechanical injury response element
	GT1GMSCAM4	7	GAAAAA	salt-induced
Other	CCAAT-box	2	CAACGG	MYBHv1 transcription factor binding site

Identification of transgenic *Arabidopsis thaliana*

The transgenic *A. thaliana* with the *pSchAK10* promoter was screened on a Kanamycin-resistant MS medium. Subsequently, PCR detection was performed on the seedlings of the transgenic *A. thaliana*. The result shows that the target band was amplified from the seedlings of the transgenic *A. thaliana* (Fig. 3), while no target band was amplified from the *A. thaliana*. It indicates that the detected transgenic *A. thaliana* were positive plants.

GUS histochemical staining analysis of transgenic *Arabidopsis thaliana*

GUS histochemical staining was performed on transgenic *A. thaliana*. The results showed that GUS driven by *pSchAK10* was mainly expressed in the leaves, petals, and root tips (Fig. 4). The staining intensity of the stem leaves was higher than that of the rosette leaves. The GUS gene was primarily localized in the veins of the rosette leaves, while it was expressed in the veins and stomata of the stem leaves. The GUS gene was predominantly expressed in the corolla of the transgenic *A. thaliana* flowering buds, with no expression detected in the receptacle or anthers. GUS activity was mainly observed in the root tip region and root hair zone. Paraffin sectioning of the root tips of the transgenic *A. thaliana* revealed that the promoter predominantly drove GUS expression in the vessel tissue of the root tips (Fig. 5).

The activity of GUS in transgenic *Arabidopsis thaliana* under abiotic stress

The activity of each promoter fragment was analyzed in *A. thaliana*. During control conditions, GUS expression was observed in transgenic plants containing the Full (1918bp), Q1 (1623bp), Q2 (1332 bp), Q3 (957bp), Q4 (576bp) and Q5 (357bp) fragments. It was observed that Full, Q1, Q2, Q3, and Q4 could be stained, while Q5 could not be impaired. The GUS activity analysis revealed that the GUS expression level of Q1 was the highest, followed by Full and Q2, while Q5 could not drive GUS expression. This result indicated that the activity of each promoter segment driving expression in *A. thaliana* was Q1>Full=Q2>Q3>Q4>Q5. The promoter fragment Q5 had lost its promoter activity (Fig. 6), possibly related to the lack of enhancer elements.

After transferring the active promoter fragments into *Arabidopsis*, their activities were detected under low-potassium, salinity, cold (4°C), and IAA treatments. The results showed that the GUS activity of Q3 and Q4 promoter fragments in transgenic *A. thaliana* was the highest, while the activities of Full, Q1, and Q2 decreased significantly. Under auxin (IAA) stress, the GUS activity of the Q1 promoter fragment in transgenic *A. thaliana* was the highest. Under low temperature, decreased significantly in the GUS activity of Full, Q1, and Q2 in transgenic *A. thaliana* (Fig. 6 and 7).

Discussion

The promoter consists of two major factors, such as the core region, which contains the core promoter element (TATA-box), and the regulatory area, which includes responsive cis-acting and enhancer elements. The type and quantity of regulatory elements directly affect the expression pattern and intensity of genes [3,8]. The *GhHAK5* gene expressed under low potassium conditions in cotton plants. The promoter sequence of this gene, *pGhHAK5*, contains various cis-acting elements associated with functions such as light induction, plant hormones, and stress responses. These elements suggest that the *GhHAK5* gene can be influenced by multiple external factors[11]. The research findings indicated that the *pSchHAK10* promoter of sugarcane contains several cis-elements, including light response, plant hormone, and stress response cis-elements, in addition to the basic TATA box and CAAT box. Plant hormone and light response cis-elements are major plant growth and development regulators. Phytohormone and light signals can induce promoter expression and regulate genes[12]. The *pSchHAK10* promoter contains not only light response and hormone cis-elements but also various stress response elements, such as LTR (low temperature), ARE (anaerobic), and GT1 (salinity). However, the promoter may be induced by stress.

Based on their expression patterns and promoters can be classified into three types, i.e., constitutive promoters, tissue-specific promoters, and inducible promoters. Constitutive promoters are expressed in all parts of the plant and are unaffected by atmospheric environmental variables. Tissue-specific promoters express only in specific tissues, while inducible promoters typically express high under specific conditions [2,13]. However, in some cases, a specific type of promoter can exhibit characteristics of other promoters. For example, the potassium transporter *HAK5* is mainly expressed in the root systems. The promoters of the *AtHAK5* gene in Arabidopsis and *OsHAK5* gene in rice drive the GUS gene to express in the root system, which tissue specificity but also can be induced by low potassium conditions to stimulate gene expression [14-16]. Most *HAK* genes in plants are expressed mainly in the root system with tissue specificity, while a few members are expressed in multiple plant tissues [17]. However, recent studies have found that the *EgHAK5* promoter in *Eucalyptus* drives GUS expression not only in the Arabidopsis root system but also in the leaf vein and embryo axis vessel tissue [16]. Present study found that the GUS driven by *pSchHAK10* was expressed in the root system and leaves of *A. thaliana*. Staining and sectioning of the root system revealed that GUS was mainly expressed in the vascular tissue of the root tip. The experimental results indicated that the *pSchHAK10* promoter is active and can promote potassium absorption efficiency in the plant root system.

The core functional region of a promoter is typically the proximal region close to the transcription initiation site of the functional gene. Still, cis-elements located at the distal end of the promoter sequence can also affect the activation activity of the promoter [18]. The CAAT-box, position, and orientation not significantly impact their functions. By adding enhancer elements, the activation activity of the promoter can be greatly increased. Four enhancers were connected in series to increase the transcription level of the *OsNAS3* gene. The resulting transcript was 30-60 times higher than the wild-type [19]. In this study, deletion analysis showed that the sequence length of the *pSchHAK10* promoter decreased, the promoter activity gradually reduced. As shown in Figure 6, transgenic *A. thaliana* of deletion mutants (Full, Q1, Q2, Q3) could be stained, while Q5 could not be impaired. The Q1 promoter fragment drove the highest expression of GUS, while the shortest Q5 promoter fragment had the lowest activity and could not drive

GUS staining. The action of Q1 and Q2 promoters were higher than those of other fragments, possibly due to more CAAT-box enhancer elements in the promoters.

In *A. thaliana*, the expression of *AtHAK5* is not only affected by abiotic stresses such as low potassium, low calcium, and salt stress [20], but also can be induced by low nitrogen and low phosphorus[2,21]. The regulation of potassium transporter gene expression is influenced by two transcription factors, such as ARF2 and RAP2.11, and can involve multiple other transcription factors[7]. The GCC-box site is an important motif in the promoter sequence of *AtHAK5* in *A. thaliana* that binds to the transcription factor RAP2.11[22]. The binding of transcription factor RAP2.11 to the ERE domain and GCC-box in the *AtHAK5* promoter is essential for activating *AtHAK5* gene expression under low potassium conditions [22]. In addition, the *GhHAK5* promoter binding site (TGTCNN) involved in transcriptional regulation under low potassium conditions is also an important motif for ARF transcription factor binding [11]. The *pSchAK10* promoter analyzed by bioinformatics contains multiple potential cis-acting elements related to abiotic stresses, including low potassium (ERE, ATTTTCATA), high salt (GT1, GGTAA), low temperature (LTR, CCGAAA), and phytohormones (TGA-element). These elements were also identified in the dehydration-responsive component binding protein (DREB1) promoter and reported to function in drought and cold-induced gene expression [7]. The *pSchAK10* promoter sequence of sugarcane does not contain GCC box sites and (TGTCNN) sites but contains an ERE structure, indicating that the expression of RAP2.11 may regulate in sugarcane. In this study, the activity of the full, Q1, and Q2 under low potassium stress significantly downregulated. At the same time, Q3 and Q4 significantly increased, indicating that there may be negative regulatory factors upstream of the promoter.

Under saline condition, high-affinity K⁺ transport proteins promote K⁺ uptake in plants, balance K⁺/Na⁺ ratio and improving salt tolerance capacity[23-25]. The promoter elements responsive to high salt stress that have been reported as GT1GMSCAM4 and GT1CONSENSUS. The GT1GMSCAM4 element has been reported to regulate salt stress expression [26]. The GAAAAA (GT-1 cis-acting element) sequence has been identified in the *SCAM-4* promoter as a core cis-acting element responsive to pathogens and salt induction[27-28]. This sequence significantly enhances salt tolerance in soybean and *Arabidopsis*. This study found that the *pSchAK10* promoter contains the GT1GMSCAM4 element (GAAAAA). The full-length *pSchAK10* promoter and Q2 contain GT-1 cis-acting elements. However, under saline stress (200 mM), the GUS enzyme activity of the full-length *pSchAK10* promoter and Q2 significantly reduced, indicating that the GAAAAA sequence in the full-length promoter and Q2 regions not enhance salt tolerance in transgenic *Arabidopsis* plants. Other unknown core sequences in the promoter may affect its salt response-ability. The significant loss in expression activity under salt stress suggests that this region may contain inhibitory cis-acting elements. Q1, Q3, and Q4 promoter fragments contain GT1GMSCAM4 elements; their GUS activities significantly increased under salt stress, indicating that the -961bp to -1918bp fragment may contain enhancer elements and other unknown cis-acting elements that respond to salt induction and enhance gene expression.

Auxin is a vital hormone for proper plant growth and development, associated in various processes, including fruit formation and abscission [29-31]. However, it is currently unknown whether *pSchAK10* can

be activated by auxin. In this study, a potential auxin response element TGA (-1381bp) was identified in the promoter of *pScHAK10* through cis-acting element prediction. It was found that auxin can significantly enhance the gene expression of Q1 and Q4 segments of the *pScHAK10* promoter, indicating that the auxin response element present in the promoter or auxin indirectly activates the promoter expression via various mechanisms[32].

The cis-acting LTR element is widely present in the promoters of genes related to the low-temperature stress response[33]. It plays a significant role in the plant's response to abiotic stresses. Cucumber *GR-RBP3* was induced by low-temperature stress, and an LTR element (-565bp) identified in its promoter region[34]. This study found that the activities of Full, Q1, and Q2 were significantly reduced, while the activity of the Q4 segment was increased under low temperatures. This result indicates that the low-temperature response element LTR in this promoter plays a negative regulatory role in the expression regulation of its gene during low temperatures. It can be functional elements related to cold stress between -576bp and -357bp in the promoter.

Conclusion

In this study, the upstream promoter of the *SchAK10* gene (*pSchAK10*) was cloned and functionally analyzed from the sugarcane variety GT42. The results of bioinformatics prediction analysis indicated that the *pSchAK10* promoter sequence includes TATA-box core elements, light-responsive elements, phytohormone-responsive elements, stress-response elements, and some specific protein-binding cis-acting elements. A fusion expression vector (*pSchAK10*-GUS) was constructed and successfully transformed into *A. thaliana*. By analyzing the expression and tissue-specific localization of GUS in transgenic *A. thaliana*, it was found that *pSchAK10* could drive the expression of the GUS gene in *A. thaliana* root, corolla, and leaf tissues, indicating that the cloned *pSchAK10* promoter is an active tissue-specific promoter. By transferring different promoter fragments into *A. thaliana* and subjecting them to stress, the analysis of GUS activity in transgenic *A. thaliana* suggests that the region between 296bp and 1561bp may be a key region for plant response to low potassium, salt, cold, and phytohormone stresses. The research findings provide theoretical basis for further elucidating the key regulatory elements of the *pSchAK10* promoter and its response mechanism to atmospheric environmental stresses.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests

Author's Contributions

Conceptualization, Luo HB and Huang CM; Methodology, Luo HB and Wei YW; Software, Xu L and Wu XJ; Supervision, Deng ZN. Wu KC. Wei YW; Data Curation, Luo HB and Ye LP; Writing Original Draft Preparation, Luo HB; Writing Review and Editing, Luo HB and Yi XP; Project Administration, Luo HB; Funding Acquisition, Luo HB and Huang CM.

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Figures

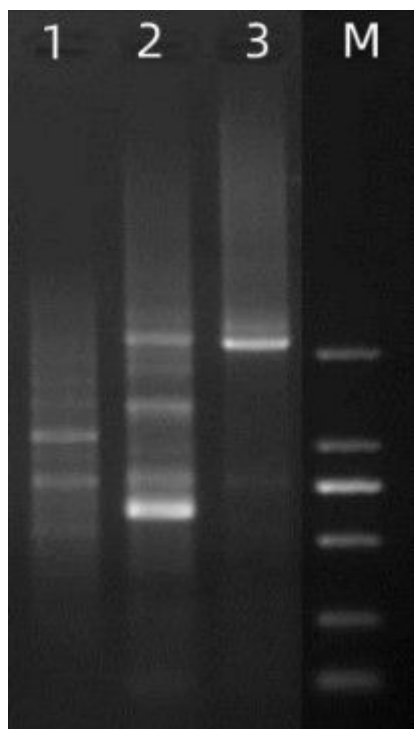


Figure 1

PCR products of *ScHAK10* promoter. 1-3 PCR products and M DNA marker (DL 2000)

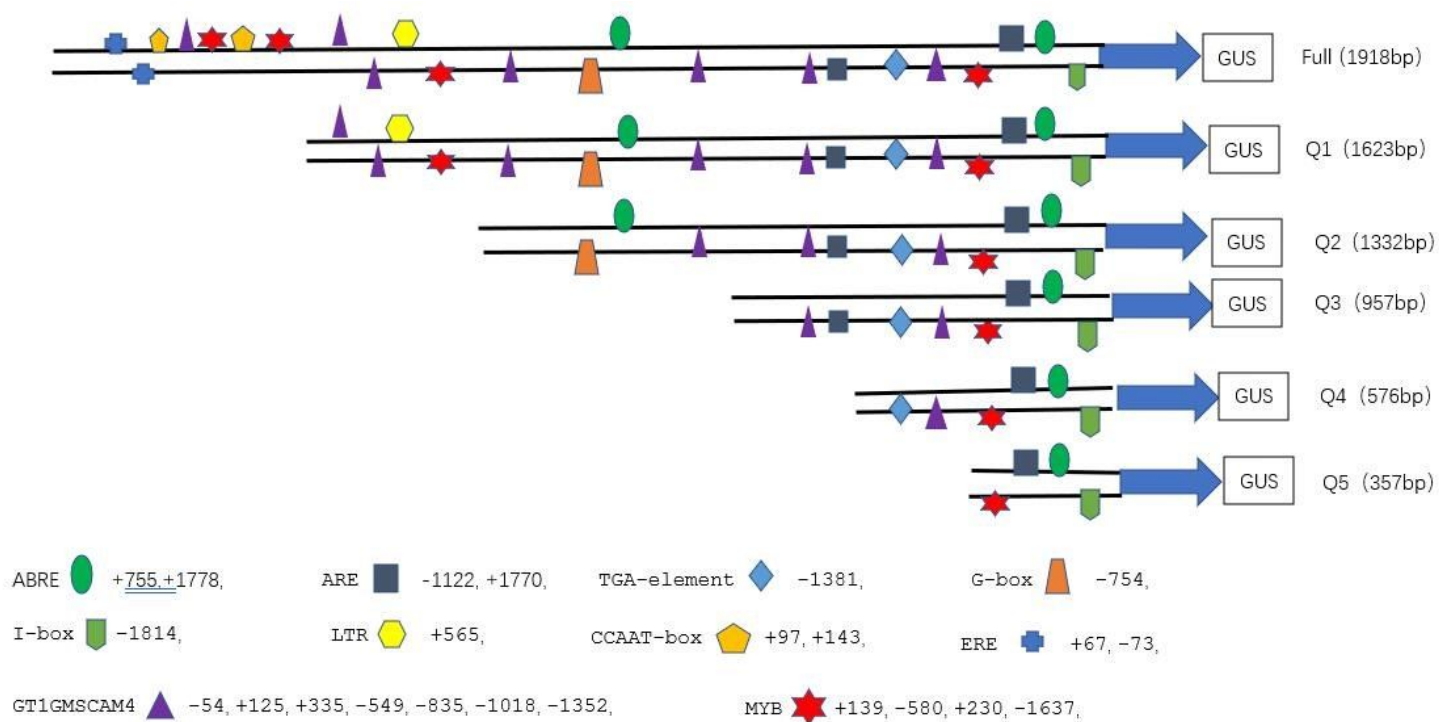


Figure 2

Schematic of the *pSchAK10* promoter deletion-GUS construct. Fragments of the promoter of varying sizes were inserted into the pBI121 vector containing the GUS reporter gene.

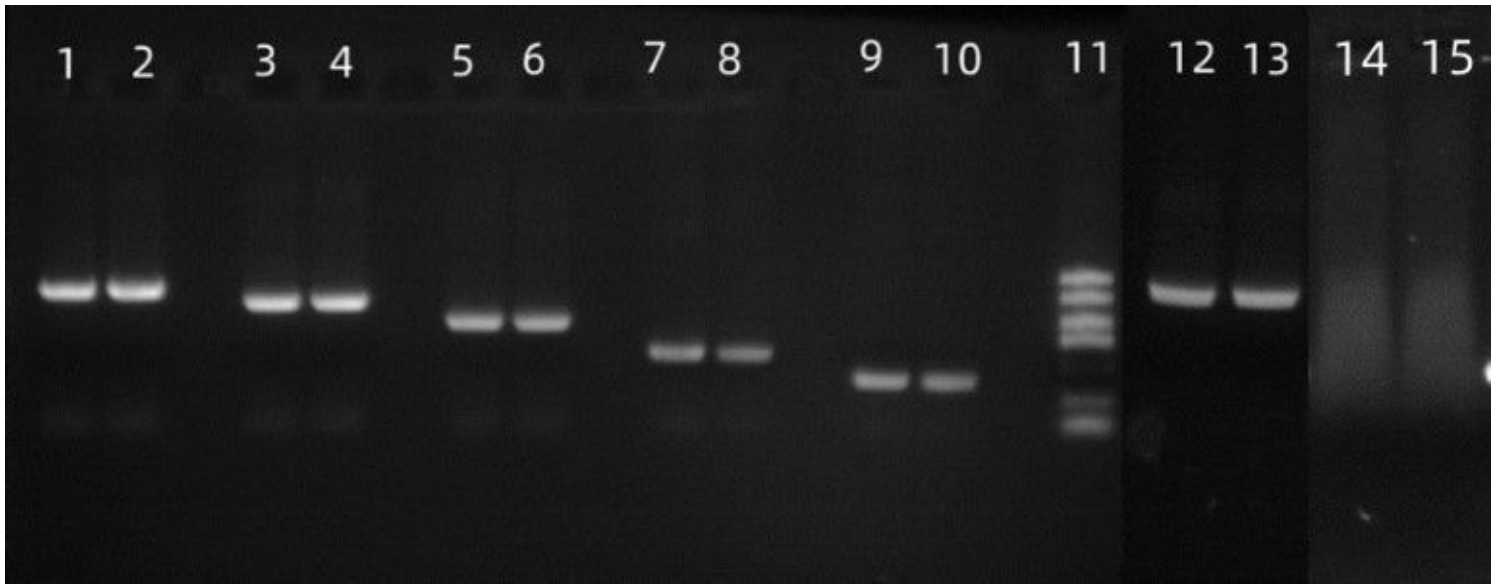


Figure 3

Enzyme restriction sites PCR products of promoter fragment. 1-2: Q1 fragments with transgenic *A. thaliana*, 3-4: Q2 fragments with transgenic *A. thaliana*, 5-6: Q3 fragments with transgenic *A. thaliana*, 7-8: Q4 fragments with transgenic *A. thaliana*, 9-10: Q5 fragments with transgenic *A. thaliana*, 11: DL 2000 DNA marker, 12-13: *pSchAK10* with transgenic *A. thaliana* and 14-15: Non-transgenic control

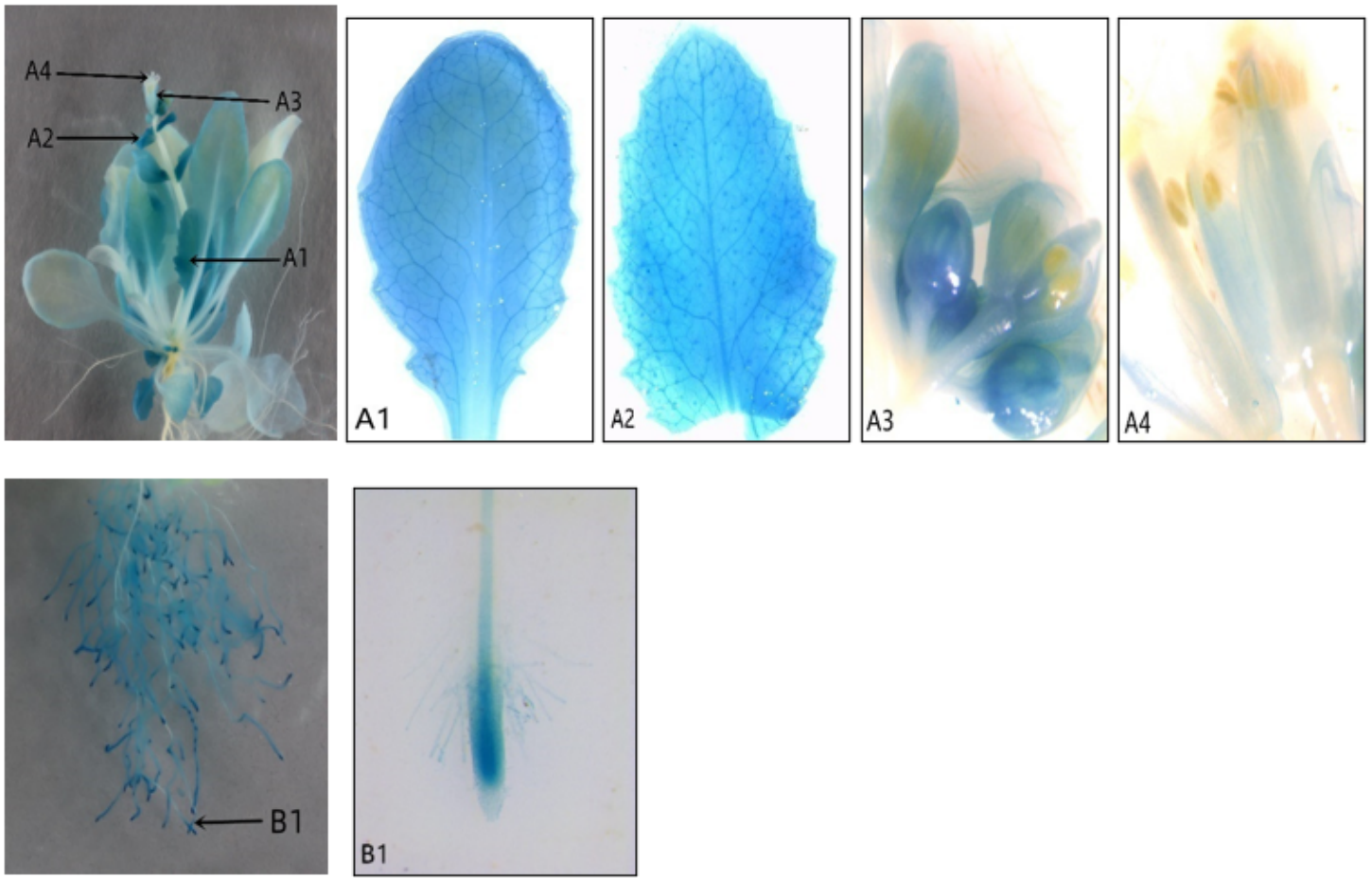


Figure 4

Histochemical staining of GUS expression in transgenic plants containing *pSchHAK10* promoters. A: *pSchHAK10* transgenic *A. thaliana*, A1: enlarged view of transgenic *A. thaliana* rosette leaf, A2: enlarged view of transgenic *A. thaliana* stem leaf, A3: enlarged view of transgenic *A. thaliana* corolla, A4: enlarged view of transgenic *A. thaliana* anther, B: *pSchHAK10* transgenic *A. thaliana* root and B1: enlarged view of transgenic *A. thaliana* root tips

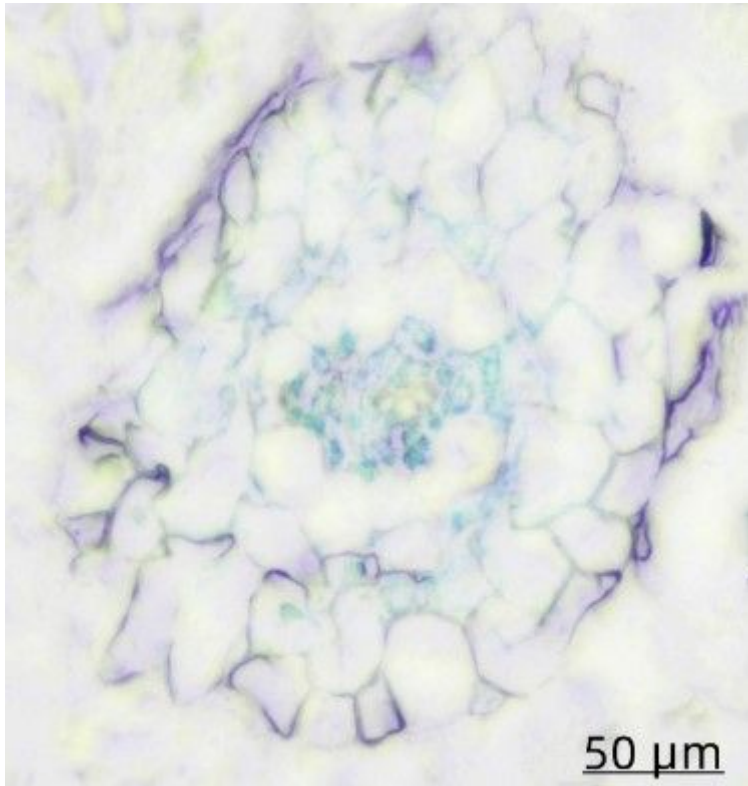


Figure 5

Paraffin sections of transgenic *Arabidopsis* root tips.

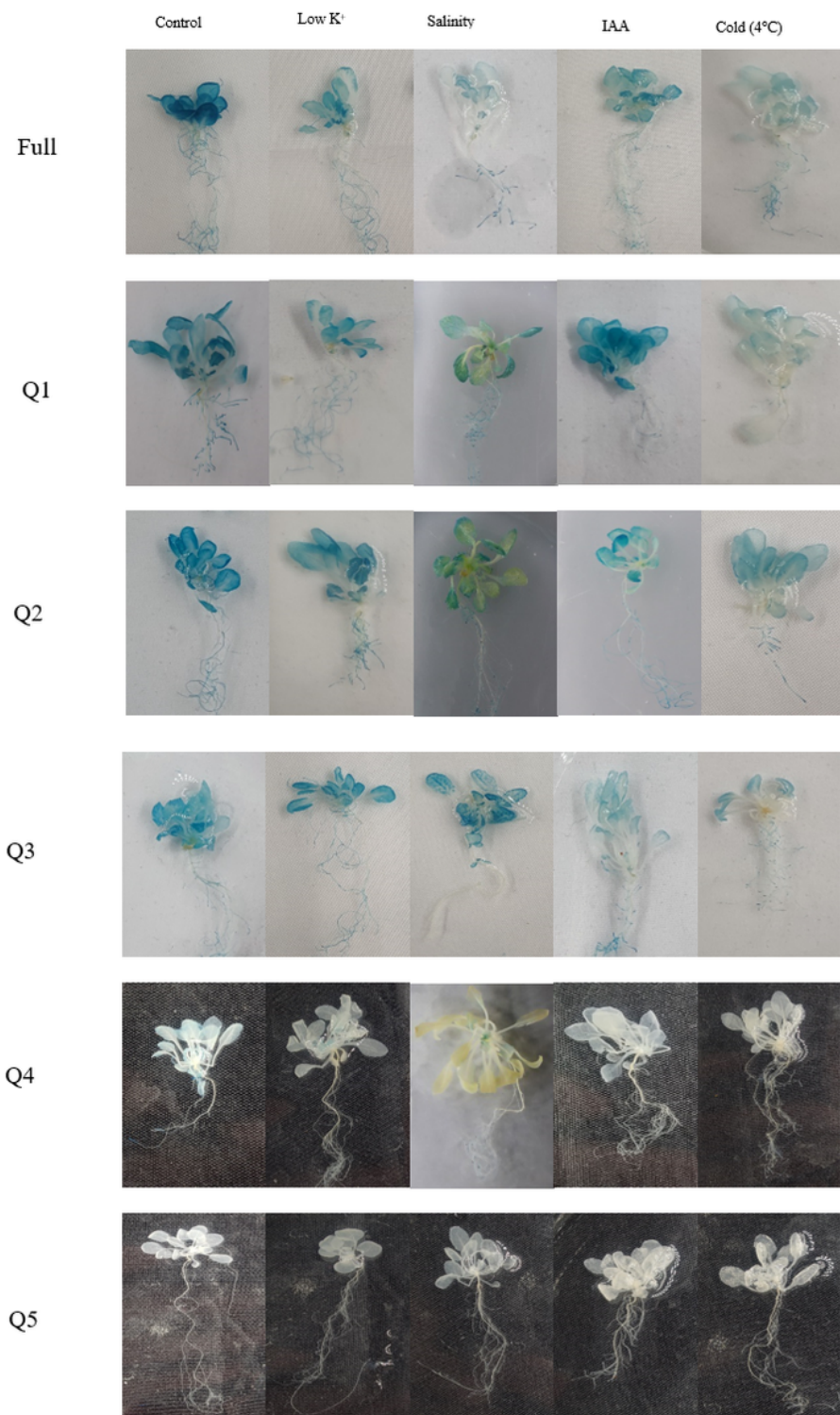


Figure 6

Histochemical assay for GUS expression in transgenic *A. thaliana* treated with low potassium, salinity, IAA and cold (4°C) stress conditions. The treated transgenic plants were incubated overnight in GUS staining solution at 37 °C. Later, treated with ethyl alcohol for 4-h to remove chlorophyll and taken photographed.

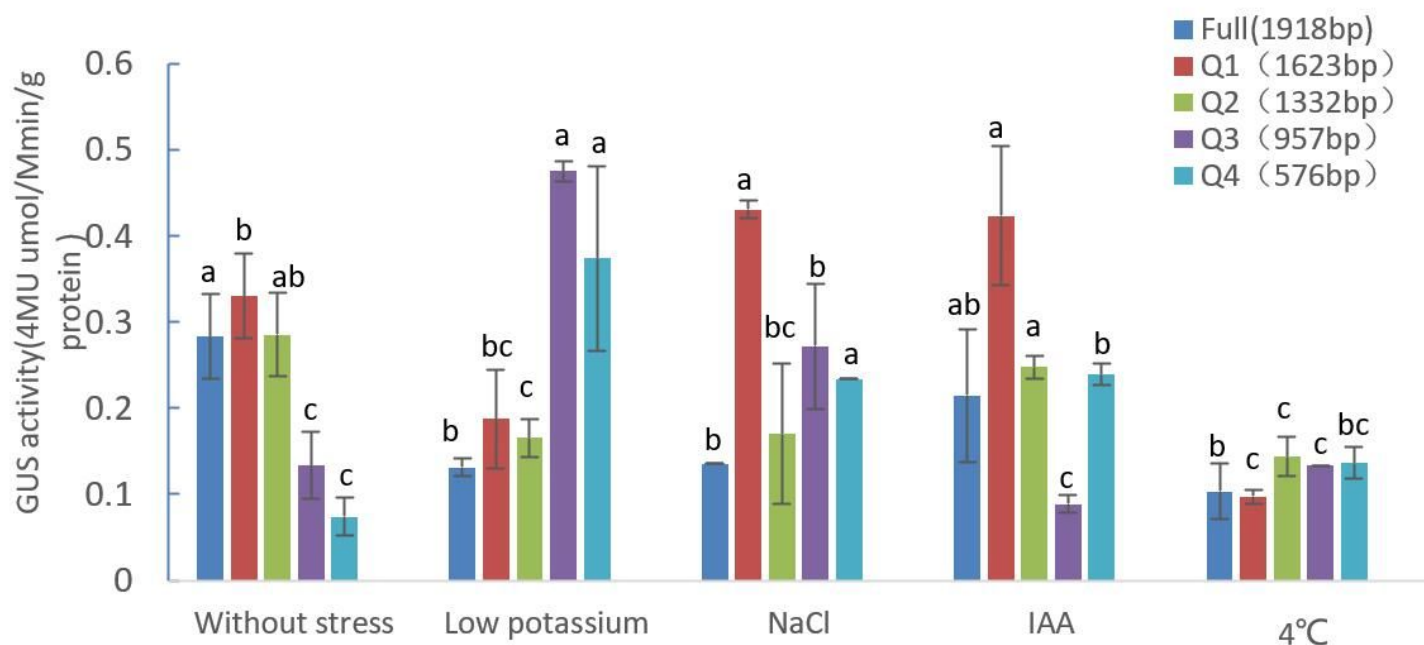


Figure 7

Analysis of GUS activity for *pScHAK10* deletions in transformed *A. thaliana* under different stress conditions. The low potassium stress condition was simulated by 50 nmol K⁺ in MS medium. Salt stress (200 mmol L⁻¹) was affected by the MS medium. 4 °C simulated the cold stress for 3 hr. The IAA (150 μmol L⁻¹) stress was simulated in MS medium. The GUS activity was expressed as nmol 4-methylumbelliferone/min/g protein. Bars represent the mean, and error bars (±) represent SD from three independent experiments. Different letters represented significant differences among treatments at $p < 0.05$.

Supplementary Files

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