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Sweet Sorghum (*Sorghum bicolor* L.) Cloning and Functional Analysis of Callose Gene *SbGlu1* in Protein Content

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Abstract The Sweet sorghum (*Sorghum bicolor* L.) Moench is a variant of grain sorghum, which origins in Africa. Due to its high sugar and tolerance, it has been considered as a potentially useful energy crop and received more attention. However, less study on sweet sorghum has been performed in physiology and molecular by Al stress. These results illustrated that the decrease of β-1,3-glucanase activity by Al could lead to callose accumulation. In POTCHETSTRM, five β-1,3-glucanase genes expression were up-regulated, and a gene expression was down-regulated. In ROMA, only one β-1,3-glucanase gene, *SbGlu1* (*Sb03g045630.1*) expressed response to Al, and the expression was higher in ROMA than in POTCHETSTRM. The expression levels of six callose synthase-like genes were very low exposure of 10 μM Al upon to 24 h in ROMA, but POTCHETSTRM exhibited the highest expression level only at 24 h. Therefore, callose synthase-like genes maybe regulate callose deposition in the later stage of Al stress in sweet sorghum. The *SbGlu1* expression positively correlated with callose content in both cultivars. The *SbGlu1* expression maybe involve in callose degradation in sweet sorghum by Al stress. The full-length cDNAs of *SbGlu1* were cloned from the root tips of both ROMA and POTCHETSTRM, respectively. The *SbGlu1* were transient expressed in onion epidermal cells for subcellular localization, showed that SbGLU1 is soluble with no specificity localization.

Keywords: *sweet sorghum, Callose, β-1,3-glucanase gene, different expressed gene, protein content*

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1. Introduction

Sweet sorghum (*Sorghum bicolor* L.) gene cloning is the first prerequisite for studying gene function. In the early 1970s, for identification and isolation Specific target gene, obtain the full length of the gene coding region sequence, clarify the gene location and elucidate the gene function [1] The technique of DNA recombination in vitro and has been put into practice through bioengineering technology, for gene work Can provide convenient conditions for research [2-5]. Plants are under stress such as bacterial invasion and animal feeding, the expression of genes and activities of enzymes in the body will be changed to complete the induction, transmission of these signals and the realization of biological effects [6]. With DNA sequencing technology, enzyme engineering technology And bioinformatics as well as Arabidopsis, canola, tobacco, tomato, potato, rice, sorghum, corn, The publication of the whole genome sequence of strawberry, grape, poplar and other plants has enabled the cloning and functional analysis of plant genes [7]. Previous research progress Plants generally resist the damage caused by insect feeding through three pathways, namely, chemotaxis, antigenicity or pest tolerance. Some plant resistance sources have multiple insect resistance pathways, and different insect resistance pathways are regulated by different resistance mechanisms. Therefore, the aphid resistance mechanism of sorghum has important guiding significance for sorghum aphid resistance breeding [8]. The resistance inheritance of sorghum to aphids was complex, with different resistance source materials controlling different number of gene loci, and the resistance genetic mechanism was controlled by single dominant and recessive genes or by a few major genes [9]. Identified anti-Al transcription factors in rice *OsART1*, a transcription factor belonging to the C_2H_2 zinc finger protein family, regulates the expression of 29 genes, these include the ALMT1 gene. The transcription factor

OsART1 in rice regulates the location of the plasma membrane of the cell Anomalous Al transporter NRAT1 gene, which belongs to Nramp (natural resistance-associated) macrophage proteins, a member of the family, are a special trivalent metal ion transporter that modulate plants the absorption of Al, and Al induces upregulation of *NRAT1* gene expression [10]. Many other plant studies have identified the member genes of MATE family located in cell membrane, which regulate Al Induce citric acid secretion in roots and improve plant Al tolerance. In addition, Al resistance has been found in Arabidopsis thaliana Sex-related C_2H_2 zinc finger protein family of transcription factors *AtSTOP1* [11]. Among the 31 genes regulated by *AtSTOP1*, MATE and FRD of MATE family members [12]. These two genes can improve plant Al tolerance under Al stress. Under Al stress, sorghum can increase its Al tolerance and citric acid content by secreting citric acid from its roots Secretion is regulated by the *SbMATE* gene on the cell membrane, whereas Al induced *SbMATE* gene expression is not rapidly, the expression of *SbMATE* gene was significantly increased after Al treatment for 2-3 days [13,14]. Sweet sorghum is a variety of grain sorghum. Al induces callose to form rapidly at root tips. The nanase gene *SB03G0456301* (*SbGlu1*) can rapidly respond to Al stress and is resistant to Al in sweet sorghum *SbGlu1* gene expression in ROMA was significantly higher than that in POTCHETSTRM, an Al sensitive cultivar Gene expression levels. It was speculated that *SbGlu1* gene induced root tip formation of sweet sorghum in Al Callose and Al tolerance may play important roles. In this study, we cloned and subcloned *SbGlu1* gene function of *SbGlu1* gene in sweet sorghum was further analyzed by cell localization and allogeneic expression in Arabidopsis thaliana.

2. Materials and Methods

2.1. Real-time Fluorescent Quantitative PCR Primer Design

Through the NCBI database (http://www.ncbi.nlm.nih.gov/) BLAST engine retrieved 6 callose synthase related genes in sorghum (*SB01G0486301*, *SB03G0234901*, *Sb03g030800*.1, *Sb03g034880.1*, *SB04G03851.1* and *Sb10g030970.1*) and 6 sorghum β-1, The 3-glucanase gene [*SB03G0454601*, *SB03G0454901*, *Sb03g045510.1*, *SB03G0456301* (*SbGlu1*), *SB08G0196701* and *Sb09g018730.1*] was compiled from the known online. The coding sequence was based on Primer design requirements of real-time fluorescent quantitative PCR, primer design software Primer 5.0 was applied Specific primers for target gene and accession No.X79378 (GenBank accession No.X79378) were designed Table 1.

2.1.1. Main Reagent

Enzymes: PrimerSTAR HS DNA polymerase (Invitrogen), rTaq (Takara), infusion Ligase, BP Clonase II and LR Clonase II (Invitrogen) Antibiotics: Bleomycin Zeo, kanamycin Kan, ampicillin Amp, Rifampicin Rif, B Acyl syringone AS, carboxyl benzyl penicillin purchased from Dingguo Company. Kit: DNA recovery and purification kit, plasmid extraction kit purchased from Trans, plant based because the DNA extraction kit was purchased from Tianroot Biological Company. Other reagents: MS medium purchased from Haibo; Basta (Glufosinate_Ammonium) LUC The substrate. Strain: Eschreichia coli DH5α (*Eschreichia coli*) from Trans, Agrobacterium tumefaciense *AGL0* and *EHA105* (Agrobacterium tumenfacience) were preserved in this laboratory. Vectors: Plant expression vectors *pEGAD* and pGWB5 were kept in our laboratory, and *pDONR* was purchased from us (Invitrogen).

Table 1. Primer pairs of callose synthase-like genes and the β-1, 3-glucanase genes for qRT-PCR

Locus	Description	Size	Primers (5'- 3')
Sb01g048630.1	Putativecallose synthase 1 catalytic subunit	139 bp	Forward: AGCAAACAGAAGGGAAAGATCA Reverse: CTAATGAACCTCCCTCCCAAG
Sb03g023490.1	F3F20.1 protein	215 bp	Forward: AAAGCATCCTTCTCACAGTACAATA Reverse: AGCCAATCAAGCAAATCCTG
Sb03g030800.1	1,3-β-glucan synthase component-like	122 bp	Forward: GAGGTCAAGCAATGGGCAG Reverse: CCCAGGGCACAATGAAGAG
Sb03g034880.1	Putative callose synthase 1 catalytic subunit	199 bp	Forward: GGAGCAAGATCATCTACGGACC Reverse: AGCATAGGACATTAGGACGAACAC
Sb04g038510.1	Putative callose synthase 1 catalytic subunit	163 bp	Forward: CAAGCAGAGTGACGCCAGAG Reverse: AACATTGACGGCTTTGAGGAC
Sb10g030970.1	Putative callose synthase 1 catalytic subunit	155 bp	Forward: GCTGTCTTGTTTGAGGTCTTGAG Reverse: GCTTGACCAGTACTTTCAGGATC
Sb03g045460.1	1,3-beta-glucanase	136bp	Forward: CTGGCATTGCTCCTCGGA Reverse: GGTTGATGCCGTTGGACTG
Sb03g045490.1	similar to Endo-1,3-beta-glucan ase	192bp	Forward: CGTGAAGCCCTACTACCCCG Reverse: GAGGGCGGGTAGGAGTTGG
Sb03g045510.1	similar to Endo-1,3-beta-glucan ase	127bp	Forward: GGTCACCAACACCTTCCCG Reverse: ACGGTAGGCGAAGTAGGGG
Sb03g045630.1	similar to Endo-1,3-beta-glucan ase	106bp	Forward: GACGGTGCAAAAGCTATCGA Reverse: GGGAAAATCGCTCCAAACA
Sb08g019670.1	similar to Endo-1,3-beta-glucan ase	181bp	Forward: ACTCCCCAGACGCCACCA Reverse: CTGCCTTCCACCTCGTTGC
Sb09g018730.1	similar to Beta-glucanase precursor	176bp	Forward: ATGGCAAGCAGGCAAGGTG Reverse: ATCGCCGTGATGCCGTTC
Sbβ-actin	similar to Actin-1	146 bp 192 bp	Forward: CGACCTTACCGACTACCTCATG Reverse: TCTTGGCAGTCTCCATCTCCT Forward: CACACTGTCCCCATCTACGAAG Reverse: GTCGTAGTCCAGGGCAATGTAG

2.1.2. Preparation of Medicine

Ampicillin (Amp) reserve solution (50 mg/mL): Weigh 0.5g Amp, dissolve in 10 ml and steam distilled water was separated after filtration and sterilization and stored at -20°C.

Kanamycin (Kan) reserve solution (50 mg/mL): Weigh 0.5g Kan and dissolve in 10mL distilled water after filtration and sterilization, they were divided and stored at -20°C.

Rifampicin (Rif) reserve solution (50 mg/mL): Weigh 0.5g Rif and dissolve in 10 mL dimethylkia sulfoxide (DMSO) was extracted and sterilized, then divided and stored at -20°C.

Car reserve solution (50 mg/mL): 0.5g Car was weighed and dissolved in 10 mL for distillation water was separated after filtration and sterilization and stored at -20°C.

Preparation of 50×TAE: Weigh 242 g tris, 37.2 g Na2EDTA·2H2O and add 800 in turn 1-L beaker of mL deionized water, stir it thoroughly with a magnetic stirrer and then add 57.1 mL acetic acid. Stir well and mix well. Add deionized water to 1000 mL. Store at room temperature and dilute when using one hundred times.

2.1.3. Preparation of medium

LB liquid medium; Weigh 5g yeast extract, 10 g tryptone, 10 g NaCl (LB solid medium added 15g AGAR powder) in turn into 1 L triangle bottle, add 800 mL distilled water, use a magnetic stirrer to stir evenly After that, adjust 1M KOH to pH 7.0, constant volume to 1000 mL, and seal at 120°C for high temperature and humid heat sterilization 20 min.

MS solid medium; MS Plant medium (sucrose and AGAR free) was purchased from Haibo Biological Company and weighed according to the method used 4.74 g MS plant medium dissolved in 800 mL distilled water, adding 10 g sucrose and 10 g AGAR powder, respectively. Use magnetic stirrers to stir evenly, adjust to pH 5.8, constant volume 1000 mL, 120°C high temperature humid heat sterilization Sterilized for 20 min, poured into petri dishes under aseptic condition in super clean table, sealed, stored at 4°C for later use.

2.1.4. Bioinformatics Analysis of Sorghum *β-1*, 3 glucanase Gene

The steps of bioinformatics analysis of sorghum *β-1*, 3 glucanase gene are as follows:

The found through sorghum genome sequence database (http://www.helmhltz-muenchen.de/)

Liang *β-1*, 3 glucanase gene *SbGlu1* (*SB03G0456301*) Coding sequence (*Coding sequence*, *CDS*) and amino acid sequence. The use of proteomics database (http://www.expasy.org/tools/scanprosite/)

The main domains of SbGLU1 protein were analyzed.

Through the NCBI database (NCBI, http://www.ncbi.nlm.nih.gov/) BLAST tool SbGlu1 gene of oats, Arabidopsis, soybean, barley, tobacco, rice and wool were found to be similar to sorghum.

Compare and analyze amino acid sequence through Clustal X and GeneDoc software, and then use the software treeview1.6.6 builds the phylogenetic tree to analyze the evolutionary relationship between sorghum and other species.

2.1.5. Statistical Analysis

The data were shown as mean ± SD. One-way analysis of variance and independent-samples T test were used in significance testing with the software of SPSS17.0. Mean values were assessed with significance set at $P < 0.05$.

Table 2. Cloning of callose gene SbGlu1 in sweet sorghum and SbGlu1 gene was amplified by RT-PCR were derived 10 μM from Al tolerant sweet sorghum and Al sensitive sorghum, respectively the root tips cDNA treated with Al^{3+} for 24 h was the template, and the PCR reaction system was as follows.

Treatment	volume (μL)
2×PrimerSTAR GC Buffer 25	25
ddH2O 17.5	17.5
dNTP Mixture(2.5mM)	4
IF-SbGlu1 F(10μM)	1
IF-SbGlu1 R(10μM)	1
cDNA	1
2.5U/μL PrimerSTAR HS DNA polymerase	0.5

3. Results

3.1. Effects of Influence of Aluminum Treatment with Different Concentration and Time on Callose Accumulation at Root Tips of Sweet Sorghum

The tolerant sweet sorghum at all treatment concentrations and at different time points accumulation of callose in the root tips of the variety ROMA was always lower than that of the Al sensitive variety POTCHETSTRM.5 microns In Al^{3+} treatment, callose accumulation between two sweet sorghum varieties was 7 times higher than that in 24 h after treatment High (Figure 1A). The 10 μM Al^{3+} treatment, sweet callose accumulation at root tip of sorghum al sensitive cultivar POTCHETSTRM was approximately the same as tolerant cultivar ROMA Brosal Callose accumulation was twice as large as that of Al, and root elongation is inhibited to a certain extent at 24 h after AL treatment (Figure 1B). There was significant difference in relative root elongation between the two cultivars. Therefore, 10 μM Al^{3+} was selected as the treatment in the subsequent test to further study the mechanism of callose accumulation induced by Al in sweet sorghum root tips. 15μM Al^{3+} treatment had the smallest difference of 1.2 times (Figure 1C). Al sensitive sweet sorghum POTCHETSTRM had callose in root tips of three concentrations amount of substance accumulation increased with the increase of treatment time. Al tolerance of sweet sorghum ROMA was 10 μM, 15 μm the accumulation of callose in root tips increased with the increase of treatment time with μM Al^{3+}, while that with 5 μM Al^{3+} Callose content in root tips was relatively small and tends to be stable between 6 and 24 h after Al treatment. Under the same treatment time, the root tip of the sweet sorghum variety ROMA increased with the treatment concentration Callose accumulation also

increased. And POTCHETSTRM, sweet sorghum susceptible to Al, was treated with 5 μM Al^{3+} Callose accumulation at 24 h was similar to that at 10 μM Al^{3+} treatment, and callose accumulation at 24 h is similar to that at POTCHETSTRM. The root relative elongation was all lower than 40% (Figure 1D). Indicating that when Al stress reached a certain degree, the sweetness was high Callose accumulation at the root tips of a beam reaches a stable level.

3.2. Effects of Aluminum Stress on Callose Synthase Activity in Root Tips of Sweet Sorghum

The effects of Al treatment on callose synthase activity in sweet sorghum root tips are shown in (Figure 2). Regardless of Al resistant products ROMA was also an Al-sensitive variety *POTCHETSTRM*. Callose synthase activity decreased from 0 h to 0 h after Al treatment 12 h showed an increasing trend.Callose synthesis of ROMA and *POTCHETSTRM* at 12 h after Al treatment. The enzyme activity was 6 and 8 times that of 0 h. The enzyme activity decreased from 12 h to 24 h. During the whole Al treatment period, callose synthase activity of Al sensitive variety POTCHETSTRM was always high In addition, callose synthase activity was observed between the two cultivars at 6 h and 12 h after sex treatment difference was significant $P < 0.05$.

3.3. Bioinformatics Analysis Results of Sorghum *SbGlu1*

The sorghum *SbGlu1* gene has a total length of 954 bp, no intron, and encodes a protein containing 318 amino acids. Amino acid sequence of Sorghum *SbGLU1*. The analysis results of GLU amino acid sequences in Sorghum (Figure 3). The picture, the ten kinds of plants amino acid sequence of GLU was highly conserved, and the C-terminus contains the structure of the 17th family of glycohydrolases Domain (*VQVVVSESGWPSAG*), which was the structural characteristic of hydrolytic protease. GLU amino acid sequence Phylogenetic tree analysis showed (Figure 4). There were three GLU phylogenetic trees for the above ten plants Clade, 1 clade of soybean, Arabidopsis and tobacco; 1 branch of Oat, bamboo; while *SbGLU1* of sorghum, *OsGLU* of rice and HvGLU of barley belong to the same evolutionary clad. Sorghum the similarity rate of SbGLU1 to rice *OsGLU* and barley *HvGLU* sequences was high (71% and 68%, respectively). These results indicated that the *AtGLU* of Arabidopsis thaliana was closely related and had the lowest similarity rate with Arabidopsis thaliana (similarity rate: 49%). The most distant relatives.

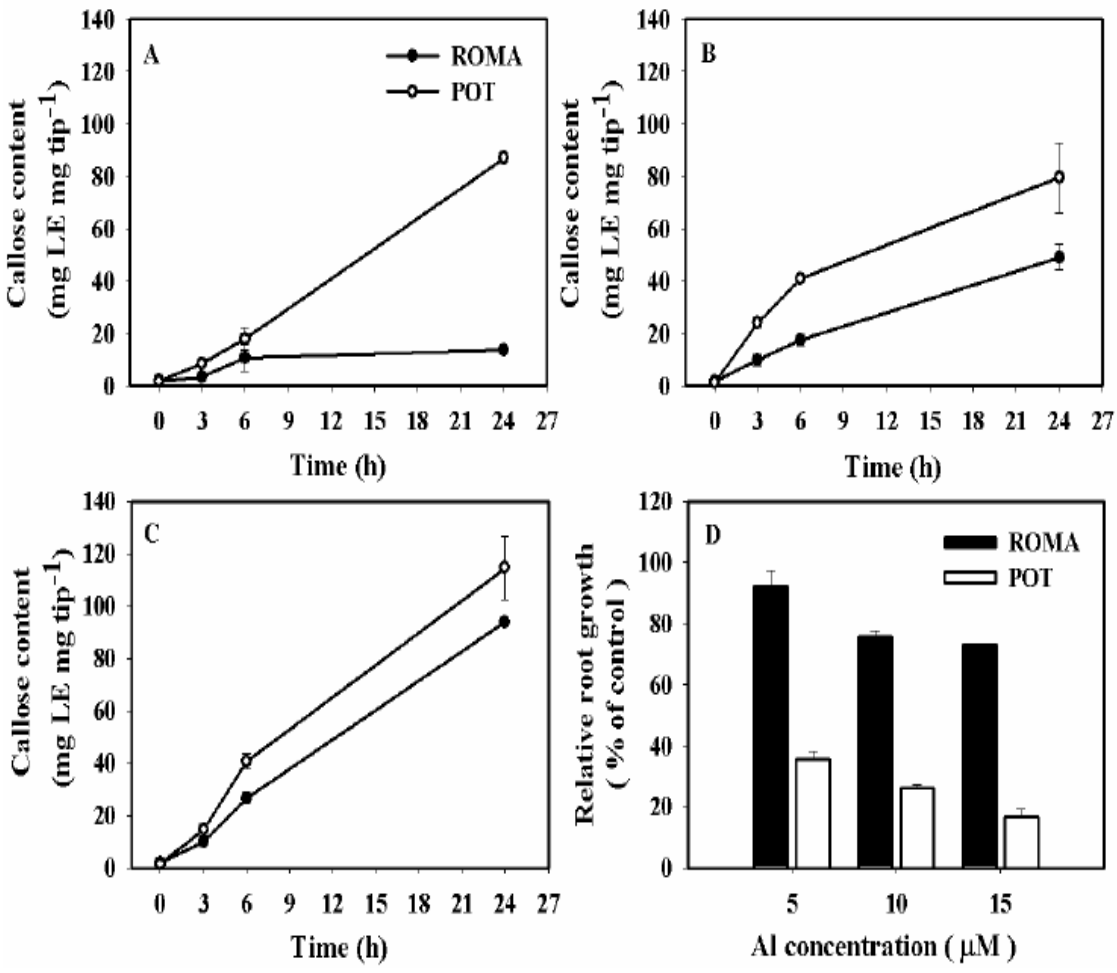

Figure 1. Effect of different Al concentration on callose content and root growth in sweet sorghum root with time-course treatment (A. 5 μM Al^{3+} Deal with; B. Treatment with 10 μM Al^{3+} ;C. 15 μM Al^{3+} treatment; D. 5, 10, 15 μM Al^{3+} treatment for 24 h Relative root elongation of sweet sorghum (Note: POT stands for POTCHETSTRM. The same as below))

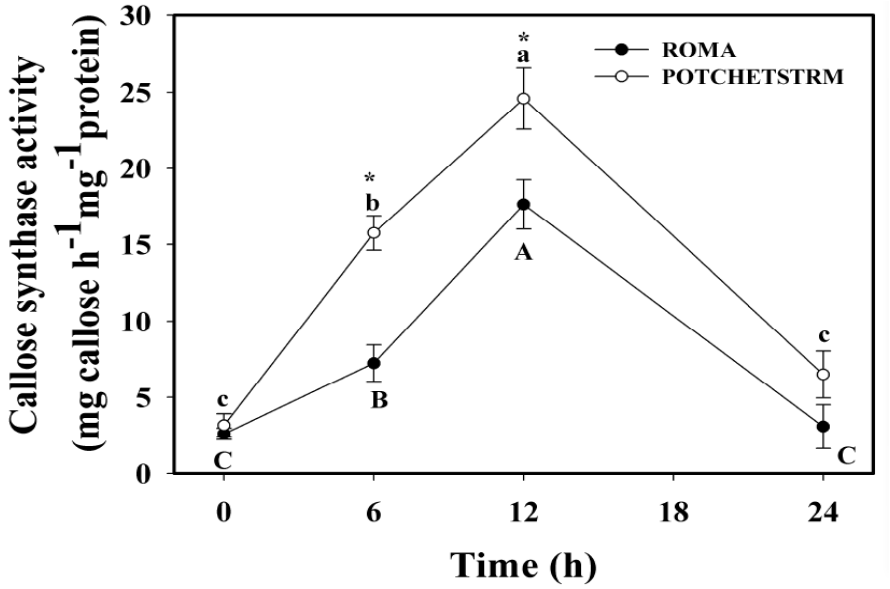

Figure 2. Effects of Al stress on callose synthase activity in the root apices of sweet sorghum

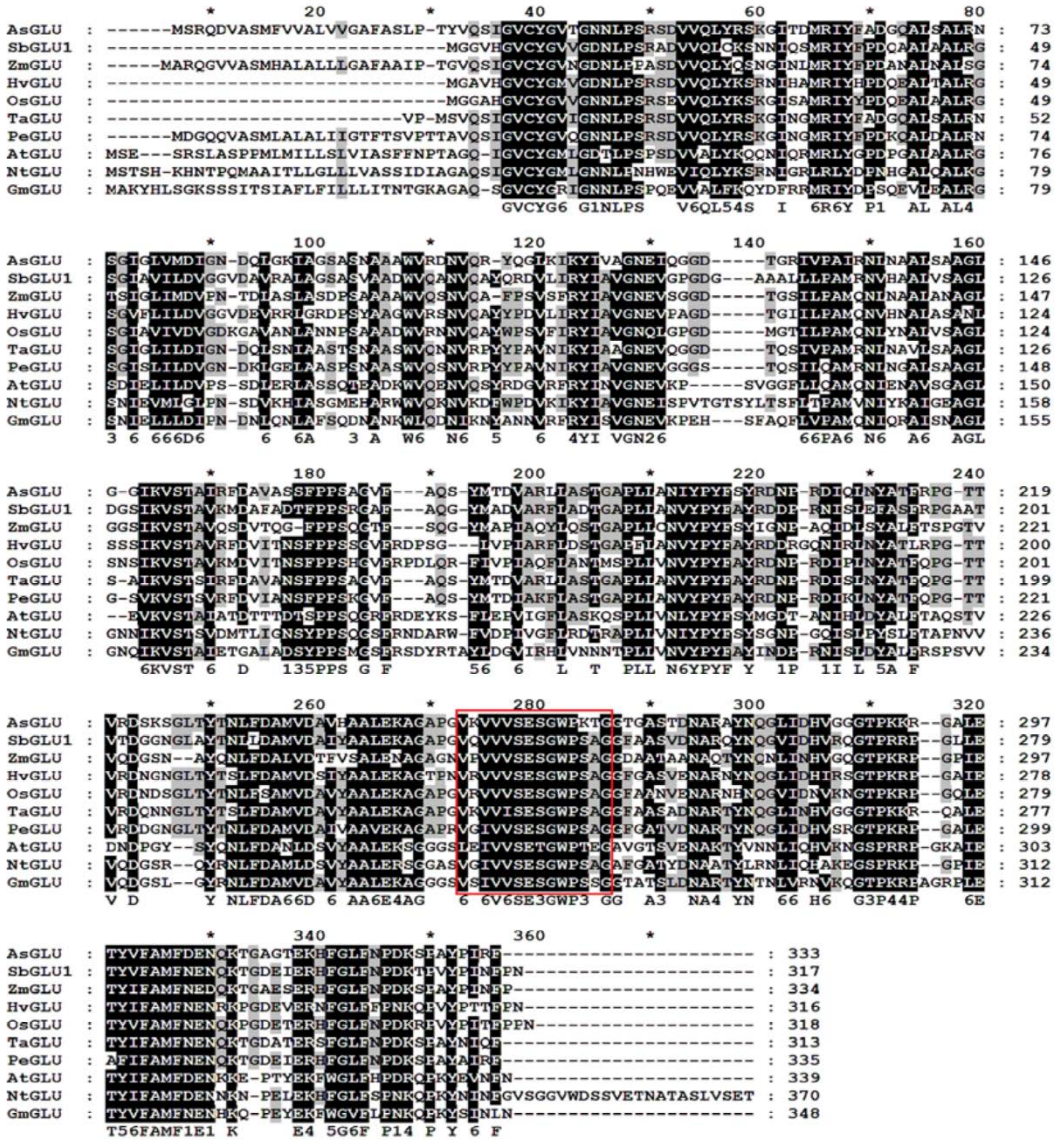

Figure 3. A multiple sequence amino acid alignment of sorghum (As : *Avena sativa* (GenBank ID: AAP33176); At: *Arabidopsis thaliana* (GenBank ID: AAM63339); Gm: *Glycine max* (GenBank ID: AAB03501); Hv: *Hordeum vulgare* (GenBank ID: 1607157A); Nt: *Nicotiana tabacum* (GenBank ID: ACF93731); Os: *Oryza sativa* (GenBank ID: AAL40191); Pe: *Phyllostachys edulis* (GenBank ID: ADG56569); Sb: *Sorghum bicolor* (GenBank ID:XP_002456922); Ta: *Triticum aestivum* (GenBank ID: AAY96422). Zm: *Zea mays* (GenBank ID: ACJ62639))

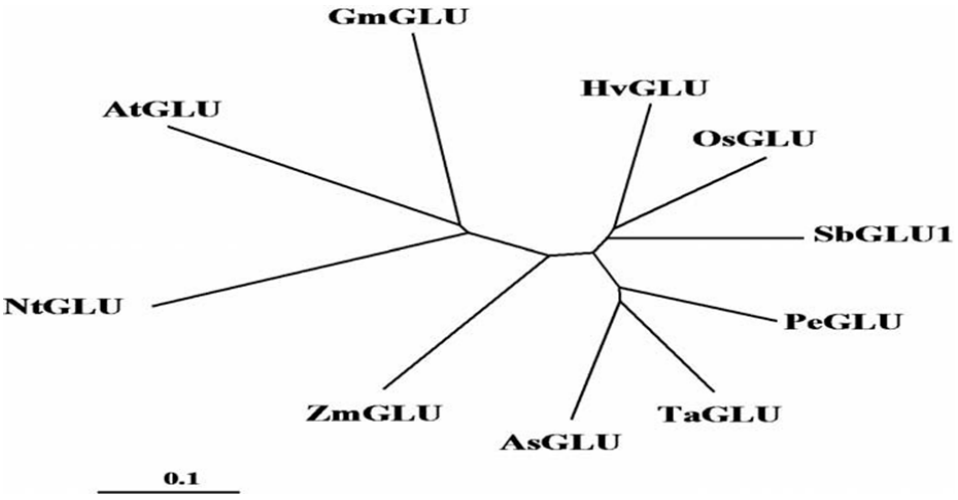

Figure 4. Phylogenetic relationship of *SbGLU1*of sorghum

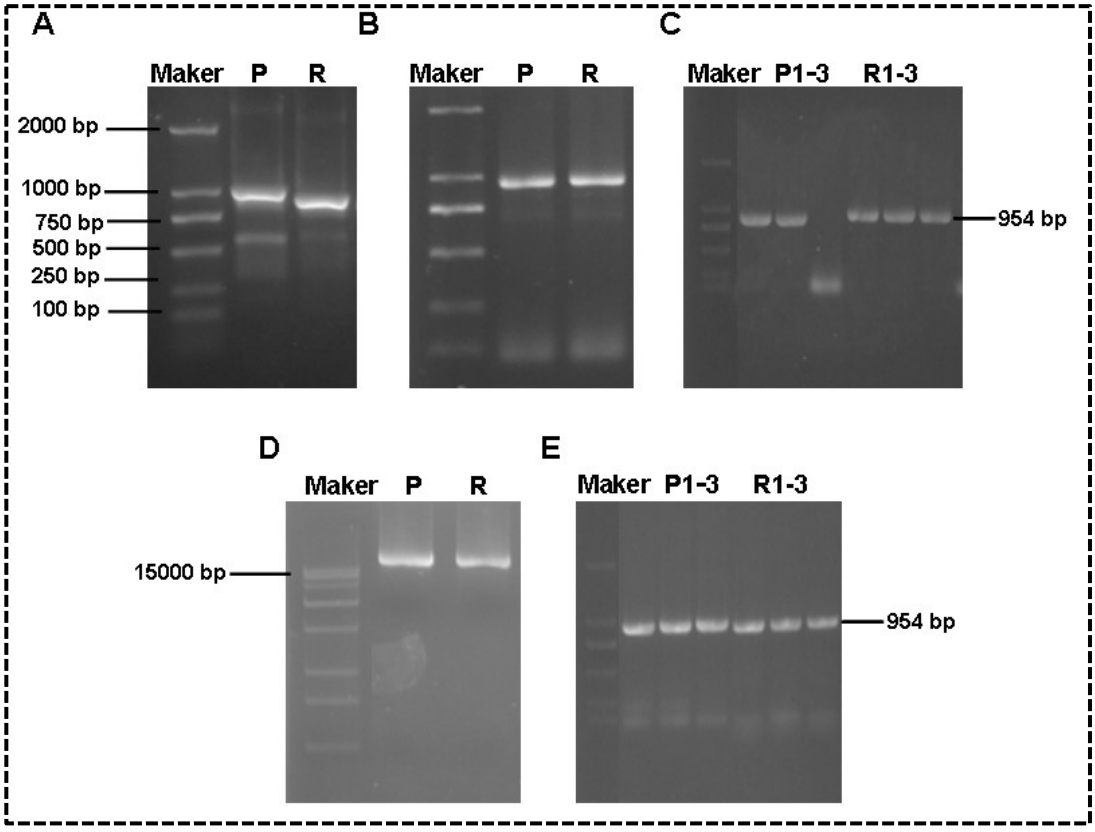

Figure 5. Electrophorogram of *SbGlu1* clone in sweet sorghum

3.4. Effects of Cloning of *SbGlu1* Gene in Sweet Sorghum

The *SbGlu1* gene of sweet sorghum was cloned to Al tolerant variety ROMA, and Al sensitive variety respectively. The *cDNA* of *POTCHETSTRM* was amplified by PCR as the template, and the electrophoretic map showed the presence of a target gene band at a position close to 1000bpMaker (Figure 5A). After purification, electrophoretic detection of pure heterozonal order recovered fragment was recombined with a linear vector (Figure 5B). Therefore, we believe that in Al tolerant cultivar ROMA and Al sensitive cultivar *POTCHETSTRM*, *SbGlu1* gene sequence is a single nucleotide polymorphism (*SNPs* (Figure 5C). However, the amino acid sequence of *SbGLU1* protein of two sweet sorghum varieties (Figure 5D). The *SbGlu1* gene from two different varieties was analyzed separately in future experiments. Select the correct sequencing Escherichia coli solution and extract two fractions respectively plasmid was transferred into Agrobacterium tumefaciae by freeze-thaw method. The liquid was amplified by PCR and displayed by electrophoresis plasmids containing *SbGlu1* gene have been successfully transferred into Agrobacterium Tumefaciae (Figure 5E) and can be used to infect Bacteriopaena transgenic Arabidopsis thaliana plants were obtained and functional analysis of *SbGlu1* gene was performed.

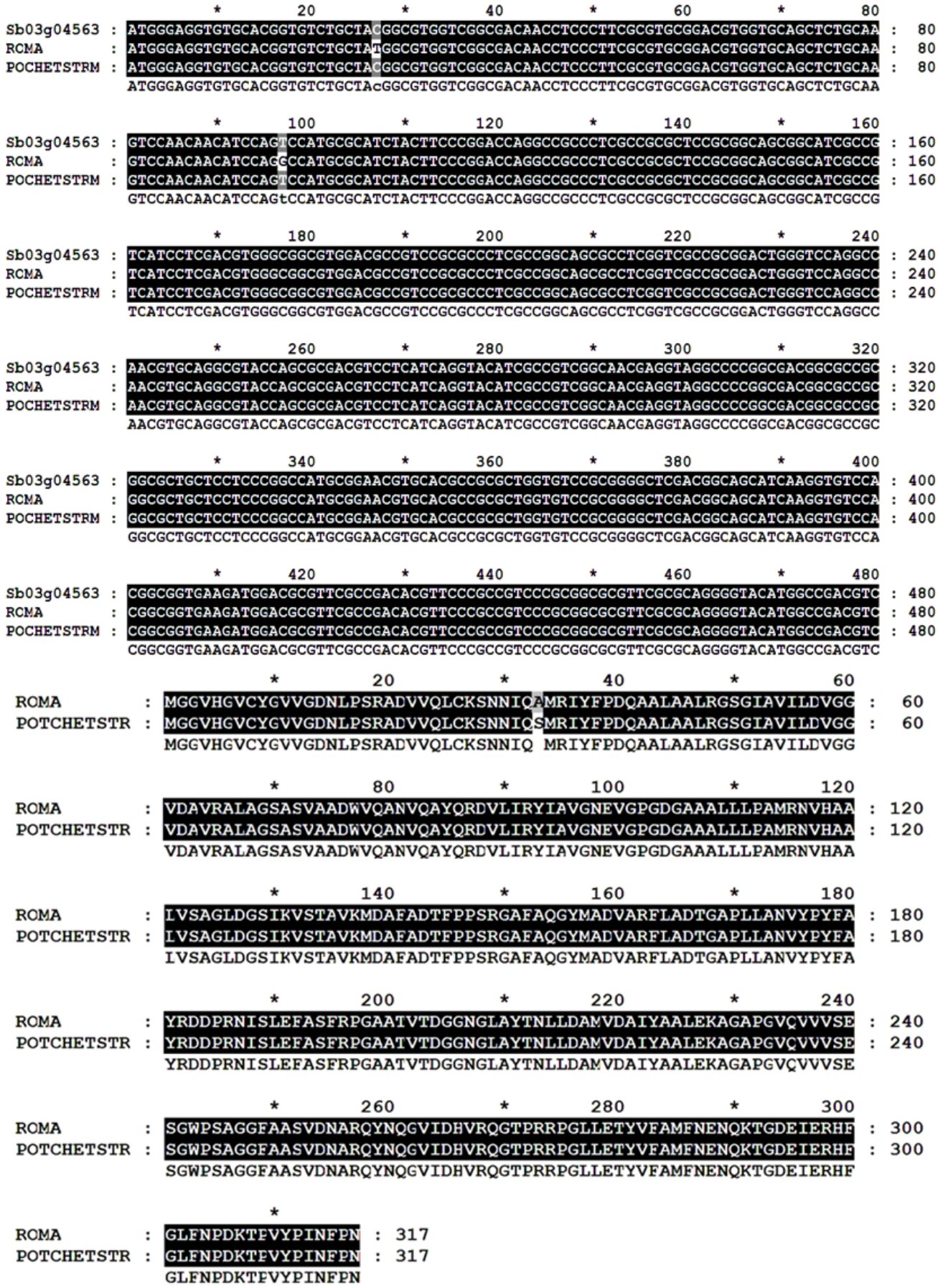

Figure 6. Aligment of *SbGlu1* sequencing, and Aligment of *SbGLU1* amino acid sequence in sweet sorghum

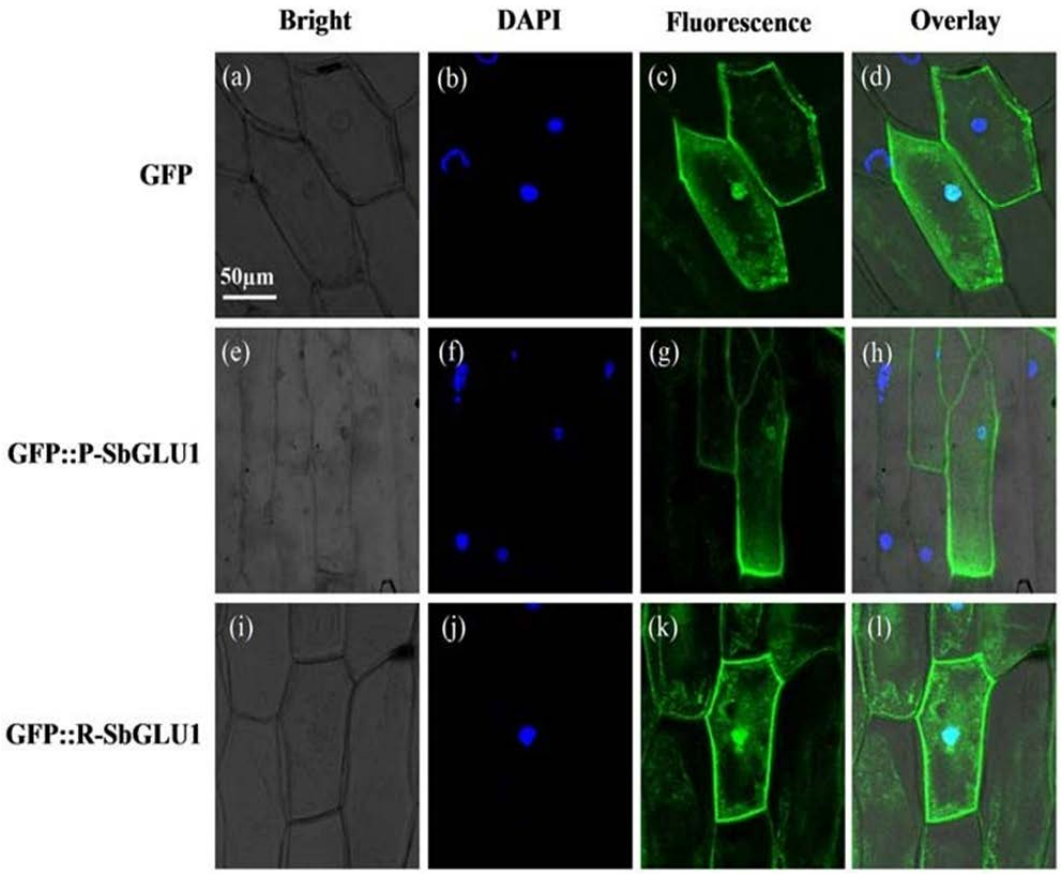

Figure 7. Subcellular localization of *SbGLU1* proteins in sweet sorghum

3.5. Aligment of SbGlu1 Sequencing, and Aligment of SbGLU1 Amino Acid Sequence

The correct one will be verified bacterial solution was sent to Beijing Huada Company for sequencing, and the comparison and analysis of sequencing results were shown in (Figure 6) from *SbGlu1* gene sequence of sensitive cultivar POTCHETSTRM and sequence queried from sorghum database SbGlu1 gene sequence from resistant ROMA was 27 '(C-T), 97' (T-G). And 817 '(C-A) were different, and the results were consistent in the three single colonies.

We suggest that the SbGlu1 gene is present in the tolerant variety ROMA and the sensitive variety POTCHETSTRM Sequences of single nucleotide polymorphism (SNPs) are polymorphism while mononucleosides difference of acid polymorphism resulted in one amino acid in the amino acid sequence of SbGLU1 protein of two sweet sorghum varieties different (-33, S-A). Two different varieties in future experiments SbGlu1 gene was analyzed separately.

3.6. Effects of *SbGLU1* Proteins in Sweet Sorghum

The N-terminus of SbGlu1 gene was fused with GFP gene, and the two genes were activated by super strong 35S promoter. GFP *SbGLU1* could be expressed by instantaneous fusion in onion epidermal cells. As shown in (Figure 7). Both control groups GFP alone or fused GFP: P: SbGLU1 and GFP: R: *SbGLU1* were distributed in cytoplasm and cytoplasm in the nucleus, *SbGLU1* protein was intracellular soluble, non-specific protein.

4. Discussion

The *SbGlu1* gene sequences of sweet sorghum Al tolerant cultivar ROMA and Al sensitive cultivar POTCHETSTRM showed changes in single nucleotide polymorphisms (SNPS), and there was no difference in Al resistance of transgenic Arabidopsis thaliana, indicating that SNPS did not affect the function of *SbGlu1* gene. This is similar to the malic acid transporter gene performed by Sasaki et al. using the Al resistant genotype *ET8* and Al sensitive genotype ES8 in wheat *ALMT1* showed similar results. A 6-nucleotide difference between the *TaALMT1-1* gene of ET8 and the *TaALMT1-2* gene of ES8 resulted in a 2-amino acid difference at the protein level, but this difference was not associated with Al resistance [15]. That more than 90% of sweet sorghum lines had copy number differences in their genomes. Such genomic structural differences enhanced the response of genes to environmental stress and stimulation, and played a role in phenotypic diversity and adaptability of sweet sorghum. In sweet sorghum recombinant inbred lines, the *MATE1* gene with three gene copy numbers showed strong Al tolerance [15,16]. A similar relationship has been reported in soybean, where variation in this gene structure enriches the genome of occulted genes associated with plant biodefense [17]. After insect feeding, genes involved in plant hormone metabolism and signaling pathways,

trauma response, control of protein and lipid binding, glutathione metabolism and cell catabolic processes are often involved in plant defense response [18,19]. Insect-resistant genotypes respond to aphid feeding by changing enzyme function, signaling and gene expression [20,21]. The plant hormone jasmonate plays a key role in resistance to biotic and abiotic stresses as a signaling molecule. Jasmonic acid and its derivative methyl jasmonate are important plant hormones for plant defense against insect feeding [22]. However, when studying the Al resistance of Hl *ALMT1* gene, Chen et al. found that the same Hl *ALMT1* gene copy number in the genome of plussum grass did not affect its gene expression [23]. Al tolerant variety ROMA and Al sensitive variety POTCHETSTRM showed no difference in the copy number of *SbGlu1* gene were differences in gene expression. This indicated that there were differences in *SbGlu1* gene expression induced by Al between the two sweet sorghum varieties in this experiment, which might not be caused by the same *SbGlu1* gene copy number, but other factors regulating gene expression. Studies have shown that the expression of *ALMT1* gene, which regulates the secretion of malic acid in wheat roots, is upstream of the gene Regulation of ABCD repeats, the more ABCD repeats, the higher gene expression and Al resistance [24]. While in susceptible soybean plants, aphids can inhibit the expression of jasmonate signaling pathway genes [25]. The transient increase of jasmonic acid in the early feeding stage of aphids can prevent aphids from colonizing on sorghum and improve the resistance to aphids feeding [26]. This study involved in styrene acrylic acid metabolic pathways of gene expression appeared to rise, phenylalanine metabolic pathway is a plant secondary metabolic pathways in a general way, and can generate trans cinnamic acid, coumaric acid intermediate and final lignin can be formed, yellow ketone, flavonoids and other secondary metabolites, involved in the salicylic acid signal pathway and lignin synthesis way [27]. The upstream promoter region of Hl *ALMT1* gene is subject to transcription factors number of cis-acting elements regulated by *HlART1* affected the expression level of this gene. Increasing the number of cil-acting elements regulated by HlART1 in the upstream promoter region of Hl *ALMT1* gene can improve the expression level of Hl *ALMT1* gene and the secretion of malic acid [28]. Similarly, regulate the secretion of citric acid in sorghum upstream repeats of *SbMATE* gene were positively correlated with Al tolerance in sorghum [28,29]. When insects feed, flavonoids, as secondary metabolites of plants, will also change some enzyme activities in insects, which will affect the growth and development of insects and make plants have a defense effect against plant-eating insects [30]. Flavonoids are also the precursors for the synthesis of tannins, which can also protect plants from damage and improve their ability to adapt to stress [31]. In all Al tolerant barley varieties, the upstream of HvAACT1 gene coding region contains a 1023 bp insertion sequence, which can improve the expression of *HvAACT1* gene and promote the secretion of citric acid in barley roots [32]. In this study, the upstream 2 kb sequence analysis of *SbGlu1* gene in sweet sorghum showed that there were certain differences in the *SbGlu1* gene promoter sequence between the Al tolerant variety ROMA and the Al sensitive variety POTCHETSTRM (Figure 5). In the 2 kb sequence upstream of SbGlu1, the Al tolerant variety ROMA had an insertion sequence of 83 bp, and the number of promoter core element TATA-box and distal regulatory enhancer element CAAT-box were significantly different between the two sweet sorghum varieties. Therefore, we believe that the upstream sequence of *SbGlu1* gene may play an important role in regulating the expression and Al resistance of this gene. In addition, in this experiment, the upstream 2 kb sequence of *SbGlu1* gene of two sweet sorghum varieties both contained elements responsive to ABA, *MeJA*, SA, GA and other plant hormones [33]. In their study on rice, they found that the *OsGlu12* gene with high sequence consistency and close genetic relationship to *SbGlu1* could respond. Our laboratory's previous studies on soybeans also showed that endogenous ABA may be involved in the signal transduction process under Al stress, which is a signal molecule that soybean senses Al toxicity and transmits between the aboveground part and the root system [34]. And SA may be an early signal molecule responding to Al stress. In addition, endogenous SA can regulate the secretion of citric acid in soybean roots under Al stress [35]. Sweet sorghum Al-tolerant variety ROMA and Al-sensitive variety POTCHETSTRM, the copy number of *SbGlu1* gene was 3 times that of internal reference genes Sb-β-actin and Sb-β-tubulin. These results indicated that the two sweet sorghum varieties had the same copy number of β-1, 3 glucanase gene *SbGlu1*. Sequence comparison of the upstream 2kb promoter region of the SbGlu1 gene of the Al-tolerant variety ROMA and the Al-sensitive variety POTCHETSTRM showed that the ROMA *SbGlu1* promoter amplified by the same primer was 67 *bp* more than that of POTCHETSTRM. The ROMA *SbGlu1* promoter sequence had an 83 *bp* insertion fragment. Predictive analysis of functional elements of the promoter sequence of SbGlu1 gene showed that in the 2 kb promoter region upstream of *SbGlu1* gene, there was a difference of 13 promoter core elements of TATA-box between the two varieties and a difference of 3 regulatory enhancer elements of CAAT-box between the two varieties. The difference in the number of these elements may be the reason for the difference in the expression of SbGlu1 gene at the transcription level between the two varieties. In addition, the promoter sequence also contained ABA, MeJA, SA, GA response elements and MYB binding drought resistance elements, suggesting that the expression of SbGlu1 gene may also be regulated by hormones and related to drought resistance.

5. Conclusions

The Al-sensitive POTCHETSTRM, Al tolerant sweet sorghum ROMA has lower synthase activity, higher *β-1, 3*-glucanase activity and higher expression of SbGlu1 gene under Al stress, which are the main reasons for less accumulation of callose at root tips. Allogeneic expression of β-1, 3-glucanase *SbGlu1* gene in Arabidopsis thaliana can reduce callose accumulation and improve Al tolerance. Due to limited time, the physiological mechanism of sensitivity to Al in sweet sorghum cultivars ROMA and

POTCHETSTRM to Al has not been thoroughly studied. This part will be gradually improved in the follow-up studies in our laboratory. We will continue to reveal the mechanism of Al induced callose accumulation in sweet sorghum root tips by analyzing the polymorphism of upstream *SbGlu1* gene and identifying the structural domains regulating gene expression, the modification of small molecular RNA and epigenetics, and the protein interacting with *SbGLU1*.

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