Plant jasmonate ZIM domain genes: shedding light on structure and expression patterns of JAZ gene family in sugarcane

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Abstract

Background: Sugarcane smut caused by Sporisorium scitamineum is one of the most severe fungal diseases in the sugarcane industry. Using a molecular biological technique to mine sugarcane resistance genes can provide gene resources for further genetic engineering of sugarcane disease-resistant breeding. Jasmonate ZIM (zinc-finger inflorescence meristem) domain (JAZ) proteins, which involved in the responses to plant pathogens and abiotic stresses, are important signaling molecules of the jasmonic acid (JA) pathway.

Results: Seven differentially expressed sugarcane JAZ genes, ScJAZ1–ScJAZ7, were mined from the transcriptome of sugarcane after inoculation with S. scitamineum. Bioinformatic analyses revealed that these seven ScJAZ genes encoded basic proteins that contain the TIFY and CCT_2 domains. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis demonstrated that the ScJAZ1–ScJAZ7 genes were tissue specific and differentially expressed under adverse stress. During S. scitamineum infection, the transcripts of ScJAZ4 and ScJAZ5 were both upregulated in the susceptible genotype ROC22 and the resistant genotype Yacheng05–179; ScJAZ1, ScJAZ2, ScJAZ3, and ScJAZ7 were downregulated in Yacheng05–179 and upregulated in ROC22; and the expression of ScJAZ6 did not change in ROC22, but was upregulated in Yacheng05–179. The transcripts of the seven ScJAZ genes were increased by the stimuli of salicylic acid and abscisic acid, particularly methyl jasmonate. The expression of the genes ScJAZ1–ScJAZ7 was immediately upregulated by the stressors hydrogen peroxide, sodium chloride, and copper chloride, whereas slightly induced after treatment with calcium chloride and polyethylene glycol. In addition, the expression of ScJAZ6, as well as seven tobacco immunity-associated marker genes were upregulated, and antimicrobial activity against Pseudomonas solanacearum and Fusarium solani var. coeruleum was observed during the transient overexpression of ScJAZ6 in Nicotiana benthamiana, suggesting that the ScJAZ6 gene is associated with plant immunity.

Conclusions: The different expression profiles of the ScJAZ1–ScJAZ7 genes during S. scitamineum infection, the positive response of ScJAZ1–ScJAZ7 to hormones and abiotic treatments, and the function analysis of the ScJAZ6 gene revealed their involvement in the defense against biotic and abiotic stresses. The findings of the present study facilitate further research on the ScJAZ gene family especially their regulatory mechanism in sugarcane.

Keywords: ScJAZ, Sugarcane-Sporisorium scitamineum interaction, Adversity stimuli, Expression profiles, Antimicrobial action

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Background

Sugarcane (Saccharum spp.), one of the most important sugar crops in the world, is also an energy crop for bioenergy production [1]. Sugarcane smut, which is caused by Sporisorium scitamineum, has become one of the most severe fungal diseases in sugarcane [2]. Smut disease induces earlier germination of buds, thinner stalks, and higher incidence of tiller and black whip, which decrease cane yield and sugar quality [3, 4]. In Queensland, Australia, smut disease has resulted in severe yield loss and even total crop failure despite suitable climatic conditions [5], which include a temperature range of 25 °C–30 °C and humidity of >80% [6]. Sporisorium scitamineum quarantine, the development of smut-resistant sugarcane varieties, and integrated field management are some of the proposed methods of controlling smut disease. Cultivating resistant sugarcane varieties is considered as the most effective measure to control smut disease compared to agronomic practices and chemical treatments [3]. However, it takes 10–12 years to obtain a valuable sugarcane cultivar through conventional cross-breeding, which is mainly attributable to its complex genetic background with a polyploid-aneuploid genome [7–9]. Recently, the continuous development of transgenic technology has provided an effective approach to determining the function of target genes and bio-orientation improvement [10]. Therefore, using molecular biological techniques to mine sugarcane resistance genes may provide excellent gene resources for further genetic engineering of sugarcane disease resistance breeding.

Previous studies have mainly focused on sugarcane resistance to smut disease from the aspects of morphology [11], physiology [12], biochemistry [13], cytology [8], genomics [14], and proteomics [15, 16]. At the molecular level, the smut pathogen in sugarcane could be detected by using conventional PCR [17, 18], TaqMan real-time PCR [19], and loop-mediated isothermal amplification (LAMP) [20, 21]. By using next-generation sequencing technology, the genomes of three strains causing sugarcane smut disease have been completed in China (strain 2014001) [14], Brazil (strain SSC39B) [22], and Germany (strain SscI8) [23], thereby providing novel insights into the pathogenic mechanisms underlying sugarcane smut disease. During S. scitamineum infection, 136 transcript-derived fragments (TDFs) [24] and 62 differentially expressed genes [25] in sugarcane were identified by cDNA-amplified fragment length polymorphism (cDNA-AFLP). Furthermore, sugarcane smut resistance-related genes such as transcription factors X1 and thaumatin (PR5) gene [26], chitinase family genes [27], β-1,3-glucanase genes [28, 29], and catalase gene [30] have been cloned and identified. Que et al. [31], Barnabas et al. [16], and Su et al. [15] have investigated the interaction between sugarcane and S. scitamineum at the proteome level. Two-dimensional electrophoresis (2DE) coupled with matrix-assisted laser desorption ionization/tandem time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) has been utilized in assessing the expression of some proteins in sugarcane after S. scitamineum challenge, and a putative effector of S. scitamineum has also been detected [16, 31].

The signal molecule jasmonic acid (JA) plays an important role in plant growth, development, secondary metabolism, and environmental stress response [32–36]. Recent studies have suggested that jasmonate ZIM (zinc-finger inflorescence meristem) domain (JAZ) proteins in the JA signaling pathway may be involved in the generation of a response to plant pathogen attacks [37]. Coronatine insensitive 1 (COI1), which plays a vital part in JA synthesis or perception, is identified in Arabidopsis thaliana mutants that are insensitive to JA [38–40]. Investigations have revealed that the COI1 gene encodes an F-box protein, which is a component of the E3-type ubiquitin ligase SCF (Skp/Cullin/F-box) complex [39, 41]. However, the mechanism by which COI1 transduces the JA signal remains unclear. JAZ family have been characterized as SCFCOI1 substrates [42–44]; however, its members also acts as repressors of JA signals, which directly interact with a JA-responsive transcription factor, the basic helix-loop-helix leucine zipper-type (bHLH zip-type) factor MYC2 [43, 45]. Therefore, the COI1-JAZ-MYC2 is considered as the first core signal module of the JA pathway [46]. JAZ proteins are also involved in other plant hormone pathways such as gibberellic acid, auxin, ethylene, and salicylic acid (SA) [47, 48].

The TIFY family includes four protein subfamilies, namely, ZIM-like (ZML), TIFY, PEAPOD (PPD), and JAZ [49]. JAZ proteins contain a conserved ZIM domain (TIF[F/Y]XG) near its N-terminal and a Jas motif (SLX2FX2KRX2RX5PY) at the C-terminal [44, 50]. The Jas motif consists of 26 amino acids and is the hallmark feature of the JAZ family that serves as a protein-protein interaction surface that is required for the repression of MYC2 and COI1 [43, 51]. Twelve members of the AtJAZ genes family have been identified in A. thaliana and their expression could be induced by JA, thereby suggesting that these play a feedback regulatory role on the activity of transcription factors that are involved in the JA signaling pathway [42, 43, 52]. In addition, the expression of AtJAZ genes are regulated by treatments against plant pathogens, sodium chloride (NaCl), and other stress factors [50, 52]. Previous studies have shown that various JAZ genes have different functions; for example, in A. thaliana, JAZ family genes are expressed during insect herbivory and mechanical damage, except for AtJAZ11 gene [53]. Demianski et al. [52] reported that eight AtJAZ genes are upregulated whereas four AtJAZ genes are not differentially expressed during
Pseudomonas syringae infection in A. thaliana. On the other hand, the expression of OsJAZ genes in Oryza sativa are tissue-specific [54]. Zhu et al. [55] showed that the overexpression of GsJAZ2 in transgenic Glycine max enhances salt resistance. Studies have also revealed that the overexpression of NaJAZd and NaJAzh genes in Nicotiana attenuata inhibits bud formation and promotes nicotine synthesis [56–58].

Current research studies on JAZ proteins mainly focus on model plants such as A. thaliana [46] and O. sativa [54]. Some of the members of the JAZ gene family have also been reported in Zea mays [59], Vitis vinifera [60], G. max [55] and N. attenuata [56–58]. However, investigations on the JAZ genes and their functions in sugarcane have not been conducted to date. In the present study, seven genes belonging to the ScJAZ family were mined from the transcriptomes of sugarcane cultivars Yacheng05–179 (smut-resistant) and ROC22 (smut-susceptible) post inoculation with S. scitamineum at 24 h, 48 h, and 120 h [61]. Sequence analysis and phylogenetic relationships were performed to analyze the gene structure and the classification of these ScJAZ genes. Their gene expression patterns in different sugarcane tissues, in smut disease, and in the presence of various defense signal compounds as stressors were examined by using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Furthermore, the full-length cDNA sequence of sugarcane ScJAZ, hereby designated as ScJAZ6, was isolated and characterized by subcellular localization, prokaryotic expression in Escherichia coli BL21, spot assay, and transient expression in Nicotiana benthamiana. The present study aimed to reveal the structure of the sugarcane ScJAZ gene as well as its expression characteristics in response to adversity stress, which may be serve as the foundation in identifying other members of the ScJAZ gene family.

Methods

Plant materials and treatments

For biotic stress, sugarcane smut-resistant and -susceptible cultivars Yacheng05–179 and ROC22, and smut whips were collected from the Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture (Fuzhou, China). Robust and healthy stems from the two sugarcane cultivars were harvested and then soaked in water for 24 h. The two-bud sets of Yacheng05–179 and ROC22 were inoculated with 0.5 µL of a suspension containing 5 × 10⁶ smut spores·mL⁻¹ in 0.01% (v/v) Tween-20, whereas the control was inoculated with 0.01% (v/v) Tween-20 in sterile distilled water instead of spores [28, 62]. After inoculation, each group containing five buds were randomly selected at 0 h, 24 h, 48 h, and 72 h, and then stored at −80 °C until total RNA extraction. Three biological replicates for each group were prepared.

For tissue-specific expression analysis, six healthy 10-month-old ROC22 plants were selected. The tissue samples, which included the root, +1 leaf (the youngest fully expanded leaf with a visible dewlap), bud, stem pith, and stem epidermis, were collected. Three biological replicates of each tissue were prepared. All samples were fixed in liquid nitrogen and stored at −80 °C until total RNA extraction.

For the abiotic treatments, the 4-month-old ROC22 plantlets were grown hydroponically for one week and then treated with eight exogenous treatments by root dipping at 28 °C with 16 h light and 8 h darkness. The simulated environmental stress conditions included plant hormone stresses of 5 mM SA, 100 µM methyl jasmonate (MeJA) and 100 µM abscisic acid (ABA), oxidative stress of 10 µM hydrogen peroxide (H₂O₂), hyperosmotic stresses of 25% polyethylene glycol (PEG) 8000 and 250 mM NaCl, and heavy metal stresses of 250 mM calcium chloride (CaCl₂) and 250 mM copper chloride (CuCl₂) [27]. Whole plantlets treated by plant hormones and CaCl₂ were collected at 0 h, 3 h, 6 h, and 12 h. Other plantlets were collected at 0 h, 6 h, 12 h, and 24 h after initiation of treatment, except for the 250 mM CuCl₂ treatment, in which collection was performed at 0 h, 24 h, and 48 h after treatment. Three biological replicates were prepared for each treatment and stored at −80 °C until total RNA extraction.

RNA extraction and first-strand cDNA synthesis

Total RNA was extracted from all the samples using TRIzol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol, and then analyzed by 1% agarose gel electrophoresis. DNase I (Promega, Madison, WI, USA) was used to remove the residual DNA. Then, 1 µg RNA was used in first-strand cDNA synthesis using a 20-mL reaction volume of the Prime-Script™ RT Reagent Kit (Perfect For Real Time) (TaKaRa, Dalian, China).

Isolation of sugarcane ScJAZ family genes

Seven ScJAZ unigenes (ScJAZ1, Sugarcane_Unigene_BMK.38081; ScJAZ2, Sugarcane_Unigene_BMK.48937; ScJAZ3, Sugarcane_Unigene_BMK.47693; ScJAZ4, Sugarcane_Unigene_BMK.45854; ScJAZ5, Sugarcane_Unigene_BMK.46432; ScJAZ6, gi34927808; and ScJAZ7, gi35024398) (Table 1), which were determined to be differentially expressed in Yacheng05–179 and ROC22 after inoculation with S. scitamineum for 24 h, 48 h, and 120 h in a previous RNA-Seq investigation [61], were selected in this study. One of the full-length ScJAZ genes, ScJAZ6 (gi34927808), which was differently expressed in the sugarcane-resistant genotype but remained unchanged in the susceptible one, was cloned (primers are listed in Table 2) from the cDNA extracted from Yacheng05–179 bud tissue by reverse...
transcription-polymerase chain reaction (RT-PCR). The amplification procedure was as follows: 94 °C for 4 min; followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min; and a final 72 °C for 10 min. The RT-PCR product of ScJAZ6 was gel-purified and cloned into a pMD19-T vector (TaKaRa, Dalian, China), and then sequenced (Shenggong, Shanghai, China).

Sequence analysis of ScJAZ family genes
The sequences of the seven ScJAZ family genes were translated and analyzed by open reading frame (ORF) Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Their signal peptides and the isoelectric points (pIs) were predicted by using ExPASy (http://us.expasy.org/tools). The conserved domain architecture of the ScJAZ proteins were predicted

### Table 1
A list of differentially expressed ScJAZ genes in Yacheng05–179 and ROC22 after challenging with Sporisorium scitamineum for 24 h, 48 h, and 120 h, respectively

| Gene name | Unigene ID | Yacheng05–179  | ROC22  |
|-----------|------------|----------------|--------|
|           |            | Log₂ fold change (T/CK) | Log₂ fold change (T/CK) |
|           |            | 24 h | 48 h | 120 h | 24 h | 48 h | 120 h |
| ScJAZ1    | Sugarcane_Unigene_BMK.38081 | 5.409 | 6.560 | 5.281 | 2.319 | 2.782 | – |
| ScJAZ2    | Sugarcane_Unigene_BMK.48937 | 2.649 | 4.206 | 2.775 | 2.968 | 2.843 | – |
| ScJAZ3    | Sugarcane_Unigene_BMK.47693 | – | 1.661 | – | 1.921 | – | – |
| ScJAZ4    | Sugarcane_Unigene_BMK.45854 | – | – | – | – | – | – |
| ScJAZ5    | Sugarcane_Unigene_BMK.46432 | – | 4.745 | 3.435 | – | – | – |
| ScJAZ6    | gi34927808 | – | 1.845 | – | – | – | – |
| ScJAZ7    | gi35024398 | – | – | – | 3.240 | 2.860 | – |

In the previous transcriptomics study, after correction, the unigenes with a false discovery rate (FDR) < 0.01 and reads per kb per million reads (RPKM) between samples of <2 (fold-change ≥ 2) were considered as differentially expressed genes [61]. A log₂ fold-change indicates the fold change of the differentially expressed genes in the transcriptome [61]. T indicates the transcriptome of sugarcane challenged with S. scitamineum. CK represents the transcriptome of the mock material. Yacheng05–179, smut-resistant sugarcane genotype; ROC22, smut-susceptible sugarcane genotype

### Table 2
Primers used in this study

| primer | Forward primer (5′-3′) | Reverse primer (5′-3′) | Strategy |
|--------|------------------------|------------------------|----------|
| ScJAZ1-Q | CCTGTTACTACCACTACTAC | ACAAGGCTTTAGATAGAGTT | qRT-PCR analysis |
| ScJAZ2-Q | CTGAAAGGAGTGAATCTCA | ATCTGACTACTAGCAAACA | qRT-PCR analysis |
| ScJAZ3-Q | TTTAATCTCCTAGTTTTCC | AATACATCTACTAGTCCTCT | qRT-PCR analysis |
| ScJAZ4-Q | TTCTACCGGCGGCAAGAT | CTTGAGGCGACACCTTCTC | qRT-PCR analysis |
| ScJAZ5-Q | CCCTCTGATCTCTGTCG | GTCTCTCCTATATACTCCCTCCT | qRT-PCR analysis |
| ScJAZ6-Q | GTGTCCTTCCCGTAACTGT | GTTGTACCCCTTCTTTTCTT | qRT-PCR analysis |
| ScJAZ7-Q | CGATTACGATGTATTCAT | ACATCTCTACGTCCTTCT | qRT-PCR analysis |
| ScJAZ6-dDNA | GGAACGAGAGCTCTGCA | GCCGCTAGGCAAAA | Gene cloning |
| ScJAZ6-Subloc | TGCTCTAGAATGAGACAGGGACTCTTG | CCGGATCGTCTCTGATGTTTCTTCTT | Subcellular localization assay |
| ScJAZ6-32a | CGGATGCAATGGAGGGAGCCTTCC | CCGTGCTCTGATGTTTCTTCTTCTT | Prokaryotic expression |
| ScJAZ6–1301 | TGCTCTAGAATGGAGGGACCTTCC | CCGTGCTCTGATGTTTCTTCTTCTT | Transient overexpression |
| GAPDH | CGCCGCACTGGAAGCA | TCTCAGGCTTCTGATGC | Reference genes |
| NtHSR201 | CGCACTCTTTCCTTGTGC | GCTCAGTTTACCTGGAGTCAGTTG | Transient overexpression |
| NtHSR203 | TGCTCTAGAATGGAGGGACCTTCC | CCGTGCTCTGATGTTTCTTCTTCTT | Transient overexpression |
| NtHSR515 | TTGGGCCGAATGATGGGTGA | TCTGACCGAATGGGAGTGG | Transient overexpression |
| NtPR1a/c | AACCTTGACGTGGGGCGACCA | GCAGTCACAACGAGCCGCAG | Transient overexpression |
| NtPR1b/c | TTAGCTCTTTCCTTGTGC | GCTCAGTTTACCTGGAGTCAGTTG | Transient overexpression |
| NtPR3 | CAGGAGAGCTTCCCTTGATTA | TCTCAGACAGGCAGCA | Transient overexpression |
| NtEFE26 | CGGACCGTGCTGCGCATAT | AAACATGCTGCCGAGTGC | Transient overexpression |
| NbAccdeaminase | TCTGAGTCTGAGTCTTGAGG | TGCACGATGCTGAGATGCTT | Transient overexpression |
| NtEF1-a | TCTGCTGTAAACAGATGGGATG | GAGATGGGAGATGGGATG | Transient overexpression |

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by using the NCBI conserved domains program (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), the SMART program (http://smart.embl-heidelberg.de/), and MOTIF search (http://www.genome.jp/tools/motif/). Psort software (http://psort.hgc.jp/form.html) was used to predict the subcellular location of the ScJAZ6 proteins. ClustalW was employed for multiple sequence alignment of the sugarcane ScJAZ proteins with 12 A. thaliana [46], 23 Z. mays [59], 15 O. sativa [54] and 11 V. vinifera [60] homologous proteins, respectively. A phylogenetic tree was constructed by the maximum-likelihood method (1000 bootstrap replicates) using the MEGA 6.06 program [63].

qRT-PCR analysis
To analyze the expression patterns of the ScJAZ family genes in different sugarcane tissues and in response to various adverse stressors, qRT-PCR was conducted using SYBR Green Master (ROX) (Roche, China) and an ABI 7500 qRT-PCR system (Applied Biosystems, South San Francisco, CA, USA). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (primers listed in Table 2) was employed as internal control [64, 65]. Beacon Designer 8.12 software was used to design the specific qRT-PCR primers (Table 2) based on the unigene sequences of ScJAZ1–ScJAZ7. Each 20-μL qRT-PCR reaction system consisted of the following: 10 μL of the SYBR Green Master Mix, 0.8 μL of each 10 μM forward and reverse primers, 1.0 μL of the cDNA template (20× diluted cDNA), and 7.4 μL of sterile distilled water. The qRT-PCR reaction conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Each qRT-PCR was repeated three times. The 2^ΔΔCt method was used to analyze the qRT-PCR data [66]. Statistical analysis was conducted using the Data Processing System (DPS) v7.05 software (China). Data were expressed as the mean ± standard error (SE). Significance (P-value <0.05) was calculated using one-way ANOVA, followed by Duncan’s new multiple range test.

Subcellular localization assay
The ORF of ScJAZ6 without a stop codon (primers are listed in Table 2) was inserted into the two restriction sites (XbaI and BamHI) of the pCAMBIA 2300-GFP vector. Then, the recombinant vector pCAMBIA 2300-ScJAZ6-GFP was transformed into the competent cells of Agrobacterium tumefaciens strain EHA105. Agrobacterium-mediated transient expression in N. benthamiana leaf was performed according to Su et al. [67]. Subcellular localization of the fusion protein was observed by using a confocal laser scanning microscope (Leica TCS SP5, Germany) after infiltration for 48 h.

Prokaryotic expression in E. coli BL21 (DE3) cells
The ORF of ScJAZ6 was amplified by using the ScJAZ6-32a primers (Table 2). The PCR product and the pET 32a (+) vector that were both digested with BamHI and HindIII were ligated. The recombinant plasmid (pET 32a–ScJAZ6) was obtained and then transformed into E. coli BL21 (DE3) competent cells. The prokaryotic expression product was gathered after the BL21 pET 32a–ScJAZ6 cells were induced by 1.0 mM isopropyl β-D-thiogalactoside (IPTG) at 28 °C for 0 h, 2 h, 4 h, 6 h, and 8 h. Moreover, the LB medium with E. coli BL21 (blank) and BL21 pET 32a (control) cells were separately induced by IPTG for 0 h and 8 h, and then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [67, 68].

As reported, the expression of JAZ gene could be regulated by various environmental stimuli such as MeJA [44], salt and alkali stresses [55], PEG [54, 60], and pathogen and pest attacks [69, 70]. To study the expression of ScJAZ6 in E. coli in response to different abiotic conditions, a spot assay was conducted in combination with treatment using PEG 8000, NaCl, and CuCl2. The E. coli BL21 cells containing pET 32a–ScJAZ6 or pET 32a (control) were cultured in LB medium at 37 °C until these reached OD600 of 0.6. Then, IPTG was added to the cultures at a concentration of 1.0 mM and further cultured at 37 °C for another 12 h. The cultured cells were diluted to an OD600 of 0.6 and then again respectively diluted to 10^-3- and 10^-4-fold by using the LB medium [68]. Ten microliters from each of the 10^-3 and 10^-4-fold dilutions was spotted onto LB plates containing the compound of PEG 8000 (15%, 30%, and 45%), NaCl (250 mM, 500 mM, and 750 mM), or CuCl2 (250 mM, 500 mM, and 750 mM) [28]. All plates were cultured at 37 °C overnight and then photographed [68].

The role of the ScJAZ6 gene in response to pathogen infection
To study the function of the ScJAZ6 gene in response to pathogen infection, the overexpression vector pCAMBIA 1301-ScJAZ6 was constructed (the primers used are listed in Table 2). The recombinant vector and the control were separately transformed into Agrobacterium strain EHA105 cells, and then cultured in LB liquid medium (supplemented with 50 μg/mL kanamycin and 35 μg/mL rifampicin) overnight at 28 °C. After incubation, the cells were collected and then re-suspended in MS liquid medium (containing 200 μM acetylsyringone) to an OD600 of 0.8. The cells were infiltrated into the eight-leaf-old N. benthamiana leaves and then cultured at 24 °C for 24 h to 48 h (16 h light/8 h darkness) [67]. The treated N. benthamiana leaves were used in ion conductivity measurements, transcripts analysis of the target gene and the nine tobacco immunity-associated
marker genes, and DAB (3,3'-diaminobenzidinesolution) staining according to Su et al. [29].

To analyze the inhibitory effect of ScJAZ6 to pathogens, two important tobacco pathogens, Pseudomonas solanacearum and Fusarium solani var. coeruleum, were cultured to an OD_{600} of 0.8 in potato dextrose water (PDW) liquid medium at 28 °C. Then, the two pathogenic bacteria were separately infected into the N. benthamiana leaves that were agroinfiltrated with pCAMBIA 1301-ScJAZ6 or pCAMBIA 1301 for 24 h. All treatment materials were cultured at 24 °C (16 h light/8 h darkness) for 7 d and then photographed. Each test was repeated three times.

**Results**

**Expression profile of ScJAZ family genes in sugarcane after inoculation with S. scitamineum**

In our previous transcriptomics study, the sugarcane smut-resistant genotype Yacheng05–179 and -susceptible genotype ROC22 were challenged with S. scitamineum for 24 h, 48 h, and 120 h [61]. Table 1 shows that five ScJAZ genes (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ5, and ScJAZ6) were upregulated by S. scitamineum in the resistant genotype, whereas in the susceptible genotype, five ScJAZ genes (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ4, and ScJAZ7) were upregulated (Table 1). These findings indicate that the ScJAZ genes could be induced by S. scitamineum in sugarcane, and their expression profiles in smut-resistant and smut-susceptible sugarcane cultivars after S. scitamineum inoculation were different.

**Phylogenetic analysis of sugarcane ScJAZ family**

The results of phylogenetic analysis of ScJAZ proteins are shown in Fig. 1. Based on the reports of Chini [46] and Zhou et al. [59], the JAZ proteins from sugarcane, A. thaliana, Z. mays, O. sativa, and V. vinifera were clustered into four branches (groups A–D). The seven sugarcane ScJAZs were clustered into two branches, group A (ScJAZ6) and group B (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ4, ScJAZ5 and ScJAZ7).

**Sequence analysis of the sugarcane ScJAZ family**

Multiple alignment analysis showed that the seven ScJAZ proteins shared only 23.63% identity at the amino acid sequence level. The domain architecture, pi, and amino acids of these ScJAZ proteins were predicted and shown in Additional file 1: Figure S1. The protein length of ScJAZ1–ScJAZ7 varied from 136 aa to 403 aa. The ScJAZ6 protein was 403 aa in length, which was the longest compared to the other six ScJAZ proteins. The pi features of ScJAZ1–ScJAZ7 were all >7, thereby indicating that these seven ScJAZ proteins are basic proteins. No signal peptide and transmembrane region were observed in the ScJAZ1–ScJAZ7 proteins. Multiple alignment identified two highly conserved sequence motifs in all seven ScJAZ proteins. The TIF[T/F/Y]XG motif was located at the N-terminal of ScJAZ, which is also known as the ZIM or TIFY domain [50]. Another Jas motif SLX2FX2KRX2RX5PY was observed at the C-terminal of the ScJAZ protein, which has been reported to play an important role in regulating jasmonate responses in A. thaliana [46].

**Tissue-specific expression patterns of ScJAZ family genes**

To study the expression patterns of the ScJAZ family genes in different tissues, the sugarcane root, bud, leaf, stem pith, and stem epidermis were used. Figure 2 shows that these seven ScJAZ genes were successfully detected in all of the five sugarcane tissues. ScJAZ3, ScJAZ4, and ScJAZ7 presented a similar gene expression pattern, and the highest expression levels were observed in the bud and leaf, whereas exhibited low expression levels in other three tissues (root, stem pith, and stem epidermis). ScJAZ1 showed the highest expression levels in the bud, leaf, and stem pith, but accumulated at relatively low levels in the root and stem epidermis. A low expression level of ScJAZ2 and ScJAZ5 was found to accumulate in the root and stem epidermis, whereas a high level of gene expression was detected in the leaf. Unlike the other six ScJAZ genes, ScJAZ6 was expressed at low level in sugarcane tissues, and the highest ScJAZ6 transcript levels were detected in the root and bud than the other tissues. These findings suggest that these seven ScJAZ genes were constitutively expressed in all five sugarcane tissues, and most of them were abundant in the bud and leaf tissues.

**Transcripts of ScJAZ family genes in sugarcane post inoculation with S. scitamineum**

The expression patterns of the ScJAZ family genes in response to S. scitamineum stress are shown in Fig. 3. The qRT-PCR results suggest that in Yacheng05–179, with elongated treatment time, the RNA expression of the four ScJAZ genes (ScJAZ1, ScJAZ2, ScJAZ3, and ScJAZ7) was downregulated after infection with S. scitamineum, whereas the other two genes (ScJAZ4 and ScJAZ6) were significantly upregulated and reached the highest expression at 1 d. Furthermore, the expression of the remaining gene, ScJAZ5, peaked at 2 d and then decreased at 3 d. However, in ROC22, the six ScJAZ genes (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ4, ScJAZ5, and ScJAZ7) were upregulated after inoculation, and only ScJAZ6 showed no change in transcript abundance after inoculation. The expression of ScJAZ1 and ScJAZ7 rapidly increased at 1 d after inoculation, which then peaked at 3 d. The expression of ScJAZ3 and ScJAZ5 showed no change after inoculation 1 d, but maintained at relatively high levels at 3 d. The expression levels of ScJAZ2 and
Fig. 1 Phylogenetic relationships between sugarcane ScJAZ proteins, and the JAZ family proteins from *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa*, and *Vitis vinifera*. The maximum-likelihood method with 1000 bootstrap replications was used. GenBank Accession Numbers were as follows: AtJAZ1 (NP_564075.1); AtJAZ2 (NP_565096.1); AtJAZ3 (NP_566590.1); AtJAZ4 (NP_157283.2); AtJAZ5 (NP_564019.1); AtJAZ6 (NP_565043.1); AtJAZ7 (NP_181007.1); AtJAZ8 (NP_564349.1); AtJAZ9 (NP_177227.5); AtJAZ10 (NP_568287.1); AtJAZ11 (NP_189930.1); AtJAZ12 (NP_189930.1); ZmJAZ1 (GRMZM2G343157); ZmJAZ2 (GRMZM2G445634); ZmJAZ3 (GRMZM2G117513); ZmJAZ4 (GRMZM2G024680); ZmJAZ5 (GRMZM2G145412); ZmJAZ6 (GRMZM2G114548); ZmJAZ7 (GRMZM2G382794); ZmJAZ8 (GRMZM2G069200); ZmJAZ9 (GRMZM2G145407); ZmJAZ10 (GRMZM2G171830); ZmJAZ11 (GRMZM2G005954); ZmJAZ12 (GRMZM2G101769); ZmJAZ13 (GRMZM2G151519); ZmJAZ14 (GRMZM2G064775); ZmJAZ15 (GRMZM2G173596); ZmJAZ16 (GRMZM2G338829); ZmJAZ17 (GRMZM2G126507); ZmJAZ18 (GRMZM2G116614); ZmJAZ19 (GRMZM2G066020); ZmJAZ20 (GRMZM2G088976); ZmJAZ21 (GRMZM2G036351); ZmJAZ22 (GRMZM2G036288); ZmJAZ23 (GRMZM2G143402); VvJAZ1 (XM_002284819); VvJAZ2 (XM_002262714); VvJAZ3 (XM_00364378); VvJAZ4 (XM_002272327); VvJAZ5 (XM_002277733); VvJAZ6 (XM_002277769); VvJAZ7 (XM_002277916); VvJAZ8 (CBI30922); VvJAZ9 (XM_002277121); VvJAZ10 (XM_002263220); VvJAZ11 (XM_002282652); OsJAZ1 (BAG20771); OsJAZ2 (ABF94310.1); OsJAZ3 (ABF94311.1); OsJAZ4 (ABF96426.1); OsJAZ5 (ABF96480.1); OsJAZ6 (BAG98147.1); OsJAZ7 (Q8H351.1); OsJAZ8 (Q69P94.1); OsJAZ9 (Q8G50.1); OsJAZ10 (Q10O81.1); OsJAZ11 (Q8GR21.1); OsJAZ12 (Q7XE1.1); OsJAZ13 (Q7XG6.1); OsJAZ14 (Q7XEA.1); and OsJAZ15 (Q94LG1.1). The red triangle represents seven sugarcane ScJAZ proteins.
**Fig. 2** Tissue-specific expression analysis of seven *ScJAZ* family genes in different 10-month-old ROC22 tissues by qRT-PCR. Data was normalized to the GAPDH expression level. All data points are the means ± SE (*n* = 3). Different lowercase letters indicate a significant difference, as determined by the Duncan’s new multiple range test (*p*-value <0.05). R: Root; L: Leaf; B: Bud; SP: Stem pith; SE: Stem epidermis.

**Fig. 3** Expression analysis of seven sugarcane *ScJAZ* family genes during sugarcane-*Sporisorium scitamineum* interaction by qRT-PCR. Data was normalized to the GAPDH expression level. All data points (with the deduction of their mocks) were expressed as the mean ± SE (*n* = 3). Different lowercase letters indicate a significant difference, as determined by the Duncan’s new multiple range test (*p*-value <0.05). Yacheng05–179, smut-resistant sugarcane genotype; ROC22, smut-susceptible sugarcane genotype.
ScJAZ4 increased and peaked at 2 d after inoculation. Comparison of the expression of the ScJAZ genes in the two sugarcane varieties indicated that the transcripts of both ScJAZ4 and ScJAZ5 were upregulated in the smut-susceptible (ROC22) and smut-resistant (Yacheng05–179) genotypes after infection throughout the whole process, whereas that of ScJAZ1, ScJAZ2, ScJAZ3, and ScJAZ7 showed opposite expression patterns, and that of ScJAZ6 showed no significant difference in ROC22 but was upregulated at 1 d and downregulated in Yacheng05–179 at 3 d. These findings suggest that the expression of the ScJAZ genes was all induced after infection with S. scitamineum regardless of genotype, except for ScJAZ6 in ROC22, but showed different expression patterns.

Expression of the ScJAZ family genes in response to various defense-related signal compounds

Figure 4 shows that the expression patterns of the ScJAZ family genes in response to phytohormones such as SA, MeJA, and ABA. The seven members of the ScJAZ gene family were expressed after the application of SA, ABA, and MeJA. Under the SA and MeJA stimuli, the ScJAZ1–ScJAZ7 genes were rapidly upregulated and reached their peak values at 3 h, and were then gradually downregulated from 6 h to 12 h after treatment. Moreover, the transcripts of all these seven ScJAZ genes significantly varied during MeJA treatment. Following ABA stress application, although the changes in gene expression levels were not higher than the other two treatments, the transcripts of the ScJAZ1–ScJAZ7 genes increased from 3 h, peaked at 6 h, and then decreased at 12 h. These results revealed that these ScJAZ family genes exhibit the same expression pattern under the individual defense-related signal compounds stresses, which elicited a positive response to SA and ABA, particularly to MeJA.

Expression of ScJAZ family genes in response to various abiotic stressors

In the case of PEG, NaCl, CuCl2, H2O2, and CaCl2 stressors, the ScJAZ genes exhibited different levels of expression (Fig. 5). In response to PEG, the ScJAZ genes
showed different expression patterns, six (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ5, ScJAZ6, and ScJAZ7) were upregulated at 24 h except for ScJAZ4 at 12 h after treatment. All seven ScJAZ genes were upregulated after H2O2 treatment, and most of these peaked at 6 h after exposure. Significant changes in the levels of expression of ScJAZ1–ScJAZ7 were observed upon NaCl treatment and reached peak values at 24 h after treatment. Meanwhile, minimal changes in the transcript levels of ScJAZ1–ScJAZ7 were observed for the initial 24 h after treatment, which then sharply increased at 48 h, thereby indicating a positive role in response to CuCl2 stress.

The expression levels of six ScJAZ genes (ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ5, ScJAZ6, and ScJAZ7) slightly increased after CaCl2 treatment, whereas the expression of ScJAZ4 decreased. Interestingly, compared to that under CaCl2 and PEG stresses, the expression of the seven ScJAZ family genes was remarkably upregulated after H2O2, NaCl, and CuCl2 treatment.

Isolation of the full-length ScJAZ6 gene from sugarcane

The present study cloned and identified the full-length sequence of the ScJAZ6 gene (GenBank Accession No. KX352246). The cDNA length of ScJAZ6 was 1776 bp,
which included an intact ORF (1212 bp, from position 202 to 1413) that encoded a 403-aa polypeptide. Its molecular mass and pI were 42.28 kDa and 9.76, respectively. The NCBI search for conserved protein domains showed that ScJAZ6 contained a TIFY domain and a CCT_2 domain, thereby indicating that ScJAZ6 belongs to the TIFY family of JAZ proteins. The TIF[Y/F]XG motif at the N-terminal of the ScJAZ6 protein was located within the TIFY domain. The Jas motif SLX_{2}FX_{2}KRX_{2}RX_{2}PY at the C-terminal was situated within the CCT_2 domain. The amino acid sequence of ScJAZ6 showed 93%, 86%, and 86% homology with that of *Sorghum bicolor*, *Setaria italic*, and *Z. mays* JAZ proteins from NCBI, respectively.

### Subcellular localization of ScJAZ6

After *Agrobacterium*-mediated transformation, the subcellular localization of the ScJAZ6 protein in *N. benthamiana* leaves was determined (Fig. 6). After infiltration for 48 h, the fusion protein of ScJAZ6::GFP was located in the cytoplasm and the plasma membrane relative to that in the control.

### ScJAZ6 expression in *E. coli*

SDS-PAGE indicated the accumulation of a 50-KDa protein after the BL21 pET 32a–ScJAZ6 cells were induced by 1 mM IPTG at 28 °C for 2 h, 4 h, 6 h, and 8 h (Additional file 2: Figure S2). The growth of the control cells (BL21 pET 32a) and the gene-expressed cells (BL21 pET 32a–ScJAZ6) on LB plates with different supplements was studied (Fig. 7). After 24 h of cultivation, the BL21 pET 32a–ScJAZ6 cells showed a faster growth rate than that of the control, which was supplemented with PEG and CuCl_{2}, but not with NaCl (Fig. 7). Interestingly, the higher concentration of CuCl_{2}, the faster the growth of the BL21 pET 32a–ScJAZ6 cells. The above results indicated that the recombinant protein of ScJAZ6 enhance the tolerance of *E. coli* BL21 cells to PEG, particularly the CuCl_{2} stimulus.

### Transient overexpression of ScJAZ6 in *N. benthamiana* leaves induces defense response

The transient overexpression of ScJAZ6 in *N. benthamiana* leaves was observed (Fig. 8). After infiltration for 1 d, a typical hypersensitive response (HR) was observed in the test leaves compared to that in the control such as enhanced conductivity (Fig. 8a) and darker DAB staining color (Fig. 8d). A significant increase in ScJAZ6 transcript abundance was observed in the *N. benthamiana* leaves at 24 h after infiltration (Fig. 8b). Moreover, the expression levels of seven tobacco immunity-associated marker genes in *N. benthamiana*, namely, the hypersensitive response (HR) marker genes *NtHSR201*, *NtHSR203* and *NtHSR515*, the SA-related gene *NtNPR1*, *NtPR-1a/c*, and the ethylene synthesis depended genes *NtEFE26* and *NtAccdeaminase*, were upregulated by the transient overexpression of ScJAZ6 (Fig. 8c). To further validate the inhibitory effect of ScJAZ6 to pathogens, *Agrobacterium* EHA105 that harbored 35S::ScJAZ6 was infiltrated into leaves of *N. benthamiana* for 24 h, then two tobacco pathogens, *P. solanacearum* and *F. solani* var. *coeruleum*, were separately injected into *N. benthamiana*. Figure 8e shows that the leaves of the control exhibited more severe disease symptom than that of the *N. benthamiana* leaves with infiltration with 35S::ScJAZ6 after inoculation with *P. solanacearum* or *F. solani* var. *coeruleum* for 7 d. These results suggest that ScJAZ6 is associated with the HR or...
immunity of sugarcane, and its overexpression in *N. benthamiana* showed an antimicrobial action on *P. solanacearum* and *F. solani* var. *coeruleum*.

**Discussion**

**Isolation of sugarcane ScJAZ family genes and phylogenetic analysis**

The JAZ subfamily belongs to the TIFY family, which is a plant-specific family of putative transcription factors, plays a significant role in regulating the development and response of plants to abiotic and biotic stresses [49, 50, 71]. To date, JAZ family proteins have been identified and characterized in several plants such as *A. thaliana* [46], *V. vinifera* [60], *O. sativa* [54], *Z. mays* [59], *G. max* [55], and *N. attenuata* [56–58]. However, the expression and function of members of the JAZ family in sugarcane are unknown. The present study aimed to identify ScJAZ family genes in sugarcane after *S. scitamineum* inoculation and to systematically analyze their sequence characters and expression profiles under various stress-related conditions.

In the present study, seven ScJAZ family genes were identified in sugarcane after *S. scitamineum* inoculation. Multiple alignment analysis showed that all these seven ScJAZ proteins shared a relatively low level of amino acid sequences identity (23.63%), and the number of amino acids varied from 136 to 403. JAZ subfamily proteins with high sequence difference have also been identified in *A. thaliana* [46] and *O. sativa* [54], suggesting that these genes may have undergone major mutations and functional divergence. Sequence analysis showed that all seven ScJAZ proteins contained a conserved ZIM domain and a Jas motif (Additional file 2: Figure S1), which is consistent to the findings of previous reports of Chung et al. [72] and Staswick et al. [73]. Previous studies also demonstrated that several *A.
thaliana] JAZ proteins engage in homomeric and heteromeric interactions that are regulated by the TIFY motif (TIFF/YXG) within the ZIM domain [72]. The function of the ZIM domain was to recruit transcriptional corepressors [74]. Similar to other plant species such as A. thaliana [46], V. vinifera [60], and Z. mays [59], domain architecture analysis showed that all these seven sugarcane ScJAZ proteins contained two conserved domains, namely, the TIFY and CCT_2 domains. The ScJAZ protein family was clustered into two clades (Fig. 1), which were in agreement with the findings in A. thaliana [46] and Z. mays [59].

Transcripts of the ScJAZ family genes are differentially expressed in various sugarcane tissues

Previous studies have shown that JAZ genes are differentially and constitutively expressed in plants such as Hevea brasiliensis [75], Z. mays [59], and O. sativa [54]. Most HbJAZ genes (HbJAZ1, HbJAZ2, HbJAZ7, HbJAZ8, HbJAZ9, HbJAZ10, and HbJAZ11) in H. brasiliensis showed higher levels of expression in the leaves than in the bark [75]. OsTIFY3 showed constitutively high expression levels in the root, callus, panicle, sheath, and flag leaf [54]. In Z. mays, ZmJAZ13 and ZmJAZ23 exhibited constitutive expression in the root, stalk, leaf, apex, silk, tassel, and seeds [59]. Similarly, in the present study, the transcripts of the seven ScJAZ family genes were differentially expressed in various sugarcane tissues (Fig. 2). ScJAZ1, ScJAZ3, ScJAZ4, and ScJAZ7 showed the highest expression level in the buds and leaves; ScJAZ2 and ScJAZ5 showed the highest levels of expression in the leaves, whereas ScJAZ6 was highest in roots and buds. These findings indicate that ScJAZ family genes are constitutively expressed in sugarcane tissues. Sugarcane buds often serve as the route of entry for smut pathogen infections [29]. Previous studies have shown that when sugarcane is infected with S. scitamineum, differential changes in the bud ultrastructure occur, i.e., the cell wall is damaged in susceptible varieties, whereas these remain intact in resistant cultivars [8]. The transcripts of most ScJAZ genes were abundant in sugarcane bud and leaf tissues, suggesting that ScJAZ genes may take part in the resistance to S. scitamineum infections.

The majority of sugarcane JAZ genes shows a positive response to S. scitamineum infections

Biotic stressors such as pathogen infection and insect herbivory negatively influences plant growth [3, 4]. Plant defense mechanisms can be mediated by hormone signaling such as JA and SA [76, 77]. Previous studies have shown that the JAZ genes are involved in plant pathogen...
resistance [52]. In *A. thaliana*, the AtJAZ1–AtJAZ12 genes are induced by *Pseudomonas syringae* infection [52]. Approximately 12 SlJAZ genes (SlJAZ1–SlJAZ12) in tomato are upregulated by *Pst* DC3000 [37]. In the present study, the transcripts of seven ScJAZ genes were induced by *S. scitamineum* infection (except for ScJAZ6 in ROC22), but showed different expression patterns between the two sugarcane genotypes after smut pathogen inoculation (Fig. 3). In the smut-susceptible genotype (ROC22), the expression of ScJAZ4, ScJAZ5, and ScJAZ6 were upregulated by infection, whereas that of ScJAZ1, ScJAZ2, ScJAZ3, and ScJAZ7 did not change with infection. In contrast, ScJAZ1, ScJAZ2, ScJAZ3, ScJAZ4, ScJAZ5, and ScJAZ7 were downregulated after inoculation, whereas ScJAZ6 showed no response to *S. scitamineum* infection in the smut-resistant genotype (Yacheng05–179). These findings indicate that the ScJAZ genes may be involved in the response to smut pathogen infection and played different roles in the two sugarcane genotypes.

**Sugarcane JAZ genes are responsive to various plant hormones and abiotic stresses**

A previous study has shown that plant hormones, including SA, JA, and ethylene, act as defense signal compounds for two types of plant-induced resistance, including systemic acquired resistance (SAR) and induced systemic resistance (ISR) [78]. Recent investigations have demonstrated that JA treatment and environmental stress could rapidly trigger the expression of JAZ genes, which in turn regulates their responses to JA [42, 43, 79]. In *V. vinifera*, *VvJAZ4, VvJAZ5*, and *VvJAZ9* are induced by both JA and MeJA [60]. In the present study, we found that the *ScJAZ1–ScJAZ7* genes were upregulated by SA and MeJA, with the latter showing a more significant induction. JAZ genes also play an important role in the JA signaling pathway in *Arabidopsis* and rice [44, 54]. Transgenic *Arabidopsis* overexpresses JAI3, which encodes a JAZ protein that harbors a short Jas motif, thereby resulting in an MeJA-insensitive phenotype [44]. Another phytohormone, ABA, also plays an important role in the response of plants to adverse environmental conditions [80]. For instance, four JAZ genes reported in *O. sativa* are responsive to ABA [80]. In this study, seven *ScJAZ* genes were all upregulated by ABA at 6 h, thereby suggesting that the expression of these *ScJAZ* genes could be regulated by ABA-dependent signaling pathways [60].

In addition to plant hormones, JAZ genes could be induced by various types of abiotic stresses such as wounding [81], herbivores [56], and salt and alkali [55]. In *Z. mays*, the expression of *ZmJAZ14* is induced by salt and PEG [59]. In the present study, all *ScJAZ* genes were immediately induced by H$_2$O$_2$, NaCl, and CuCl$_2$, and slightly induced by CaCl$_2$ and PEG (Fig. 4), thereby suggesting that the *ScJAZ* genes may play roles in different types of stress pathways in sugarcane. A recent study has shown that the overexpression of *OsTIFY11a* in rice results in an improvement in stress tolerance such as drought, salt, and low temperature [44].

**The functional verification of ScJAZ6: Subcellular localization, prokaryotic expression, and transient overexpression**

To date, several plant JAZ genes have been characterized in *A. thaliana* [46], *O. sativa* [54], *N. tabacum* [56], and *Glycine soja* [82]. In this study, *ScJAZ6* was upregulated by *S. scitamineum* induction in the resistant genotype Yacheng05–179, but remained unchanged in the susceptible genotype ROC22 (Fig. 3). As reported, the expression of recombinant plant proteins in *E. coli* cells enhances cell growth when under high temperature (47 °C), NaCl, carbofuran, CdCl$_2$, CuCl$_2$, and UV-B stressors [83]. The expression of the *ScJAZ6* gene resulted in a positive response to the SA, ABA, MeJA, H$_2$O$_2$, NaCl and CaCl$_2$ stressors (Figs. 4 and 5). Moreover, the transcripts of *ScJAZ6* were also significantly upregulated by CuCl$_2$ (Fig. 5), which coincided with the results of the spot assay in that the expression of the recombinant protein of *ScJAZ6* in *E. coli* BL21 cells resulted in better growth under CuCl$_2$ stress (Fig. 7). Interestingly, the higher concentration of CuCl$_2$, the better the cell growth, thereby suggesting that *ScJAZ6* could enhance the tolerance of sugarcane to CuCl$_2$. Subcellular localization of *ScJAZ6* in *N. benthamiana* showed that 35S::*ScJAZ6::GFP* was located in the cytoplasm and the plasma membrane (Fig. 6). However, previous studies have shown that *ZmJAZ14* and *GsJAZ2* were only located in the nucleus [55, 59]. Cell death could efficiently restrict pathogen growth and development and trigger some reaction such as the induction of R gene expression, ion fluxes, stimulation of ROS, and defense-related hormones [84–86]. In this study, during transient expression of *ScJAZ6* in *N. benthamiana* leaves, the darker DAB staining color was indicative of the accumulation of H$_2$O$_2$ in the *N. benthamiana* leaves after 24 h of infiltration (Fig. 8d). As Levine et al. [87] reported, H$_2$O$_2$ played a key role in evaluating a localized hypersensitive response. Furthermore, the immunity-associated marker genes involved in the HR, SA, JA, and ethylene signaling pathways were upregulated (Figs. 8c and d), which in turn induces a defense response in *N. benthamiana*, and an antimicrobial action against the pathogenic bacteria, namely, *P. solanacearum*, and *F. solani* var. *coeruleum* (Fig. 8e). These results showed that the expression of the *ScJAZ6* gene is closely related to plant immunity and HR, which is in agreement with the findings of Levine et al. [87] and Ron and Avni [88].
Conclusions
Transcriptome analysis of *S. scitamineum*-resistant and -susceptible sugarcane genotypes respectively challenged with *S. scitamineum* identified seven sugarcane JAZ family genes that encoded proteins harboring a N-terminal ZIM domain and C-terminal Jas motif. The ScJAZ1–ScJAZ7 genes were constitutively expressed in the sugarcane root, bud, leaf, stem pith, and stem epidermis tissues. Transcript expression of these ScJAZ genes were observed after infection with *S. scitamineum*, regardless of genotype (except for ScJAZ6 in ROC22), but were differentially expressed. In the case of phytohormones and various abiotic stresses, the seven ScJAZ family genes were all upregulated by the SA, ABA, MeJA, H2O2, NaCl, and CuCl2 stimuli. In addition, the overexpression of ScJAZ6 in *E. coli* BL21 cells enhanced its growth under CuCl2, NaCl, and PEG stimuli. Moreover, the transient overexpression of ScJAZ6 in *N. benthamiana* leaves resulted in enhanced conductivity, darker DAB staining color, and increased expression levels of the seven tobacco immunity-associated marker genes, as well as an antimicrobial activity. The findings of the present study may be utilized in future research investigations on the function of JAZ family genes in sugarcane, and may also serve as a basis for the elucidation of the mechanism underlying sugarcane immunity.

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Availability of data and materials
The data supporting the conclusions of this article are within the paper and its Additional files. All sequencing reads which contain the seven ScJAZ unigenes are deposited in the National Center for Biotechnology Information under the BioProject number PRJNA395694 with the Sequence Read Archive (SRA) study accession SRP115507.

Authors’ contributions
YCS and YXQ conceived and designed the experiments. FL, TTS, LW and WHS performed the experiments. FL, SWG and YCS analyzed the data. FL and YCS wrote the manuscript. YCS, YXQ and LPX revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable. This is to confirm that no specific permits were needed for the described experiments, and this study did not involve any endangered or protected species.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Abbreviations
2DE: Two-dimensional gradient polyacrylamide gels; aa: Amino acids; ABA: Abscisic acid; BHHL zip-type: Basic helix-loop-helix leucine zipper-type; CaCl2: Calcium chloride; cDNA-AFLP: cDNA-amplified fragment length polymorphism; COII: Coronatine insensitive 1; CuCl2: Copper chloride; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; H2O2: Hydrogen peroxide; HR: Hypersensitive reaction; IPTG: Isopropyl β-D-thiogalactoside; ISR: Induced systemic resistance; JAZs: Jasmonate ZIM domains; LAMP: Loop-mediated isothermal amplification; MALDI-TOF-TOF/MS: Matrix-assisted laser desorption/ionization time of flight mass spectrometry; MeJA: Methyl jas monotone; NaCl: Sodium chloride; ORF: Open reading frame; pI: isoelectric point; PEG: Polyethylene glycol; PR: Pathogenesis-related protein; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; RNA-seq: RNA sequencing; RT-PCR: Reverse transcription-polymerase chain reaction; SA: Salicylic acid; SAR: Systemic acquired resistance; SCF: Skp/Cullin/F-box; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SE: Standard error; TDFs: Transcript-derived fragments

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