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Full Length Research Paper

Molecular cloning and expression analysis of a zeta-class glutathione S-transferase gene in sugarcane

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Glutathione S-transferases (GSTs) play an important role in stress tolerance in plants. This is the first report of cloning and characterization of a zeta-class *GST* gene in sugarcane (GenBank Accession number: GQ246461). Sequence analysis showed that the cDNA sequence of *Sc-GST* gene was 829 bp, contained a 621 bp open reading frame (ORF), the 5' untranslated region (UTR) of 65 bp and 3'UTR of 143 bp, plus the typical AATAA region and poly (A) tail. It encoded the 206 amino acid residues with a molecular mass of 23.1 KD and isoelectric point of 6.10. Protein domain prediction and multiple sequence alignment demonstrated that the conserved domain in Sc-GST at N-terminus was SSCXXRXRIA, while that at C-terminus was quebec platelet disorder (QPD), both of which were specific for zeta-type GST in eukaryotes. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and enzyme activity assay indicated that the prokaryotic expression product was a fusion protein with a molecular weight of about 30 KD, which also possessed GST enzyme activity. It was revealed in real-time quantitative polymerase chain reaction (qPCR) analysis that the *Sc-GST* gene had induced expression under H_2O_2 and *Ustilago scitaminea* stresses, while it was inhibited and then induced by salicylic acid (SA) stress, suggesting that it is a type of stress-tolerant gene playing a certain role in sugarcane resistance response.

Key words: *Saccharum officinarum*, glutathione S-transferase, homology, prokaryotic expression, real-time quantitative PCR.

INTRODUCTION

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a group of multifunctional enzymes, which play a key role in cellular detoxification. They can catalyze the conjugation of the tripeptide glutathione (GSH; γ-Glu–Cys–Gly) into a variety of hydrophobic, electrophilic, and usually cytotoxic substrates, and thus reduce glutathione (GSH) and protect the cellular macromolecules from attack of these toxic compounds. Since the first *GST* gene was cloned from *Zea mays*, a number of *GST* genes or its homologous sequences had been identified and annotated from a variety of plants (Shimabukuro et al., 1970; Basantani and Srivastava, 2007; Chi et al., 2010). According to their protein homology and genomic structure, the GSTs in plants were classified into 8 types, including the phi, tau, theta, zeta, lambda, dehydroascorbate reductase (DHAR) and tetrachlorohydroquinone dehalogenase (TCHQD) which were soluble (cytoplasmic) plus the microsomal one (Dixon et al., 2002a; Moons, 2005; Basantani and Srivastava, 2007). Among them, two classes, phi and tau, were specific to plants and belonged to the most numerous and the abundance of these enzymes; three smaller classes, zeta and theta (also found in animals and fungi) and lambda were widely distributed in eukaryotes, while both types of DHAR and TCHQD were only recently

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Abbreviations: GST, Glutathione S-transferase; **IPTG,** isopropyl-β- D-thiogalactopyranoside; **DHAR,** dehydroascorbate reductase; **TCHQD,** tetrachlorohydroquinone dehalogenase; **HR,** hypersensitive response; **SAR,** systemic acquired resistance; **QPD,** quebec platelet disorder; **qPCR,** quantitative polymerase chain reaction; **SA,** salicylic acid.

reported in *Arabidopsis*, rice and soybean (Dixon et al., 2002a). Besides, the *GST* gene was a kind of multi-gene family, and so far it had been found that, *Arabidopsis thaliana* had 48 *GST* genes, *Glycinemax* had 25 *GST* genes, *Zea mays* had 42 *GST* genes and *Oryza sativa* had 59 *GST* genes, respectively (Dixon et al., 2002b; Moons, 2003; Soranzo et al., 2004; Chi et al., 2010).

Previous studies have demonstrated that plant GSTs play an important role in various stress responses. Several researches revealed that, the expression of GSTs in plants was highly responsive to biotic and abiotic stress and a wide variety of stress-associated chemicals, including those arising from pathogen attack, oxidative stress and heavy-metal toxicity, such as microbes, plants, insects and vertebrates and other synthetic and natural auxins, salicylic acid, methyl jasmonate, abscisic acid and H_2O_2 from the toxic effects of electrophilic compounds (Dixon et al., 2002a; 2002b; Moons., 2005). Tobacco seedlings which over-expressed a tobacco tau class *GST* gene were found to be more tolerant to chilling and osmotic stress than wild-type plants (Roxas et al., 1997), while a *GST* gene discovered in black grass participated in oxidative stress tolerance (Cummins et al., 1999). A tomato tau class *GST* gene was reported to confer resistance to oxidative stress (Kampranis et al., 2000). Cho et al. (2007) revealed, the expression of a phi class *GST* gene from *O. sativa* appeared to play a role in the conjugation of herbicide and GPOX activity. Expression profiling of the GST family in sorghum and other higher plants indicated that this gene family played a role not only in stress-related biological processes but also in the sugar-signalling pathway (Chi et al., 2010). All the earlier mentioned researches represent an era of attempting to assign functions for GSTs in endogenous metabolism and development.

Sugarcane is an important commercial or cash crop of tropical and sub-tropical regions. Kurama et al. (2004) identified three expressed sequence tag (EST) clusters homologous to *GST* consensus sequence in sugarcane EST genome through the sequencing of ESTs of a sugarcane database derived from libraries induced and not induced by pathogens. A comparison of the deduced amino acid sequence of these three sugarcane EST clusters with cloned *GST* genes in GenBank database revealed a high degree of similarity with the phi class *GST* gene. Expression analysis by reverse transcriptase polymerase chain reaction (RT-PCR) showed that all these EST expressed in sugarcane leaves inoculated with *Puccinia melanocephala*. To our knowledge, this is the only report about *GST* gene identification and characterization in sugarcane. Until now, there is still no report about the molecular cloning and characterization of the full-length *GST* gene in sugarcane, which should be helpful in knowing the stress tolerance mechanism and improvement of sugarcane varieties by molecular breeding approach. The objective of this study was to clone and characterize one full-length *GST* gene in sugarcane.

MATERIALS AND METHODS

Sugarcane variety FN 22, sugarcane stem full-length cDNA library, *Escherichia coli* DH*5α* and BL21 (DE3), and the prokaryotic expression vector were provided by Key Laboratory of Sugarcane Genetic Improvement, Ministry of Agriculture, People's Republic of China. Restriction enzyme *EcoR* I, *Sal* I, T_4 DNA ligase, Taq enzyme, DNA and protein molecular marker, SYBR®Premix Ex Taq™ were purchased from TaKaRa (Japan); Isopropyl-β-D-thiogalactopyranoside (IPTG) and Reverse Transcription kit were from Promega Corporation (USA); and the instrument used in the real-time qPCR analysis was the ABI PRISM7500 real-time PCR system.

Strong and evenly growing sugarcane stalks were soaked and sterilized in water with 0.1% potassium permanganate for 24 h and then planted into autoclaved fine sand in 10 trays, with 40 shoots in each tray. These trays were then placed into the illumination incubator where each day they received daylight for 12 h at 29°C and nightlight for 12 h at 26°C with a light intensity of 440 mol m^{-2} s^{-1}. When growing to the height of 15 cm, they were transferred into the greenhouse for cultivation and sprayed with Hongland nutrient solution. In the six leaves period, evenly growing stalks were selected and then the sand was removed. After rehydration for 2 days, the stalks were cultivated in Hongland nutrient solution for one week and then treated as follows: In group one, 5 m mol·L^{-1} SA and 10 mmol·L^{-1} H_2O_2 were sprayed onto the leaves, respectively (Que et al., 2009a). In group two, spore suspension with concentration of 5×10^6 spores ml^{-1} was inoculated through stabling bud tissues (Que et al., 2009a). The sampling times were 0, 12, 24, 36, 48, 60 and 72 h for SA and H_2O_2 treatment and 0, 12, 24, 36, 48 and 60 h for *Ustilago scitaminea* treatment. All the samples collected were immediately fixed in liquid nitrogen, and stored in a refrigerator at -85°C until RNA extraction.

Obtaining and sequence analysis of full-length cDNA of *Sc-GST* gene

The large-scale sequencing and bioinformatics analysis were conducted for the full-length cDNA library of sugarcane stem (Private bulletin). The library clones which contained EST highly homologous to *GST* gene in National Center for Biotechnology Information (NCBI) database were selected and sequenced completely to obtain sugarcane full-length *GST* gene.

For the full-length cDNA sequence of *GST* gene, open reading frame (ORF) was predicted with the online tool ORF Finder from NCBI (http://www.ncbi.nlm.nih. gov/gorf/gorf.html). The BLAST program in NCBI was used for the homology analysis. Besides, the ProtParam (http://cn.expasy.org/tools /protparam.html) was applied to analyze the basic properties of the encoding protein. Multiple sequence alignment and phylogenetic analysis were performed with DNAMAN software (version 6.0) and MEGA3.1. InterProScan (http://ebi.ac.uk/InterProScan) and SMAR (http://smart.embl-heidelberg.de) were used to analyze the putative domain of the encoding protein of *GST* gene. The gene sub-cellular localization was carried out with SubLoc V1.0 (http://www.bioinfo.tsinghua.edu.cn/SubLoc/) and the signal peptide prediction was performed with SignalP3.0 (Error! Hyperlink reference not valid.). SOPMA was adopted for the secondary protein structure prediction (http://npsa-pbil.ibcp.fr/cgi-bin/npsaautomat.pl?page=npsa_sopma.html) and the analysis of the repetitive sequence of the amino acid was performed with the REP searcher (http://www.embl-heidelberg.de/~andrade/papers/rep/search.html).

Construction of prokaryotic expression vector of *Sc-GST* gene

pET29a (+) was used as the prokaryotic expression vector and the primer pairs amplifying the ORF of *Sc-GST* were designed. The primer sequences were as follows, of which the underlined parts were the restriction endonuclease site of *EcoR* I and *Sal* I, respectively: *Sc-GST*-F: 5'-TTGGATCCATGGCAGAGGCGGAG-3′; *Sc-GST*-R: 5′-TTGTCGACGGAAGAGGAAGGGG-3′.

The primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services CO., Ltd. With plasmid DNA extracted from the library clone containing *Sc-GST* gene as the template, the PCR amplification was carried out in a 50 µl PCR reaction. It was composed of 5µl 10 × PCR buffer, 4 µl 2.5 mmol·L^{-1} deoxynucleotide triphosphates (dNTPs); 2 µl 10 µmol·L^{-1} of forward and reserves primers, respectively; 2 µl plasmid DNA; 0.25 µl Taq enzyme (5 U·µl); ddH_2O was added as the supplement. PCR amplification program was pre- denatured for 5 min at 94℃; denaturation for 30 s at 94℃, annealing for 30 s at 55℃ and extension for 1 min at 72℃, 30 cycles; followed by final extension for 10 min at 72℃. When the reaction finished, 1% agarose gel electrophoresis was performed and the target PCR product was recovered. After receiving the double digestion with *Eco*R I and *Sal* I, the obtained ORF sequence was ligated by T_4 DNA ligase into pET29a (+) which had also executed the double digestion with *Eco*R I and *Sal* I. The recombinant was then transferred into *E. coli* DH5α. The plasmid DNA of several clones selected by blue/white colony screening was extracted. A positive clone validated by PCR and double digestion was just the prokaryotic expression vector termed the pET29a-*ScGST* (Que et al., 2009b).

SDS-PAGE analysis of prokaryotic expression product and enzyme activity assay

pET29a-*ScGST* and empty pET29a (+) were both transformed into *E. coli* BL21 (DE3). The single colony was inoculated into the LB medium containing the ampicillin (100 µg·ml^{-1}) for overnight shake culture at 150 r/min at 37℃. In the following day, 1% of the medium was inoculated into the LB medium containing 100 µg·ml^{-1} ampicillin and shake cultured. When OD_{600} reached 0.4 to 0.6, 1 ml of LB liquid medium was collected as the control, and the remaining medium was added with IPTG with the final concentration 1 mmol·L^{-1}. The LB medium with pET29a-*ScGST* (BL21) was induced for 2, 4 and 6 h, respectively at 37℃, and 1 ml medium was collected at each time point; the LB medium with empty pET29a (+) (BL21) was induced in IPTG for 6 h and 1 ml medium was collected. The medium collected was used for the SDS-PAGE with 25 µl sample loading and also for GST enzyme activity assay according to Habig et al. (1974). When the electrophoresis finished, the gel was colored with Coomassie brilliant blue and imaged (Que et al., 2009b).

Expression profile of *Sc-GST* gene under various exogenous stresses

25S rRNA was selected as the control gene in the real-time qPCR analysis (Que et al., 2009c). According to the sequence of *Sc-GST* and *25S rRNA* (BQ536525), two pairs of real-time quantitative polymerase chain reaction (qPCR) primers were designed with GenScript Online PCR Primers Design Tool (http://www.genscript.com/cgi-bin/tools/primer_genscript.cgi). The forward and reverse primers of *Sc-GST* gene were 5′-CTCGACTTCGAGATCATCCC-3′ and 5′-AGGTAGAGCA GGTAGGACGC-3′, respectively. For the *25S rRNA*, they were 5′-GCAG CCAAGCGTTCATAGC-3′ and 5′-CCTATTGGTGGGTGAACAATCC-3′, respectively.

The 20 µl reverse transcription system contained 4 µl 25 mmol·L^{-1} $MgCl_2$, 2 µl 5 × RT reaction buffer, 2 µl 10 mmol·L^{-1} dNTPs, 0.5 µl RNase inhibitor, 0.5 µl Random primers (0.5 µg), 15 U AMV reverse transcriptase and 4 µl total RNA (1 µg) as the template and RNase free H_2O as the supplement. Reverse transcription program was as follows: it was firstly kept at room temperature for 10 min, then incubated at 42℃ for 15 min and 95℃ for 5 min, and then kept at 0 to 5℃ for 5 min. In real-time qPCR amplification, the RT-PCR product was used as the template and the total volume of the reaction system was 25 µl, including SYBR Primix Ex Taq^{TM} (2×), 12.5 µl; Rox Reference DYE II, 0.5 µl; forward and reverse primers of 10 µmol·L^{-1}, 0.5 µl each; cDNA template, 2.5 µl; and sterile water, 8.5 µl. Three replicas were set for each sample. The PCR reaction conditions were pre-denatured at 95℃ for 10 s, and then 40 cycles with 94℃ for 5 s and 60℃ for 25 s. When the reaction was completed, the melting curve was analyzed. The method of $2^{-\Delta\Delta CT}$ was adopted to analyze the real-time qPCR results (Livak and Schmittgen, 2001; Que et al., 2009d).

RESULTS

Cloning and sequence analysis of *Sc-GST* gene

Through the large-scale sequencing and bioinformatics analysis, one EST, which had 87% homology to a *GST* gene from *Z. mays* (EU970141), was obtained from sugarcane stem cDNA library. The library clone containing this EST was then sequenced completely and a full-length cDNA sequence of *GST* gene in sugarcane was obtained and termed as *Sc-GST*. It was submitted to the NCBI with the GenBank Accession number of GQ246461. The *Sc-GST* had a full length of 829 bp, contained a ORF of 621 bp, 5' UTR (untranslated region) of 65 bp and 3'UTR of 143 bp, and the typical AATAA and poly (A) tail could also be found in the 3'UTR (Figure 1).

The primary structure of the protein predicted was shown in Figure 1. The ORF of *Sc-GST* gene encoded 206 AA residues with the molecular weight of encoding protein of 23.1 KD and isoelectric point of 6.10. Protein domain prediction showed that it had the conserved domain both at the N- and the C-terminus (Figure 2).

Sequence homology analysis of *Sc-GST* gene

The blastn alignment analysis indicated that, the homology between the *Sc-GST* gene and the zeta-type *GST* gene in *Z. mays* (EU970141) was 84% (671/796). It also showed that the homology of the *Sc-GST* encoding protein with that of the zeta-type *GSTs* in *Z. mays* (ACG42259), *O. sativa* (ABI17930) and *Brassica napus* (AAO60040) were 87% (196/224), 71% (161/225) and 54% (122/222), respectively. Therefore, it could be initially determined that the *Sc-GST* gene obtained in this study was of zeta type. Furthermore, through the Conserved Domain Database (CDD) search of the protein conserved domain in NCBI, it could be found that the GST protein encoded by the obtained *Sc-GST* had the typical characteristics of the zeta-type GST structure both at the C- and N-terminus, which further determined that this *Sc-GST* was of zeta type (Figure 3).

The zeta-type GSTs from different species were

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1      CTCTCGCTCT CGCGTGTTTC AGCGAAGCGG CTAGGCAATC GTCAGTTCGT CACTGCAATC
61     TGCAAATGGC AGAGGCGGAG GCGACGACGG GGCGGCTGAG GCTGTACTCG TACTGGCGTA
           M  A   E  A  E   A  T  T  G   R  L  R   L  Y  S   Y  W  R  S
121    GCTCATGCTC CCACCGCGCC CGCATCGCTC TCAATCTCAA AAGTGTGGAT TACGAGTACA
         S  C  S   H  R  A   R  I  A  L   N  L  K   S  V  D   Y  E  Y  K
181    AGGCGGTGAA CCTTCTCAAG GGCGAGCAGT CTGATCCAGA ATTCGTCAAG CTTAATCCTA
         A  V  N   L  L  K   G  E  Q  S   D  P  E   F  V  K   L  N  P  M
241    TGAAGTTCGT CCCTGCGTTG GTTGATGGCG ATCGTGTAAT CGGTGACTCT TATGCGATAG
         K  F  V   P  A  L   V  D  G  D   R  V  I   G  D  S   Y  A  I  A
301    CATTGTATTT GGAGGACAAG TACCCAGAAC CTCCTCTTCT ACCTCAAGAC CTTCAAAAGA
         L  Y  L   E  D  K   Y  P  E  P   P  L  L   P  Q  D   L  Q  K  K
361    AGGCTTTGAA TCACCAGAGG TTCATTGACC AAAAGGTTGG TGCTGGGGAG AGTGTCTTGT
         A  L  N   H  Q  R   F  I  D  Q   K  V  G   A  G  E   S  V  L  W
421    GGACTCAACA ACAAATCGAG AGAGGTTTCA CAGCTATTGA GAACCTAATA CAACTAAAAG
         T  Q  Q   Q  I  E   R  G  F  T   A  I  E   N  L  I   Q  L  K  G
481    GATGTGCTGG GAAGTATGCA ACAGGAGATG AAGTCCAACT GGCGGATGTA TTCCTTGCAC
         C  A  G   K  Y  A   T  G  D  E   V  Q  L   A  D  V   F  L  A  P
541    CCCAGATCTA TGCAGCCATT GAACGCACTA AAATTGATAT GTCAAATTAC CCCACGCTTT
         Q  I  Y   A  A  I   E  R  T  K   I  D  M   S  N  Y   P  T  L  S
601    CTAGGCTTCA CTCGGAGTAC ATGGCACACC CTGCATTTGT AGCAGCGCTC CCTGGCAGGC
         R  L  H   S  E  Y   M  A  H  P   A  F  V   A  A  L   P  G  R  Q
661    AACCAGACGC CCCTTCCTCT TCCTAGGAAC TGCACCCTGT TTGCTCCGTC GTATTGTTCC
         P  D  A   P  S  S   S  *
721    TCTGGATATA TAGATTTGGA GTTGCTCCGG TTACTTCTGT AAGAATAATA AGTACAGAAT
781    TTCGTCTGCA AAGTCAAATA TTTTCGCAAA AAAAAAAAAA AAAAAAAAA
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Figure 1. Nucleotide sequence of *Sc-GST* gene and primary structure of Sc-GST protein. Capital and small letters represent amino acid and nucleotide, respectively; *represents stop codon; the underline shows the polyadenylation signal AATAA.

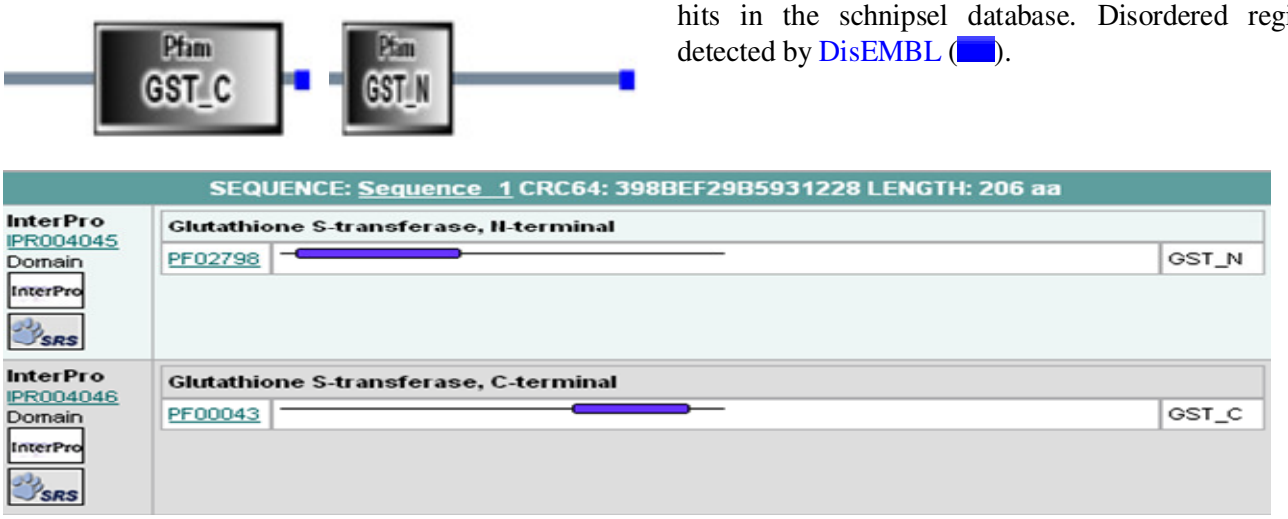

Figure 2. Putative domain of Sc-GST protein.

searched and downloaded from the NCBI, and DNAMAN was used for the multiple sequence alignment towards the amino acid of these zeta-type GSTs. The results of multiple sequence alignment were shown in Figure 4, in which the shaded part was the conserved region. Multiple sequence alignment showed that the N-terminus of all proteins was highly conserved with the typical SSCXXRXRIA domain, which was specific in zeta type

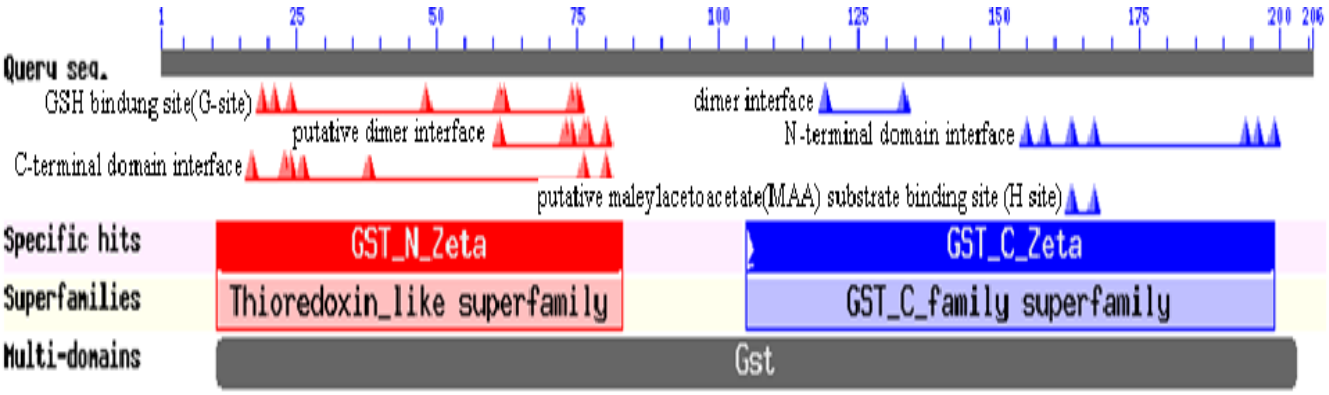

Figure 3. Protein domain analysis of Sc-GST protein.

GST in eukaryotes. These proteins also had the quebec platelet disorder (QPD) conserved region at the C-terminus, which again indicated that the Sc-GST was of zeta type.

Using the MEGA3.1 software, the phylogenetic tree of the Sc-GST and the zeta-type GSTs from other species was constructed. Figure 5 showed that these zeta-type GST proteins consisted of two branches, one from animals and the other from plants. In the branch of plant, the Sc-GST and the GST of the *Z. mays* were classified into one group while that from *A. thaliana* and *B. napus* were classified into the other group, showing that the homological evolutionary relationship between the two within one group was close. Besides, it could be found that the relationship between the zeta-type GST of Gramineae and Cruciferae in the category of plant was not that close, maybe they evolved from the same ancestor along different pathways.

Construction of the *Sc-GST* gene prokaryotic expression vector

After the PCR product from the ORF amplification of *Sc-GST* gene had been recovered, it received the double digestion with *Bam*H I-*Sal* I. The target fragment was then recovered to ligate by T_4 DNA ligase into the pET29a (+) which also received the double digestion by *Bam*H I and *Sal* I, and the positive recombinant pET29a-*ScGST* was then transformed into the *E. coil* DH*5a*. The fragment with the same size as that of the target could be obtained from pET29a-*ScGST,* through the identification with PCR amplification and *Bam*H I + *Sal* I digestion. The results of the agarose gel electrophoresis are shown in Figure 6, indicating the successful construction of the prokaryotic expression vector pET29a-*ScGST*.

SDS-PAGE analysis of the prokaryotic expression product and enzyme activity assay

The SDS-PAGE electrophoresis results of the prokaryotic expression products are shown in Figure 7. It showed that the Sc-GST protein began to express in the second hour with the weight of 30 KD, which was basically consistent with the deduced molecular weight of the fusion protein of 30.1 KD, including *Sc-GST* encoding protein of 23.1 KD plus the His • Tag peptides of 7 KD, while no target protein was expressed in the empty vector. Enzyme activity assay indicated that the prokaryotic expression product possessed the GST enzyme activity. From the aforementioned, it indicated again that this prokaryotic expression vector was successfully constructed, without wrong coding or reading-frame shift of the amino acid sequence, and *Sc-GST* gene had the successful expression in the BL21 (DE3).

Expression profile of *Sc-GST* gene under different kinds of exogenous stresses

Real-time PCR was used to examine the expression profile of *Sc-GST* gene under various exogenous stresses. Figure 8 shows that the expression profile of *Sc-GST* gene varied with different types of stresses. Under the stress of SA, the expression of *Sc-GST* gene was firstly inhibited, which lasted at the time points of 12 and 24 h, and the expression of *Sc-GST* gene was induced but not obviously at the time points of 36, 48 and 60 h with the expression value almost equal to that at 0 h. At the time point of 72 h, the expression of this gene was inhibited again. Under the H_2O_2 stress, the expression of *Sc-GST* gene was induced during the whole period with the "increasing-declining-increasing" trend. At the time point of 12 h, the expression was 1.7 times that of the control at 0 h, and decreased to be equal to that of the control at the time point of 24 h. It was up-regulated gradually after the time point of 36 h and reached the peak at the 72 h time point which was 5.6 times of that of control at 0 h. After the *U. scitaminea* inoculation, the *Sc-GST* gene expression in sugarcane was constantly induced, and the induction was rather strong at the time point of 12 h with the expression 6.9 times that of control

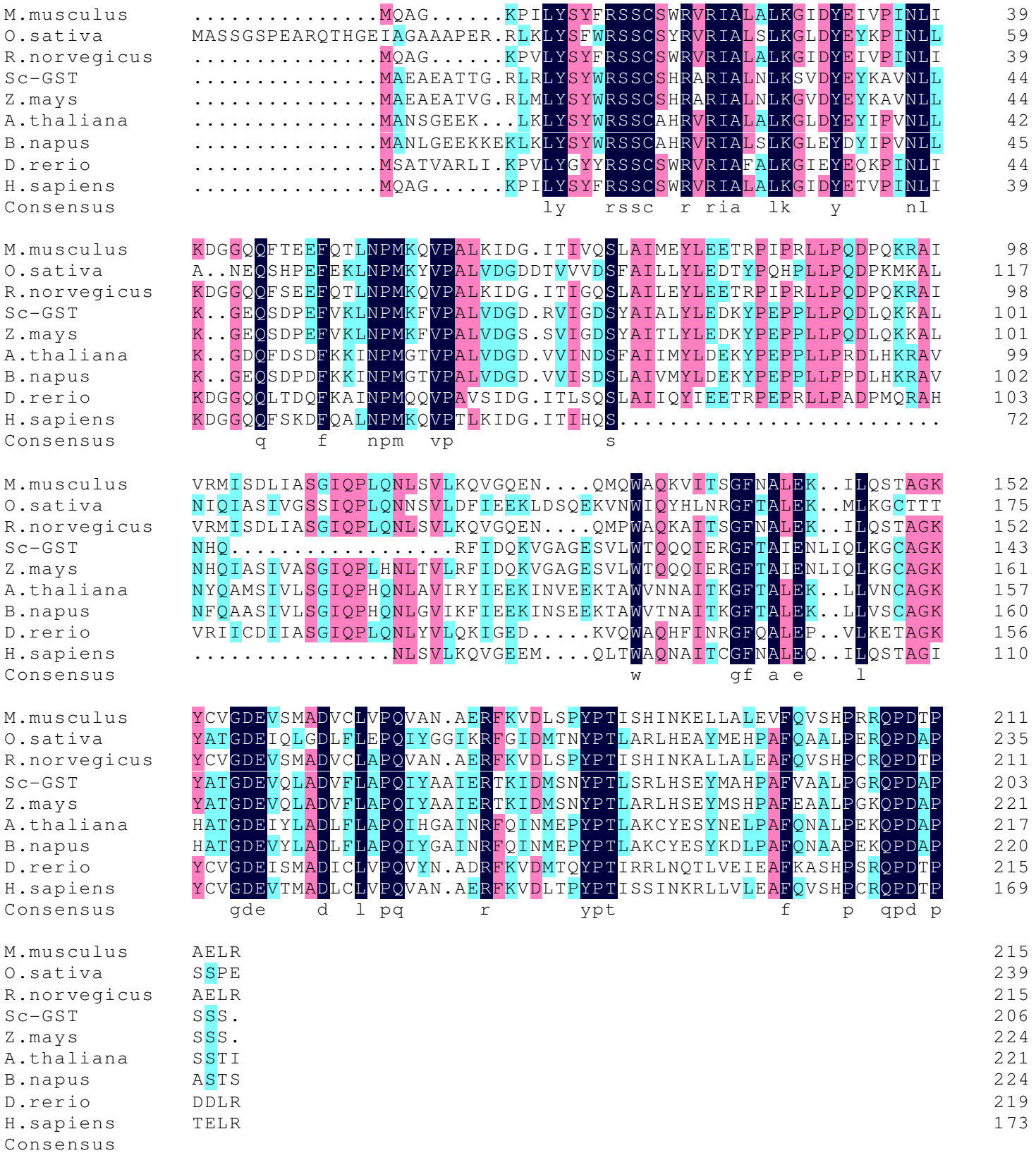

Figure 4. Multiple sequence alignment of zeta class GST proteins isolated from different species. The big frame shows the conservative domain of zeta GST; the small frame shows the QPD domain. *M. musculus* (NP_034493); *O.sativa* (ABA92395); *Rattus norvegicus* (NM_001109445); *Z. mays* (ACG42259); *A. thaliana* (AAO60039); *B. napus* (AAO60040); *Danio rerio*,(NM_001002481); *Homo sapiens* (NM_145871).

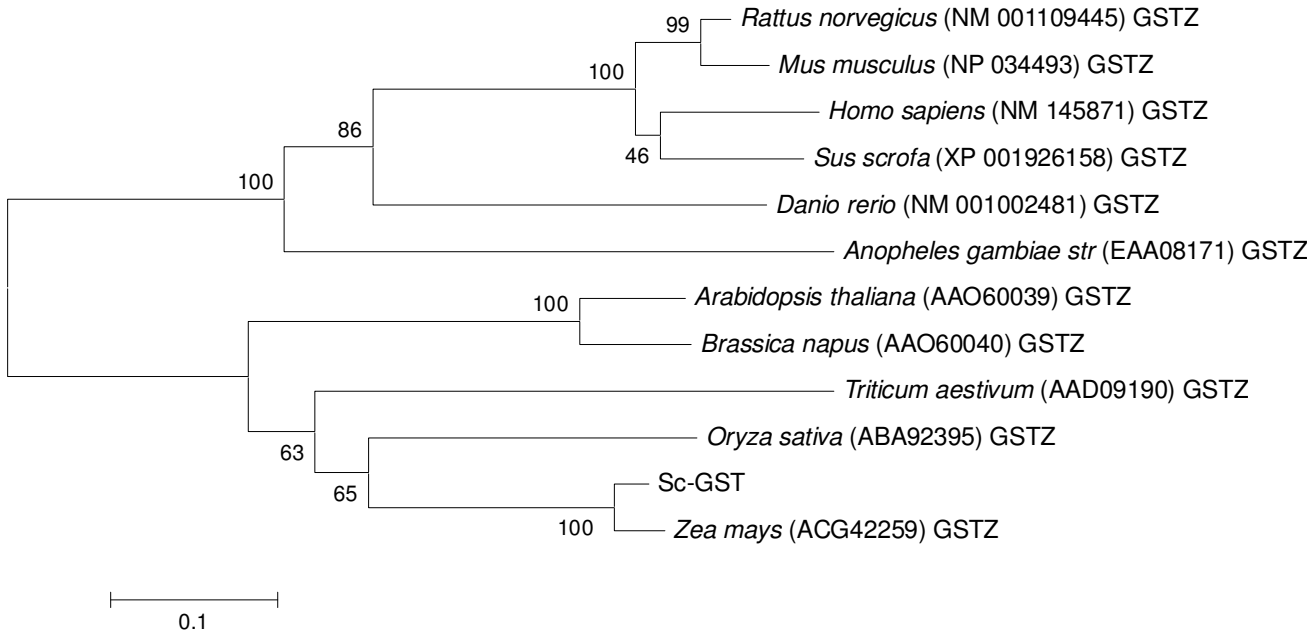

Figure 5. Phylogenetic tree of zeta class GST isolated from different species.

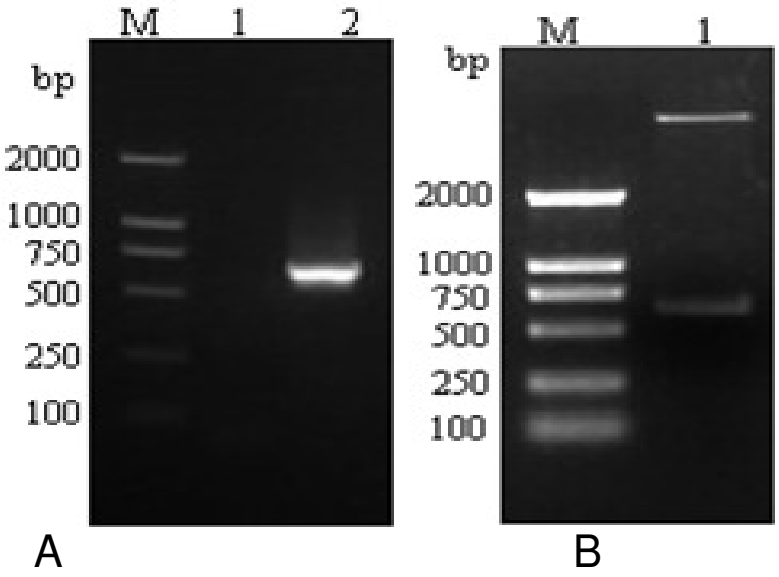

Figure 6. Validation of pET29a-*ScGST* by PCR and double digestion with *Bam*HI+*Sal*I. A: M, DL2000; 1, negative control; 2, PCR production. B: M, DL2000; 1, digested with *Bam*HI+*Sal*I.

at 0 h. Although the expression was still induced during the following time points, it began to decrease gradually. Finally, at the time point of 60 h, the expression was up-regulated again, reaching 5.7 times that of the control. Based on the real-time qPCR analysis, it could be found that the *Sc-GST* had the induced expression under the stress of H_2O_2 and *U. scitaminea*, while it was inhibited and then induced under the stress of SA, which was in accordance with the previous studies, indicating that the biotic and abiotic stress could induce the expression of *GST* (Dixon et al., 2002a; Dean et al., 2005; Marrs, 1996; Gonneau et al., 1998). From the aforementioned, it was inferred that the *Sc-GST* gene might play a certain role in the mechanism of sugarcane detoxification and thus

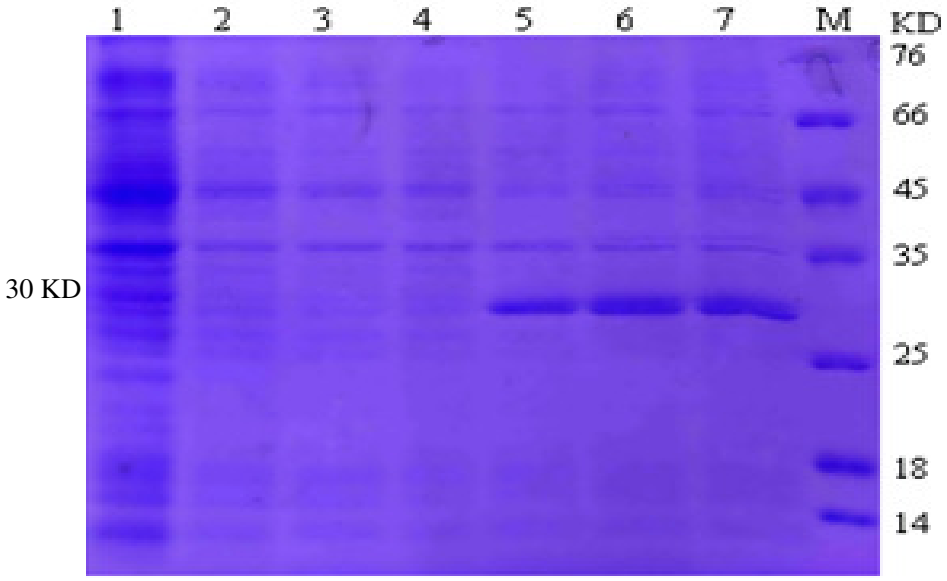

Figure 7. The expression of pET29a-*ScGST* in BL21. M, protein maker; 1, BL21 without induction; 2, pET29a (+) induction for 6 h; 4, pET29a-*ScGST* without induction; 5, 6 and 7, pET29a-*ScGST* induction for 2, 4 and 6 h, respectively.

disease resistance.

DISCUSSION

To date, GSTs were found to diverge significantly in terms of overall sequence, nucleotide or amino acid, even within a certain class. Within the phi and tau classes, the sequence identity in both classes could be as low as 40% and the identity between the two classes was even less than 25% in plants. However, as demonstrated by the crystallization and X-ray diffraction of GST proteins from bacterial, plant and animal, all GSTs characterized so far displayed a striking level of structural conservation (Dixon et al., 2002b). It is noteworthy that, the structures of all known GSTs exhibit a two-domain fold, of which one was a conserved N-terminal domain for glutathione binding and thioredoxin folding and the other was even more divergent C-terminal four-helix bundle fold domain containing the determinants for second-substrate specificity (Sheehan et al., 2001). DNA sequences corresponding to zeta-class GSTs had also been described in mammals, plants and *Caenorhabditis elegans* (Board et al., 1997). In the present study, a full-length *GST* gene was obtained from the sugarcane stem cDNA library, and was termed as *Sc-GST*, with GenBank Accession number GQ246461. The encoding protein of this gene had 87% homology with the zeta-type GST protein in *Z. mays* (ACG42259). In comparison with the zeta-type *GST* genes cloned in *Z. mays* (ACG42259), *O. sativa* (ABI17930) and *B. napus* (AAO60040), the homologies of the putative amino acid sequences were relatively high, indicating that the *GST* gene was rather conservative among these species. Protein domain analysis showed that, the structure of this *Sc-GST* encoding protein had the characteristics of that of the zeta-type GST, the typical SSCXXRXRIA domain at its N-terminus and the QPD region at its C-terminus, which was specific in zeta-type GST. From the aforementioned, it indicated that the *Sc-GST* gene obtained in this study belongs to the zeta class type.

Despite the numerous associations of GSTs with stress responses and even much greater progress that has been made in the function survey of GSTs in a range of plants recently, the function of GSTs enzymes in sugarcane remains elusive. Whereas, plant GSTs had well described a variety of functions, which included defending the invasion of the exogenous and the endogenous toxin (Edward and Dixon, 2000; Dixon et al., 2002a; Moons, 2003), acting as the binding protein or the ligand (Zettl et al., 1994; Gonneau et al., 1998), protecting the tissue from injury (Berhane et al., 1994; Roxas et al., 1997; Cummins et al., 1999; Dixon et al., 2002a), regulating the stable redox status and the programmed aging of cell (Kampranis et al., 2000; Moons, 2005) as well as acting as the stress signaling protein (Loyall et al. 2000) or catalyzing the isomerization reaction (Dixon et al., 2000). In plants, the expression of most *GST* genes was constitutive, while that of some were tissue-specific and could also be regulated by biotic and abiotic stresses (Dixon et al., 2002a). Coldness, heat, heavy metals, salt, polyethylene glycol (PEG), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), naphthalene acetic acid (NAA), H_2O_2 and methyl viologen (MV), which is also termed paraquat, may all induce the expression of a certain type of *GST*. Previous researches had also

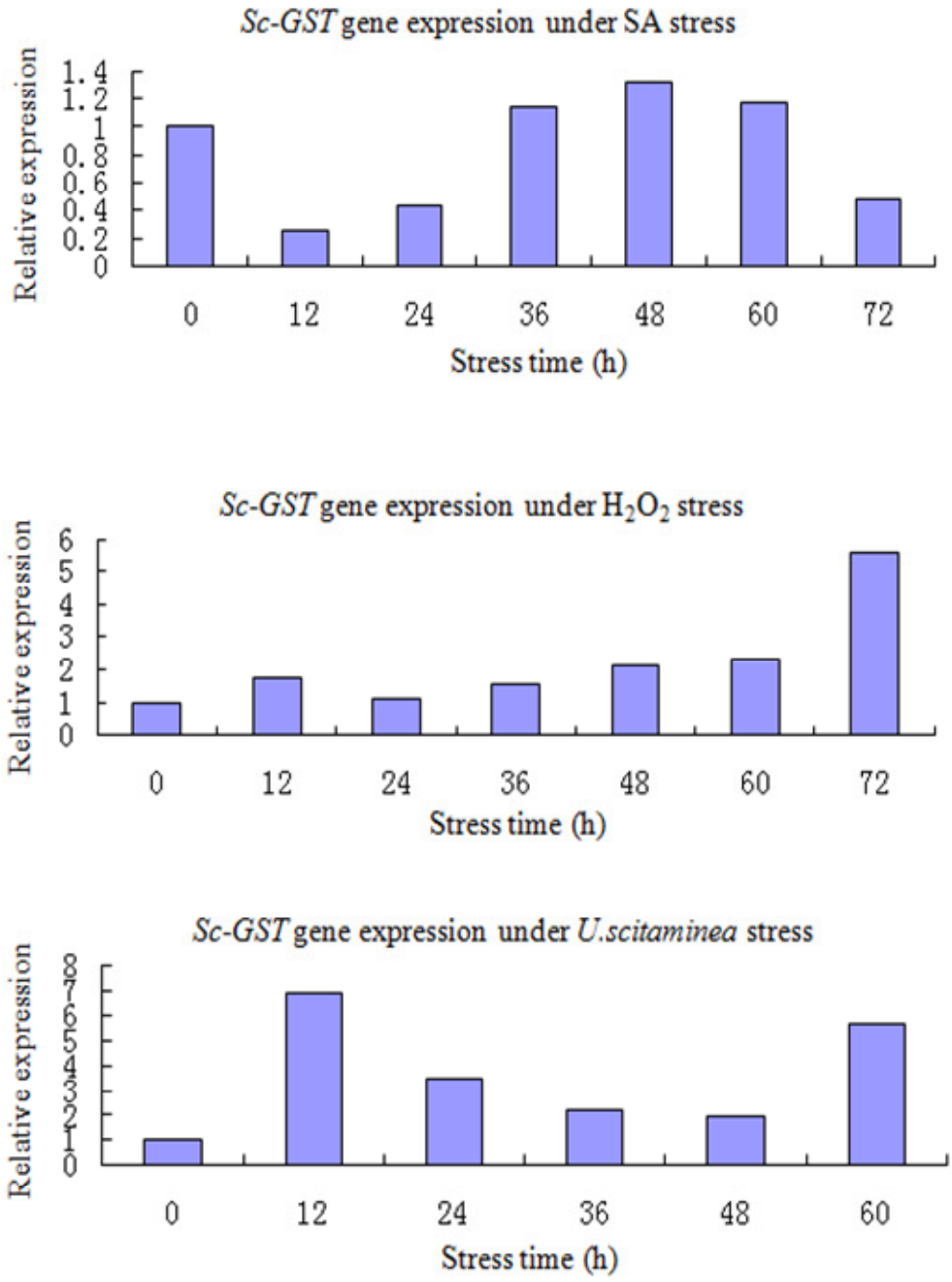

Figure 8. Expression profile of *Sc-GST* gene under various exogenous stresses.

revealed that, the expression of the zeta-type *GST* genes could be induced by the cell division, indole-3-acetic acid (IAA) and cytokinin (Marrs, 1996; Gonneau et al., 1998). Several GSTs were known to be induced by plant hormones, suggesting that plant hormones could regulate GSTs or GSH-conjugating activity was regulated by plant hormones (Marrs, 1996; Gonneau et al., 1998). SA and H_2O_2 were proven to play important roles in signal transduction and resistance response of plant at early stages, such as hypersensitive response (HR) and systemic acquired resistance (SAR) (Scott et al., 1999).

In the present study, with the application of pET29a (+)/BL21 (DE3) prokaryotic expression system, the fusion protein expression of *Sc-GST* gene was successfully induced and the protein was found to have GST-specific enzyme activity. Real-time qPCR analysis demonstrated that the expression of *Sc-GST* gene was induced both by H_2O_2 and *U. scitaminea* stress, and firstly inhibited and then induced by SA treatment. Although further study is necessary, the expression of *Sc-GST* gene was supposed

to be regulated by all these three kinds of exogenous stresses and *Sc-GST* gene was thus assumed to play a certain role in sugarcane disease resistance, which was similar to the results from previous studies in several other plant species (Dixon et al., 2002a). Free oxygen radicals, such as O_2^-, H_2O_2 and OH^-, would be generated under the stress of H_2O_2 and *U. scitaminea*. These radicals had strong toxin and could cause great harm to plant cells. While the expression of *Sc-GST* gene was greatly induced under the stress of H_2O_2 and *U. scitaminea*, it was reasonable to assume that *Sc-GST* gene was a type of stress-tolerant gene playing a certain role in detoxification and thus disease resistance in sugarcane. This is the first report of cloning and characterization of a full-length zeta-class *GST* gene in sugarcane. The results should be helpful in the ongoing exploitation of resistance genes and their application in sugarcane.

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