Isolation and expression analysis of Cu/Zn superoxide dismutase genes in sugarcane and the wild species Saccharum arundinaceus

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ABSTRACT
Saccharum arundinaceus is one of the most important species of sugarcane related species, and with strong resistance. Superoxide dismutases (SODs) play an important role in stress tolerance in plants. In this paper, two novel full-length cDNA sequence of the Cu/Zn SOD gene, denoted as SoSOD-1a and SoSOD1a (GenBank Accession number KJ002569 and KT327179), were isolated from S. arundinaceus and sugarcane, respectively. SoSOD-1a cDNA is 689 bp in length, including 33 bp of 5’-untranslated region (UTR) and 35 bp of the 3’-UTR, and a 621-bp open reading frame (ORF) encoding a 206 amino-acid sequence of the protein which has a conserved domain of superoxide dismutase belonging the SOD family. The SoSOD1a sequence is 690 bp, containing a 600 bp ORF, a 36-bp 5’-UTR and 54-bp 3’-UTR. It encoded the 199 amino-acid sequence of the protein which also has a conserved domain of superoxide dismutase belonging the SOD family. Between these two sequences, there was 97.5% similarity, 17 single nucleotide polymorphism (SNP) sites and 4 small insertion/deletion fragments; 95.5% protein similarity and 10 amino-acid mutation sites. In homologous evolutionary analysis, the Cu/Zn SOD genes from different plant species were rather conservative. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that the prokaryotic expression product was a fusion protein. Real-time quantitative polymerase chain reaction analysis demonstrated that the expression profile of SaSOD-1a and SoSOD1a were different under various drought stress duration. It was also suggested that SOD1a has a different drought response mode in sugarcane and the wild species S. arundinaceus.

Introduction
Superoxide dismutase (SOD; EC1.15.1.1) is a ubiquitous enzyme belonging to a family of metalloenzymes, which catalyse the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. The reaction is continued by catalase (CAT) and peroxidase (POD), converting the hydrogen peroxide to water and molecular oxygen, thus preventing the formation of hydroxyl radicals, which are highly destructive to the cell [1]. On the basis of the metal cofactor used, SODs are classified into four groups: iron SOD (Fe-SOD), manganese SOD (Mn-SOD), copper-zinc SOD (Cu/Zn-SOD) and nickel (Ni-SODs), which are localized in different cellular compartments [2,3].

Under stress, both the increase in SOD enzyme activity and higher expression of SOD transcripts have been reported by various research groups [4]. Higher transcript levels of Cu/Zn-SOD have been reported by Xu et al. [5] in cassava, under exposure to the reactive oxygen species (ROS)-generating reagent methyl viologen or to H$_2$O$_2$. Lu et al. [6] found that overexpression of Cu/Zn-SOD could enhance tolerance and recovery of potato plants from drought stress. Since salt and drought stress result in ROS production, plants have exhibited an increase in SOD activity under these stresses as a step towards stress alleviation [4,7,8].

The first plant SOD gene was cloned from maize in 1987 [9]. From that day on, a number of SOD genes have been cloned in several plant species, such as tomato [10], tobacco [11], rice [12] and peach [13]. In sugarcane, Kurama et al. [14] obtained the Mn-SOD promoter and six Cu/Zn-SOD expressed sequence tags (EST) using sugarcane EST sequence, and analysed them by real-time quantitative PCR. Que et al. [15] obtained the full-length sequence of sugarcane Mn-SOD, and found that smut treatment could significantly induce its expression. From the afore-mentioned, it is of particular interest to clone and characterize the full-length SOD gene in sugarcane and a closely related wild species, which should be helpful to reveal the stress resistance mechanism in sugarcane, especially the oxidative stress resistance. This result may be used as a theoretical basis for breeding...
stress-resistant sugarcane varieties with the aid of biological techniques.

Sugarcane (Saccharum officinarum L.) is the most important source of sugar and energy crop in the world. In China, sugarcane is the largest perennial tropical crop, and was mainly planted on the mountain slopes and hilly lands where the irrigation conditions are poor and the drought stress is intensive [16]. So, the improvement of drought-tolerant sugarcane varieties is an effective approach to solve the problem. Saccharum arundinaceus, a family member of genus Saccharum, is a high biological yield wild resource known for having strong adaptability, stress resistance and intensive tillering [17]. S. arundinaceus has already been used in cross-breeding of sugarcane as valuable breeding material [18,19]. Hence, it is expected that mining the elite gene resources in S. arundinaceus using modern biotechnologies could help to improve the stress resistance of sugarcane.

Materials and methods

Plant materials

The test material, the cultivated sugarcane variety ROC22 and the wild species S. arundinaceus Sa-79, were collected and identified by our research team. ROC22 is the variety most widely planted in China and Sa-79 is a very good drought-tolerant material. A potted experiment was carried out at the base of Zhanjiang Experimental Station, Chinese Academy of Tropical Agriculture in May 2013. Routine management procedures were adopted, and favourable water and fertilizer conditions were maintained. Stay seedlings were grown up to the 5–6 leaf stage; consistently growing plants were taken and divided into 2 groups: a group of normal water supply and another group of natural drought stress. The sampling times were 0, 1, 2, 3, 4, 5 and 6 d of treatment. All the samples collected were immediately fixed in liquid nitrogen and stored in a refrigerator at −70 °C until RNA extraction.

Obtaining and sequence analysis of full-length cDNA

The total RNA was extracted using the Easy Pure Plant RNA Kit (TransGen Biotech Co., Ltd, Beijing, China) by following the product manual. The first-strand cDNA was synthesized from 11 μL RNA template with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) according to the manufacturer’s protocols with oligo (dT)18 as a primer.

Using a full length cDNA sequence of the SOD gene (XM_002445626.1) from sorghum as the probe, we searched the homologous sequences against an EST library of genus Saccharum with BLAST (Basic Local Alignment Search Tool) at the NCBI (National Center of Biotechnology Information) database. EST sequences from Saccharum with higher identity with the SOD gene from sorghum were screened preliminarily. After further screening, several EST sequences containing the complete coding region were assembled to obtain the putative complete coding reference sequences for the cloning of SOD homologous genes from S. officinarum and S. arundinaceus. Two pairs of specific primers were designed according to the reference nucleotide sequences of SOD genes (Table 1). S1 was used for the first round of PCR amplification, and S2 for the second round. The cDNA was used as template to amplify the specific fragments. The total volume of the PCR reaction was 20 μL (1 μL of cDNA template, 10 μL 2 × Taq PCR Mastermix (TIANGEN Biotech Co., Ltd, Beijing, China), 0.5 μL each primer and 8 μL H2O). PCR procedures using primers S1 and S2 were as follows: 94 °C 5 min, 94 °C 30 s, 60 °C 30 s, 72 °C 60 s, 30 cycles, 72 °C 10 min, and a hold at 4 °C (TaKaRa TP600, TaKaRa Bio Inc., Japan). Then, 5 μL of PCR products mixed with 1 μL of loading dye buffer were loaded into 1% agarose gel wells for electrophoresis.

The ProtParam (http://cn.expasy.org/tools/protparam.html) was applied to predict the basic properties of the encoded protein. Multiple sequence alignment and phylogenetic analysis were performed with DNAMAN software (version 6.0). The gene sub-cellular localization was predicted with SubLoc V1.0 (www.bioinfo.tsinghua.edu.cn/SubLoc/) and the signal peptide prediction was performed with SignalP3.0 (www.cbs.dtu.dk/services/SignalP/). SOPMA was adopted for the secondary protein structure prediction (https://npsa-prabi.ibcp.fr/).

Sequence alignment, protein prediction and evolutionary analysis of homologous sequences were performed using DNASTAR 6.0 software. Alignments of the

| Table 1. Description of five primer sets used for cloning. |
|-------------|-------------|-----------------|-----------------|
| Use         | Primer name | Primer sequence (5’→3’) | PCR fragment size (bp) |
|-------------|-------------|--------------------------|------------------------|
| Gene cloning| C1          | Forward: CAAGACCCCTCCAAAAGTCCReverse: GAAGACAAAGGCCACACAG          | 734                     |
|             | C2          | Forward: AAAGTCCCCAAAGGCCCGCRReverse: AACAGTGAAACGCACACTGC          | 700                     |
| Vector construction | V         | Forward: ATGGCCGCGCAGTCCTTCT  | 621                     |
|             |             | Reverse: CTCAATGGTGCAGCCCAA  |                         |
|             |             | qPCR                      |                         |
|             | Q           | Forward: GAGCGAGGAGCTACACAC  | 205                     |
|             |             | Reverse: CCCACAGGATGGCCAAACA |                         |
|             | 25S         | Forward: ATACACCGCACCAGGTCTCAGAAG  |                         |
|             |             | Reverse: CTCACAGGCGCAATCTTTTCC  |                         |
obtained sequences and the predicted protein sequences were done by BLAST search after accessing the NCBI website (http://www.ncbi.nlm.nih.gov/). The molecular formula, molecular weight and isoelectric points (Pl) of amino acid residues were determined using Protparam software. The hydrophobicity of proteins was analysed by ProtScale, the signal peptides and transmembrane domains were predicted by Interpro. The phylogenetic tree was build using MEGA 6.0 software by the neighbour-joining method.

**Construction of prokaryotic expression vector of**

**SaSOD-1a and SoSOD1a gene**

pEASY-E1 (TransGen Biotech, Beijing, China) was used as the prokaryotic expression vector and the specific primer pairs amplifying the open reading frame (ORF) region of SOD1a were designed. The primer set V was used according to Table 1.

The primers were synthesized by Invitrogen Corporation (Shanghai, China). With plasmid DNA extracted from the library clone containing the SaSOD-1a and SoSOD1a gene as the template, the PCR amplification was carried out. The PCR reaction and amplification program were the same as those employed in the gene cloning strategy. When the reaction finished, 1% agarose gel electrophoresis was performed and the target PCR product was recovered. Then, the obtained ORF sequence was ligated into pEASY-E1 by following the product manual. The recombinant plasmid was then transferred into Escherichia coli Trans1-T1. The plasmid DNA of several clones selected by blue/white colony screening was extracted. Two positive clones validated by PCR were just the prokaryotic expression vectors which were named as pEASY-E1-SaSOD-1a and pEASY-E1-SoSOD1a.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of prokaryotic expression product**

pEASY-E1-SaSOD-1a, pEASY-E1-SoSOD1a and empty pEASY-E1 were transformed into E. coli BL21 (DE3). A single colony was inoculated into LB (Luria–Bertani) medium containing ampicillin (50 µg/mL) to obtain an overnight shake culture at 37 °C. On the following day, 1% of the medium was inoculated into the LB medium containing 50 µg/mL ampicillin and the culture was grown on a shaker. When the optical density at 600 nm (OD600) reached 0.4 to 0.6, 1.0 mL of the culture broth was collected as the control, and the remaining suspension was added IPTG (isopropyl β-D-1-thiogalactopyranoside) with a final concentration of 1.0 mmol/L. The cell culture with pEASY-E1-SaSOD-1a and pEASY-E1-SoSOD1a was induced for 4 h, at 37 °C, and 1.0 mL of culture was collected; the cell culture with empty pEASY-E1 was induced with IPTG for 4 h and 1.0 mL culture was collected. The cell culture collected was used for SDS-PAGE with 20 µL sample loading. When the electrophoresis ended, the gel was stained with Coomassie brilliant blue and imaged.

**Expression profile of SaSOD-1a and SoSOD1a under different drought stress duration**

According to the sequence of SaSOD-1a and SoSOD1a, one pair of real-time qPCR primers were designed (Table 1). For the purpose of real-time qPCR analysis, 25S rRNA was selected as the control gene [19]. In real-time qPCR amplification, the RT-PCR product was used as the template and the total volume of the reaction system was 20 µL, including 10 µL SYBR Primix Ex TaqTM (2 ×); 0.5 µL Rox reference dye II; 0.5 µL forward and reverse primers (10 µmol/L, respectively); 1 µL cDNA template and 7.5 µL of sterile water. Three replicas were set for each sample. The PCR reaction conditions were pre-denaturation at 94 °C for 5 min, and then 30 cycles with 94 °C 30 s, 60 °C 30 s and 72 °C 30 s (ABI PRISM 7700, ABI, USA). When the reaction ended, the melting curve was analysed. The method of $2^{-\Delta\Delta CT}$ was adopted to analyse the real-time qPCR results [20].

**Results and discussion**

**Cloning and sequence analysis of SaSOD-1a and SoSOD1a gene**

The EST library of Saccharum was searched on the NCBI website with the cDNA sequence of the SOD gene (XM__002445 626.1) from sorghum. A number of Saccharum EST sequences related to the SOD gene were found, and three of them showed higher similarity with the cDNA sequence of the SOD gene from sorghum (Table 2). They contained the complete coding region of the SOD gene. These three sequences were spliced and assembled to obtain a 997 bp nucleotide sequence which was used as the putative reference sequence for primer design in the cloning of SOD homologous gene in S. arundinaceus and S. officinarum.

**Table 2. Characterization of three sugarcane ESTs.**

| GenBank accession number | Position | Length (bp) | Similarity (%) | Expected value (£) |
|--------------------------|----------|-------------|----------------|-------------------|
| CA172003.1               | 40–688   | 649         | 98%            | 0.0               |
| CA172317.1               | 183–842  | 660         | 97%            | 0.0               |
| CA113926.1               | 622–968  | 348         | 94%            | 2e-149            |
Two pairs of primers, C1 and C2, were used (Table 1) to perform PCR amplification with cDNAs of Sa-79 and ROC22. In the second round of PCR amplification, a single strip appeared using either pair of primers, with the size of about 700 bp (Figure 1). The PCR products with primer C2 were recovered, cloned and sequenced, and two cDNA sequences were obtained. These sequences were further aligned with those downloaded from NCBI, and these two cDNA sequences including the start and stop codons were assembled. They were denoted as \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \), respectively. These two sequences were submitted to Genbank under accession numbers KJ002569 and KT327179.

The length of the cDNA sequence of \( \text{SaSOD-1a} \) was 689 bp, including 33 bp of the 5'-untranslated region (UTR) and 35 bp of the 3'-UTR, and a 621-bp open reading frame (ORF) encoding 206 amino acids. The \( \text{SoSOD1a} \) sequence was 690 bp long, containing a 600 bp ORF, a 5'-UTR of 36 bp and a 3'-UTR of 54 bp. It encoded 199 amino acids. The cDNA sequences of \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \) showed high similarity. Their similarity was 97.5%.

Polymorphism analysis suggested that the differences between \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \) were two insertion/deletion sites (Table 3) and 13 single nucleotide sites in the coding regions. The numbers of T/C, A/G, A/C, A/T and C/G SNPs were 8, 1, 2, 1 and 1, respectively (Table 4). The phenomenon of allelic variation is common in crops such as rice, wheat and maize [21,22]. Allelic variation occurs as a result of ancestral gene evolution and mutation. In this study, allelic variation is a change in the corresponding protein coding sequence, which may result in alterations in phenotype and function.

### Protein sequence analysis and function prediction

The \( \text{SaSOD-1a} \) sequences encoded 206 amino acids and \( \text{SoSOD1a} \) sequences encoded 199 amino acids. The molecular weights of the two proteins were predicted to be 20.78 and 20.10 kDa, and their pl was 5.237 and 5.408, respectively. Further sequence alignment showed that the predicted amino acid sequences of the proteins encoded by \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \) both had 10 variation sites (Figure 2). These mutational changes may alter the protein structure. However, it is inconclusive whether the enzymatic activity is also changed.

BLASTP search and protein function prediction were performed through NCBI for the two protein sequences. The results showed that both protein sequences contained a conserved structural domain of Cu/ZnSOD, the \( \text{SaSOD-1a} \) conservative structure domain of 55–198 amino acids, whereas that of \( \text{SoSOD1a} \), 54–195 amino acids (Figure 3). It indicated that this protein probably has the function of catalysing the conversion of superoxide radicals to molecular oxygen.

The amino acid sequence alignment of plant and nonplant superoxide dismutases indicates a high homology among the proteins. The plant SODs have very similar sequences, but there are variations that unambiguously differentiate chloroplastic and cytosolic forms. This suggests that the plant proteins originated in a common ancestor and later differentiated according to the cell-compartment in which they are now found [23]. In this study, the predicted \( \text{SaSOD-1a} \) protein was found to have high homology (95.5%) with \( \text{SoSOD1a} \).

| Gene   | Sites          | 27 | 51 | 71 | 98 | 199 | 209 | 234 | 246 | 343 | 361 | 369 | 435 | 532 |
|--------|----------------|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| \( \text{SaSOD-1a} \) | C               | C  | C  | T  | T  | G   | T   | G   | T   | T   | A   | C   | T   | T   |
| \( \text{SoSOD1a} \)  | A               | T  | A  | C  | A  | C   | C   | C   | C   | C   | T   | C   | C   | C   |

Table 3. Insertion/deletion sites of \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \).

| Gene   | Nucleotide sites |
|--------|------------------|
| \( \text{SaSOD-1a} \) | 132–134 |
| \( \text{SoSOD1a} \)  | 589–595 |

Table 4. Allelic variations of \( \text{SaSOD-1a} \) and \( \text{SoSOD1a} \).
Figure 2. Alignment of predicted protein sequences of SaSOD-1a and SoSOD1a.

Figure 3. Size and location of conserved protein domains encoded by SaSOD-1a (a) and SoSOD1a (b).

Figure 4. Multiple sequence alignment of Cu/Zn SOD proteins isolated from different plant species. SbSOD (EES15166.1); SsSOD (AIY26425.1); ZmSOD (BAI50562.1); TaSOD (AAB67991.1); BdSOD (KQJ99399.1); OsSOD (EA243665.1).
Evolutionary analysis of SaSOD-1a and SoSOD1a

On comparing the deduced amino acid sequences with those from other plants, SOD was found highly conserved in the plant kingdom (Figure 4). Like other protein sequences, SaSOD-1a and SoSOD1a also contained a 54 amino-acid site which was rather conserved in the evolution. This indicated that the gene encoding Cu/Zn SOD may have potential function in the stress-tolerance mechanism in plants.

The phylogenetic tree was made by the neighbour-joining method with multiple alignments of amino acids from different species. It showed that the proteins encoded by SaSOD-1a and SoSOD1a were the closest to SOD from sorghum, but distant from Brassica rapa, Theobroma cacao, soybean and so on. The species were grouped into two clusters based on SOD sequences. The proteins encoded by SaSOD-1a and SoSOD1a belonged to the first cluster and showed high identity (Figure 5).

Prokaryotic expression of SaSOD-1a and SoSOD1a gene

After the PCR product from the ORF amplification of SaSOD-1a and SoSOD1a gene was recovered, the target fragment was then recovered and ligated into the prokaryotic expression vector pEASY-E1. The fragment of the same size as that of the target fragment could be obtained from the positive recombinant with PCR amplification. The results of the 1% agarose gel
electrophoresis suggested successful construction of the prokaryotic expression vector pEASY-SaSOD-1a and pEASY-SoSOD1a.

The expression of the target protein encoded by the SaSOD-1a and SoSOD1a genes began within the first hour and reached a peak at the fourth hour. The molecular weight of the protein expressed in pEASY-SaSOD-1a and pEASY-SoSOD1a was about 23 kDa; that was the molecular weight of 20.78 and 20.10 kDa of the SaSOD-1a and SoSOD1a encoding protein plus the molecular weight of about 0.87 kDa of the His Tag peptides. No target protein was expressed in the empty vector (Figure 6). This indicated again that the prokaryotic expression vector was successfully constructed, without wrong coding or reading-frame shift of the amino acid sequence, and SaSOD-1a and SoSOD1a were successfully expressed within the E. coli BL21 (DE3) cells.

Expression profile of SaSOD-1a and SoSOD1a under different stress duration

Environmental stress conditions, such as drought, salinity, cold stress, heat shock or heavy metals, cause excessive accumulation of ROS such as the superoxide anion in plant cells [24–27]. SODs (EC 1.15.1.1) represent the first line of defense against oxygen toxicity by catalysing the dismutation of superoxide anion radical into hydrogen peroxide and oxygen [28]. In the present study, real-time qPCR analysis of SaSOD-1a and SoSOD1a gene expression under various durations of drought stress was conducted. The expression profiles of SaSOD-1a and SoSOD1a were different under various drought stress durations. Under drought stress, the expression of SaSOD-1a gene was up-regulated. At the time point of 120 h, the expression was 9.3 times that of the control at 0 h, but it was slightly inhibited at the time point of 144 h. On the contrary, the expression of SoSOD1a gene was down-regulated. The expression value reached about 1.8 at 24 h, but the gene was hardly expressed at 96 h.

Conclusions

In this study, we cloned and identified two Cu/Zn-SOD genes from S. arundinaceus and S. officinarum, and the recombinant protein was shown to result in better growth of E. coli. The expression of SaSOD-1a in S. arundinaceus was higher significantly than that of SoSOD1a in S. officinarum. With the increase of drought stress time, the expression of SaSOD-1a was up-regulated, but SoSOD1a was down-regulated. From these observations, we could conclude that S. arundinaceus has a stronger ability to remove superoxide radical than common sugarcane cultivar.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidant, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91:179–194.

[2] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7:405–410.

[3] Mittler R, Vanderauwera S, Gollery M, et al. Reactive oxygen gene network of plants. Trends Plant Sci. 2004;9:490–498.

[4] Negi NP, Shrivastava DC, Sharma V, et al. Overexpression of CuZnSOD from Arachis hypogaea alleviates salinity and drought stress in tobacco. Plant Cell Rep. 2015;34(7):1109–1126.

[5] Xu J, Duan XG, Yang J, et al. Coupled expression of Cu/Zn-superoxide dismutase and catalase in cassava improves tolerance against cold and drought stresses. Plant Signal Behav. 2013 [cited 2017 Mar 29];8(6):e24525. DOI:10.4161/psb.24525

[6] Lu YY, Deng XP, Kwak SS. Over expression of CuZn superoxide dismutase (CuZn SOD) and ascorbate peroxidase (APX) in transgenic sweet potato enhances tolerance and recovery from drought stress. Afr J Biotechnol. 2010;9:8378–8391.

[7] Kaya C, Ashraf M, Dikilitas M, et al. Alleviation of salt stress-induced adverse effects on maize plants by exogenous application of indoleacetic acid (IAA) and inorganic nutrients – a field trial. Aust J Crop Sci. 2013;7:249–254.

[8] Shiriga K, Sharma R, Kumar K, et al. Expression pattern of superoxide dismutase under drought stress in Maize. Int J Innov Res Sci Eng Technol. 2014;3(4):11333–11337.

[9] Cannon RE, White JA, Scandalias JG. Cloning of cDNA for maize superoxide dismutase 2 (SOD2). Proc Natl Acad Sci USA. 1987;84:179–183.

[10] Perl-Treves R, Nacmias B, Aviv D, et al. Isolation of two cDNA clones from tomato containing two different superoxide dismutase sequences. Plant Mol Biol. 1988;11(5):609–623.

[11] Bowler C, Alliotte TM, De Loose M, et al. The induction of manganese superoxide dismutase in response to stress in Nicotiana plumbaginifolia. EMBO J. 1989;8(1):31–38.

[12] Kamikawa H, Morita S, Tokumoto M, et al. Molecular cloning and characterization of a cDNA for an iron-superoxide dismutase in rice (Oryza sativa L.). Biosci Biotech Biochem. 1999;63(2):302–308.

[13] Baqnoli F, Giannino D, Caparrini S, et al. Molecular cloning, characterisation and expression of a manganese superoxide dismutase gene from peach (Pr unus persica [L.] Batsch). Mol Genet Genomics. 2002;267(3):321–328.

[14] Kurama EE, Fenille RC, Rosa JRVE, et al. Mining the enzymes involved in the detoxification of reactive oxygen species (ROS) in sugarcane. Mol Plant Pathol. 2002;3(4):251–259.

[15] Que YX, Liu JX, Xu LP, et al. Molecular cloning and expression analysis of a Mn-superoxide dismutase gene in sugarcane. Afr J Biotechnol. 2011;11(3):552–560.

[16] Chen RK, Xu LP, Lin YQ. Modern sugarcane genetic breeding. Beijing: China Agriculture Press; 2011.

[17] Tai PYP, Miller JD. A core collection for Saccharum spontaneum L. from the world collection of sugarcane. Crop Sci. 2001;41:879–885.

[18] Mary S, Nair N, Chaturvedi PK, et al. Analysis of genetic diversity among Saccharum spontaneum L. from four geographical regions of India, using molecular markers. Genet Resour Crop Evol. 2006;53:1221–1231.

[19] Pan YB. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Techn. 2006;8:246–256.

[20] Que YX, Xu LP, Xu S, et al. Selection of control genes in real-time qPCR analysis of gene expression in sugarcane. Chin J Trop Crops. 2009;3:274–278.

[21] Jeppe RA, Thomas L. Functional markers in plants. Trends Plant Sci. 2003;8(11):554–560.

[22] Zhang W, Dubcovsky J. Association between allelic variation at the phytoene synthase 1 gene and yellow pigment content in the wheat grain. Theor Appl Genet. 2008;116(5):635–645.

[23] Fink RC, Scandalias JG. Molecular evolution and structure-function relationships of the superoxide dismutase gene families in angiosperms and their relationship to other eukaryotic and prokaryotic superoxide dismutases. Arch Biochem Biophys. 2002;399:19–36.

[24] Hasegawa PM, Bressan RA, Zhu JK, et al. Plant cellular and molecular responses to high salinity. Plant Physiol Plant Mol Biol. 2012;80:255–272.

[25] Zhu JK. Salt and drought stress signal transduction in plants. Annu Rev Plant Biol. 2002;53:247–273.

[26] Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004;55:373–399.

[27] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7:405–410.

[28] Fridovich I. Superoxide radical and superoxide dismutase. Annu Rev Biochem. 1995;64:97–112.

[29] Leclercq J, Martin F, Sanier C, et al. Overexpression of a cytosolic isofrom of the HbCuZnSOD gene in Hevea brasiliensis changes its response to a water deficit. Plant Mol Biol. 2012;80:255–272.

[30] Malan C, Greyling MM, Gressel J. Correlation between Cu/Zn superoxide dismutase and glutathione reductase, and environmental and xenobiotic stress tolerance in maize inbred. Plant Sci. 1990;88:157–166.

[31] Morita S, Tsukamoto S, Sakamoto A, et al. Differences in intron-mediated enhancement of gene expression by the first intron of cytosolic superoxide dismutase gene from rice in monocot and dicot plants. Plant Biotechnol Rep. 2012;29:115–119.