IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

Clone and Sequence Analysis of Cu/Zn-SOD Gene from *Saccharum arundinaceum* Retz.

To cite this article: Yanli Yao *et al* 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **81** 012001

Clone and Sequence Analysis of Cu/Zn-SOD Gene from *Saccharum arundinaceum* Retz.

Yanli Yao, Junjun He, Shulian Xing, Xiaowen Hu, Lei Xu, Yang Liu*

Zhanjiang Experiment Station, Guangdong Dryland Water-saving Agricultural Engineering and Technology Research Center/ Tropical Dry Farming Research Center, Chinese Academy of Tropical Agricultural Sciences, Guangdong Zhanjiang, 524013

*Corresponding author: Yang Liu; Email: lyfull@163.com

Abstract. *Saccharum arundinaceum* has strong drought tolerance, the abundant stress tolerance gene resource could be used in sugarcane breeding programs. Superoxide dismutase (SOD) is a crucial enzyme in reactive oxygen species (ROS) action, which are likely to function as components of a stress-signaling pathway. To investigate the gene of SOD, by using a homologous cloning strategy, a Cu/Zn-SOD cDNA sequence(NCBI accession number XM_ 002445626.1) in Sorghum was as probe to retrieve, aligned and spliced with *Saccharum* EST database, a full length of Cu/Zn-SOD gene named SaSOD-1b (NCBI accession number: KJ 002570) from *Saccharum arundinaceum* was cloned. The cloned gene sequence was 692bp, including 136bp 5' untranslated regions (UTR), 162bp ORF and 135bp 3' UTR, encoded 206 amino acids. By bioinformatics analysis, the cloned genic protein molecular weight was 20.78kDa, and pI was 5.32, the amino acid sequence owned the Cu/Zn-SOD conserved domain, and it had high similarity with other plants' Cu/Zn-SOD. The phylogenetic tree analysis indicated that the cloned gene was most closely relative with Sorghum Cu/Zn-SOD gene, this study could laid a foundation for further understanding the relation between SOD and *Saccharum arundinaceum* drought resistance**.**

1. Introduction

Drought, salt and low temperature are the main abiotic stress types limiting plants growth and development, they are usually nominated as osmotic stress because they change the osmotic pressure of plant cell. Abiotic stress influences cell's water absorption and water distribution, and then limit cell's growth and development, finally severely reduce crop production. Therefore, how to improve crop's production and save water resource by intensifying stress resistance has become the key to the sustainable and efficient development of agriculture. Recently, molecular breeding by gene engineering to improve crop abiotic stress resistance has become an effective way to increase crop production.

Plant cells inevitably produce and reply on ROS as signal molecule in cell life cycle, meanwhile, to maintain cells' stable physical status, redundant ROS molecules would be eliminated by the anti-oxidative enzymatic system distributing in every part of the cell (Apel and Hirt 2004;Gechev et al 2006; Kwak et al 2006). The main relevant enzymes to clean out ROS are superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Mittler et al 2004; Miller et al 2010). SODs is the only enzyme to catalyze superoxide ROS, with its principal effect of transforming O_2^- into H_2O_2, later then, H_2O_2 is reduced into H_2O and O_2 by peroxide scavenger enzymes.

Saccharum arundinaceum is the wild plant of *Saccharum* related genera, with abundant gene resources to resist abiotic stress, it's the ideal material to clone relevant stress-resistant gene. The study took *Saccharum arundinaceum* as material, which was *Saccharum officinarum*'s wild resource collected from Hainan Island, achieved full-length gene sequence by homologous clone; implemented bioinformatics analysis, studied the gene expression under drought stress and normal water supply, with the purpose of providing gene resource for *Saccharum officinarum* and other plants' genetic improvement on stress-resistant adjustment by gene engineering.

2. Materials and Methods

2.1 Materials

Saccharum arundinaceum used in the expriement were *Saccharum officinarum*'s wild plants collected and conserved by the research group. Adopt potted experiment, regular water and fertilizer management, select plants with consistent growing status and separate them into 2 groups 2 months after germination: one group with normal water supply, the other with drought stress treatment. Sample the leaves of 1d, 2d, 3d, 4d, 5d, 6d, 7d after curling, and the samples were stored in -70℃ refrigerator after liquid nitrogen quick freeze treatment.

2.2 Total RNA extraction and cDNA fisrt strand synthesis

Saccharum arundinaceum total RNA extraction method referred to the instruction of TRAN EasyPure Plant RNA Kit. Detect total RNA purity by 1% agarose gel electrophoresis. Take total RNA as template for cDNA first strand synthesis; follow the instruction of Thermo RevertAid First Strand cDNA Synthesis Kit.

2.3 Gene Clone

According to GenBank Sorghum Cu/Zn-SOD gene sequence (Accession number：XP_002445671.1), retrieve, align and splice with *Saccharum* EST database, design 2 pairs of specific primer, and amplify the specific fragment by nested PCR. For the first round of Cu/Zn-SOD gene amplification, the forward primer was 5'-CAAGACCCTCCCAAAGTCC-3'; the reverse primer was: 5'-GAAGACAA AAGGCCACCAAG-3'. For the second round of Cu/Zn-SOD gene amplification, the forward primer was 5'-AAAGTCCCAAAGGCCGCCG-3'; the reverse primer was: 5'-AACAGTGAAGCTGCAA CTGC-3'. Take *Saccharum arundinaceum* cDNA as template, amplify by PCR, reaction process was: initial denaturation for 5 min at 94℃; denaturation for 30s at 94℃, anneal for 30s at 60℃, extend for 1min at 72℃; finally, extend for 10min at 72℃. Detect by 1% agarose gel electrophoresis, recycle the target fragment and ligate to the clone vector pEASY^{TM}-T5. Entrust INVITROGEN Trading (Shanghai) Co., Ltd to detect two directional sequencing.

2.4 Bioinformatics Analysis

Log in NCBI (http://www.ncbi.nlm.nih.gov/), retrieve and analyze *SaSOD-1b* gene sequence and amino acid sequence by Blast. Analyze protein physiochemical properties and secondary structure by ExPASy Protparam and SOPM program. Compare and analyze the amino acid sequence predicted by *SaSOD-1b* gene through DNASTAR MegAlign program. Construct phylogenetic tree for *SaSOD-1b* gene amino acid sequence and other species by MEGA6.06.

3. Results and Analysis

3.1 Cu/Zn-SOD gene PCR amplification

Utilize the designed specific primer, take *Saccharum arundinaceum* cDNA as template to implement double PCR amplification， detect PCR product by 1% agarose gel electrophoresis. The results showed that, the target band near 700bp (Fig. 1) amplified by PCR was basically consistent with the expectation. Recycle the target band and ligate to the clone vector T5, transform the screened positive

clone and entrust a sequencing company to sequence, then a comprehensive cDNA sequence including initiation codon and termination codon was acquired and nominated as *SaSOD-1b* (accession number: KJ002570). The full length of gene sequence was 692bp, including 36bp 5' UTR and 35 bp 3'UTR, and one 621bp open reading frame, encoding 206 amino acids.

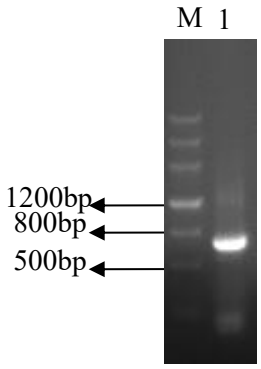

Figure 1. PCR fragments amplified with C2 prime sets on a 1% agrose gel
M: DNA Marker III; 1: PCR result of Cu/Zn-SOD gene

3.2 SaSOD-1b gene encoded protein properties and function prediction

Log in ExPASy Protparam (http://web.expasy.org/protparam/), on-line predict *SsSOD-1b* physio-chemical properties, the results showed that *SaSOD-1b* encoded protein's molecular formula was $C_{910}H_{1440}N_{260}O_{289}S_4$, with molecular weight 20.78KDa, and pI 5.32; it was composed of 18 amino acids, with the highest content of Ala (14.6%), the lowest of Met and Cys the lowest (1.0%), and the instability index of 16.58，which elaborated that the protein is stable protein. The general average hydrophobicity index was 0.172, predict *SaSOD-1b* secondary structure by SOPMA, the results showed that, SaSOD-1b contained 46 a-helix, 86 random coils, 56 extended strands, 18β-turn, the contents were 22.33%, 41.75%, 27.18% and 8.74% respectively.

Compare and analyze in CCD database of NCBI, the results showed that, SaSOD-1b protein contained 2 binding sites: Cu^{2+} binding site (the 98th, 100th, 115th, 172th site) and Zn^{2+} binding site (the 115th, 123th, 131th, 135th site) and the Cu/Zn-SOD conserved domain composed by the amino acids from codon 62th to codon 198th (Fig. 2).

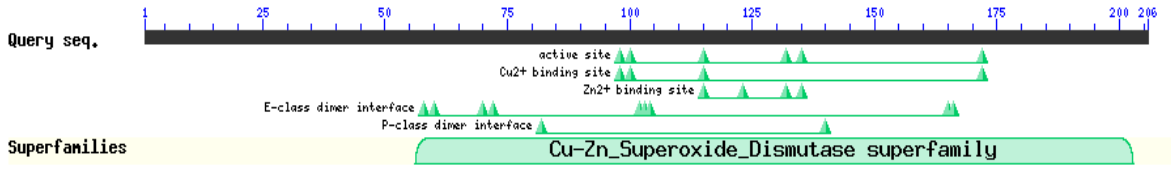

Figure 2. Size and location of conserved protein domains encoded by SaSOD-1b

3.3 SaSOD-1b Protein Homology Analysis

Log in NCBI (http://www.ncbi.nlm.nih.gov/) retrieve the amino acid encoded by *SaSOD-1b* gene by Blastp, it was found that the consistency between SaSOD-1b protein and *Sorghum bicolor*，*Saccharum spontaneum* L., and *Zea mays* L. were 97%, 96%, 93%, respectively. It was inferred that the cloned gene was *Saccharum spontaneum* L. Cu/Zn-SOD gene. Utilize MEGE6.06 Construct/Test Maximum Likelihood Tree to implement Phylogenetic tree analysis on different plants' Cu/Zn-SOD amino acid sequence, it was seen from Fig. 3 that, *Saccharum spontaneum* L. Cu/Zn-SOD protein had the closest genetic relation with gramineous plants; the next was crucifer, and was not closely related to other plants.

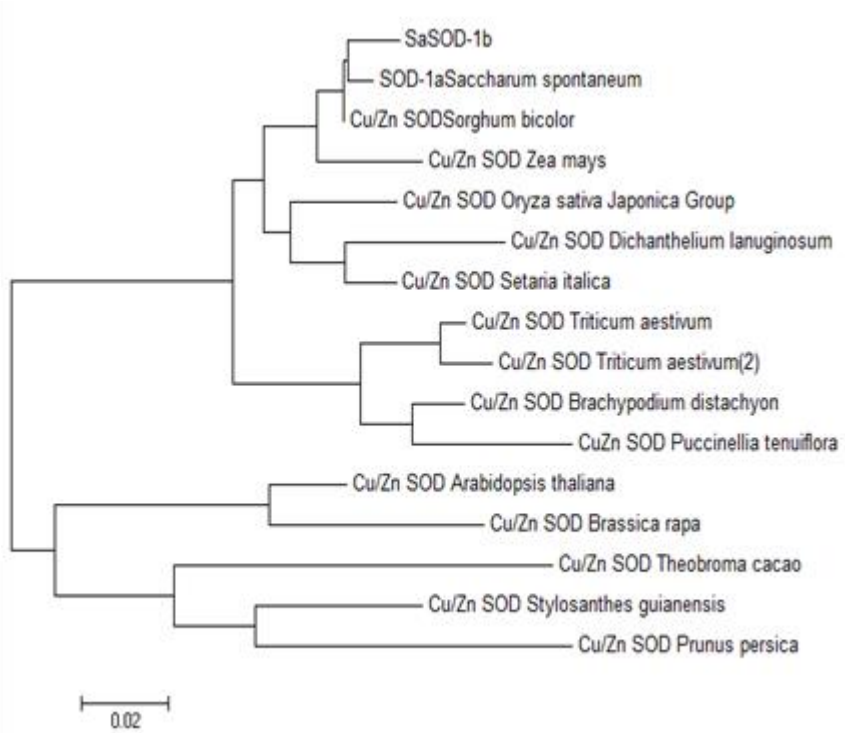

Figure 3. Phylogenetic analysis of SaSOD-1b proteins with other related proteins

4. Conclusion and Discussion

With the rapid development of plant genome study, more and more genes are being cloned. According to plant protein evolutionarily conserved domain, design primer by homology sequence to clone homology gene from different plants has become a new strategy for plant gene clone. The Study acquired *Sorghum bicolor* Cu/Zn-SOD gene cDNA sequence from GenBank to clone the full length cDNA sequence of *Saccharum arundinaceum* Retz. Cu/Zn-SOD (*SaSOD-1b*) gene, through homologous analysis, the results showed that the homology of *SsSOD-1b* and Cu/Zn-SOD gene sequence registered in GenBank were over 80%. Bioinformatics analysis further confirmed gene properties and information, including the encoded protein's physiochemical properties, hydrophilicity, hydrophobicity, and secondary structure, etc. *SaSOD-1b* gene had many conserved sites, Cu^{2+} and Zn^{2+} metal binding sites of protein molecule were rather conserved. *SaSOD-1b* gene acquirement laid a foundation for further studying on protein's advance structure, seeking the potential function of *Saccharum arundinaceum* Retz. Cu/Zn-SOD, improving plants' stress-assistance and studying on gene transfer.

Acknowledgments

This work was supported in part by Institute Research Grant of CATAS-ZES (zjky201503)

References

[1] Apel K and Hirt H 2004 *Annu Rev Plant Biol.* **55** 373
[2] Bowler C,Slooten L, Vandenbranden S, De Rycke R, Botterman 3, Sybesma C, Van Montagu M and Tnze D 1991 *EMBO J* **10** 1723-32
[3] Foyer CH and Shigeoka S 2011 *Plant Physiol* **155** 93-100
[4] Gechev TS, Breusegem FV, Stone JM, Denev I and Laloi C 2006 *BioEssays* **28** 1091-101
[5] Kwak JM, Nguyen V and Schroeder JI 2006 *Plant Physiol* **141** 323-9
[6] Miller G, Suzuki N, Ciftci-Yilmaz S and Mittler R 2010 *Plant Cell Environ* **33** 453-67
[7] Mittler R,Vanderauwera S,Gollery M and Breusegem FV 2004 *Trends Plant Sci* **9** 490-8