**RESEARCH ARTICLE**

Haina ZHANG, Chengjin GUO, Cundong LI, Kai XIAO

# Cloning, characterization and expression analysis of two superoxide dismutase (SOD) genes in wheat (*Triticum aestivum* L.)

**Abstract** Superoxide dismutases (SODs) play an important role in catalyzing the conversion of $O_2^-$ to $H_2O_2$, which can reduce the amount of harmful reactive oxygen specie (ROS) generated by the adverse environments, and alleviate the damage to plants. As one class of SODs, CuZnSODs have vital functions in preventing the ROS-generated cell damage and the death in aerobically growing organisms. In this study, two novel CuZnSOD genes in wheat, referred to *TaSOD1.1* and *TaSOD1.2* were identified, cloned and characterized. *TaSOD1.1* and *TaSOD1.2* were 780 bp and 1121 bp, respectively, predicting all to encode 201 amino acids. A 45-aa length of transit peptide at the N-terminal and a 79-aa conserved CuZn-SOD domain were respectively located in TaSOD1.1 and TaSOD1.2. Phylogenetic analysis indicated that the query SODs, most of CuZnSODs, could be classified into four subgroups. Compared with the control (CK), the abundance of *TaSOD1.1* transcripts did not change under drought, salt, low and high temperature conditions, but the *TaSOD1.2* transcripts were strongly induced by the above abiotic stresses, which was in accordance with the elevated SOD activities in leaves in the above stress treatments to some extent, suggesting its involvement in the plant's acclimation and tolerance to the above abiotic stresses by possibly reducing the amount of the harmful ROS from enhancement of the SOD activity.

**Keywords** wheat (*Triticum aestivum* L.), superoxide dismutase (SOD), characterization, expression

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## 1 Introduction

Plants, as well as other aerobic organisms, are continuously subjected to potentially destructive reactive oxygen specie (ROS), including superoxide ($O_2^-$), lipid peroxides (ROO·), $H_2O_2$, and the highly reactive hydroxyl radical (OH·) (Kliebenstein et al., 1998). The amount of ROS generated in the metabolic processes could be increased by environmental stimuli, causing the damage of cellular components.

$O_2^-$ is an abundant ROS that is formed by univalent electron transfer to $O_2$ and can contribute to the synthesis of OH·. Therefore, the reduction of its generation is essential for alleviation of plant damage under an abiotic stress (Hu et al., 2007; Moradi and Ismail, 2007). Superoxide dismutases (SODs) play an important role in catalyzing the conversion of $O_2^-$ to $H_2O_2$, which can reduce the amounts of harmful ROS, OH·. In total, three different classes of SOD activity have been identified by the active site-metal cofactors (Fe, Mn, or Cu and Zn). The primary sequences of FeSOD and MnSOD apoproteins are related, whereas CuZnSOD is distinct (Bowler et al., 1992). It is clear that the plant SOD isoenzymes also differ in their subcellular location. Typically, MnSOD is mitochondrial (Wolfe-Simon et al., 2006), FeSOD is plastidic, and CuZn-SOD may be plastidic or cytosolic (Bowler et al., 1992). There are also reports on the peroxisomal and extracellular SODs (Streller and Wingsle, 1994; Bueno et al., 1995).

The importance of SOD in the acclimation of plants under the abiotic stresses has been demonstrated in several studies. Mutations in human and mouse CuZnSOD have been linked to the disease familial amyotrophic lateral sclerosis, which results in premature neuron death (Rosen et al., 1993). The protective role of SOD in plants has been explored by transgenic approaches, primarily through overexpression or by correlation of SOD expression to the degree of oxidative stress resistance (Bowler et al., 1994; Alscher et al., 1997; Scandalios, 1997; Du et al., 2006). These results indicate a vital role for CuZnSOD in

preventing the ROS-generated cell damage and the death in aerobically growing organisms.

Literatures have shown that the existence of three classes of SOD enzymes could be encoded by a small gene family. In Arabidopsis, the total of seven SOD genes, including three CuZnSODs, one MnSOD and three FeSODs, have been isolated through the analysis of the large numbers of cDNA and genomic DNA sequences (Newman et al., 1994; Rounsley et al., 1996; Delseny et al., 1997; Kliebenstein et al., 1998). As of today, there have been five wheat SOD genes, including two CuZnSODs (*SOD1.1* and *SOD1.2* (Wu et al., 1996)) and three MnSODs (*SOD3.1* and *SOD3.2* (Wu et al., 1997); and another one with GenBank accession number AF092524) have been isolated and characterized. In this study, two novel CuZnSOD genes in wheat, referred to *TaSOD1.1* and *TaSOD1.2*, which had high similarities with *SOD1.1* and *SOD1.2*, respectively, were identified from the international bioinformatics website of TIGR (www.tigr. org) based on the gene indices tool using CuZnSOD as the keywords. Based on the sequences of *TaSOD1.1* and *TaSOD1.2*, the two novel wheat CuZnSOD genes were cloned and characterized. Meanwhile, the expression patterns of *TaSOD1.1* and *TaSOD1.2* under drought, salt, low and high temperature conditions were detected. It was found that the abundance of *TaSOD1.2* transcripts were induced by the above abiotic stresses, which was in accordance with the elevated SOD activities in leaves in the above stress treatments to some extent, indicating its involvement in plant's acclimation and tolerance to the above abiotic stresses by possibly reducing the amounts of harmful ROS from the enhancement of SOD activity.

## 2    Materials and methods

### 2.1    Identification of two novel wheat CuZnSOD genes

Based on the TIGR Gene Indices program in the international bioinformatics website (www.tigr.org), two wheat CuZnSOD genes (tentative consensus (TC) numbers were TC250697 and TC250698, respectively) were identified when using superoxide dismutases as the keywords. The BLAST analysis in National Center for Biotechnology Information (NCBI) using these two wheat CuZnSODs to be the queries showed no wheat CuZnSOD genes with same sequences released, except two homologous genes of *SOD1.1* and *SOD1.2* (Wu et al., 1996). Therefore, they were named *TaSOD1.1* (TC250697) and *TaSOD1.2* (TC250698), respectively.

### 2.2    Cloning of *TaSOD1.1* and *TaSOD1.2*

The open reading frames (ORFs) of *TaSOD1.1* and *TaSOD1.2* were the reverse transcriptase- polymerase chain reaction (RT-PCR) amplified with the specific primer pairs. Because the Expressed Sequence Tags (ESTs) composing the TC of *TaSOD1.1* (TC250697) and *TaSOD1.2* (TC250698) all contained an EST released from drought stress cDNA libraries (EST ID WHE2013_A08_A15ZS for *TaSOD1.1*, and EST ID WHE0283_D07_G13ZS for *TaSOD1.2* in TIGR website, respectively), the wheat (cv. Shixin733) seedlings, after 36 h of 10% polyethylene glycol (PEG) treatment, were used for *TaSOD1.1* and *TaSOD1.2* amplifications. The total RNA of PEG treated leaves was extracted by TRIzol reagent (Invitrogen) following the instructions. The single strand of cDNAs transcribed by AMV reverse transcriptase (TaKaRa) was used for the templates for *TaSOD1.1* and *TaSOD1.2* amplifications. The primers for *TaSOD1.1* were 5′- CACCTCCACCACCAACCCCCAAAAG (forward) and 5′- AATGGCGTCGTTACAAGTATGACTG (reverse), and the primers for *TaSOD1.2* were 5′- CTTCCGCTTCCGACGACAGCCATG (forward) and 5′- ACCAGAGATGGAAACCAGCGACTA (reverse). PCR was performed in a total volume of 20 µL in a reaction consisting of 2 µL single strand of cDNAs, 20 pmol each primer, 250 µmol·L$^{-1}$ of each of the four dNTPs, a buffer and 1 unit of Taq DNA polymerase (TaKaRa). The PCR was performed at 94°C for 5 min, followed by thirty cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min. After that, an extra extension of 72°C for 10 min was performed. The RT-PCR products of *TaSOD1.1* and *TaSOD1.2* were sequenced after TA cloned in the pUCm-T vector and mobilized in Escherichia coli (*E. coli*) DH5α (Sangon).

### 2.3    Characterization analysis of TaSOD1.1 and TaSOD1.2

The open reading frames and deduced amino acids of TaSOD1.1 and TaSOD1.2 were analyzed online by an ExPASY tool (htpp://expasy.org/tools/dna.htmal). The transit peptides in TaSOD1.1 and TaSOD1.2 were predicted based on the TargetP (www.cbs.dtu.dk/ services/TargetP). The CuZn-SOD conserved domains in TaSOD1.1 and TaSOD1.2 were explored by the NCBI BLASTp program (www.ncbi.nlm.nih.gov/ struncture.cdd). For the construction of phylogenetic tree of TaSOD1.1, TaSOD1.2 and other related SODs in plant species, the SODs in plant species with high similarities with TaSOD1.1 and TaSOD1.2 were first identified by the BLAST search in NCBI using TaSOD1.1 and TaSOD1.2 as the queries. The phylogenetic tree of TaSOD1.1 and TaSOD1.2 and other related SODs was drawn by the DNAStar tool. In the mean time, multiple alignments of TaSOD1.1 and TaSOD1.2 and several other SODs in plant species with high similarities were also performed by the DNAStar tool.

## 2.4 Expression analysis of *TaSOD1.1* and *TaSOD1.2* under drought, salt, low and high temperature conditions

Wheat seeds (cv. Shixin733) were used in this study. First, the seeds were sterilized on surface, and then germinated at a culture chamber in darkness at 25°C. Three days later, the germinated seeds were then transferred in a growth chamber at the temperature of 25°C/20°C with a photoperiod of 12 h, and grown in pots holding vermiculite. The germinated seeds and seedlings were irrigated with Murashige and Skoog (MS) nutrient solution once every 3 days so as to maintain a suitable supply of water and nutrients to the plants. Fifteen days after seed germination, the seedlings were conducted with drought, salt, low and high temperature treatments. For drought and salt stress treatments, the plants were irrigated with the MS solution supplemented with 10% polyethylene glycol (PEG) 6000 or supplemented with 100 mmol·L⁻¹ NaCl for 36 h, respectively. For low and high temperature treatments, the plants were transferred to a growth chamber set a temperature of 4°C and 35°C for 36 h, respectively. Leaves were harvested at the indicated time and were immediately frozen in liquid nitrogen, stored at −80°C until their use.

The total RNA in leaves of control and 36 h under drought, salt, low and high temperature stresses were isolated with RNA extraction kit (TRIzol) reagent (Invitrogen) according to the manufacturer's instructions. For the detection of transcripts of *TaSOD1.1* and *TaSOD1.2* under different treatments, the same RT-PCR procedure and primer pairs were used as those in *TaSOD1.1* and *TaSOD1.2* cloning mentioned above. The products of 25 and 40 cycles were used to identify the abundance of *TaSOD1.1* and *TaSOD1.2* transcripts under different treatments. For each treatment, the triplicates of RT-PCR with consistent results were performed.

## 2.5 SOD activity assay in wheat leaves under the control of drought, salt, low and high temperature

The leaves were harvested at a time course of 6 h, 12 h, and 36 h in the drought, salt, low and high temperature treatments. About 0.2 g sample each was ground to powder in a mortar with liquid nitrogen. 4 mL phosphate buffer solution (PBS, pH 7.8) was used to extract the soluble proteins that contained the SODs. After 15 min of 10000 × g centrifugation at 4°C, the supernatant was used for the assay of SOD activity. The procedure of SOD activity assay was performed according to the description by Cakamsk and Marschner (1992). For each time point, four assay replicates with consistent results were used in this study. The leaves at each time point without treatments were used as control (CK).

# 3 Results

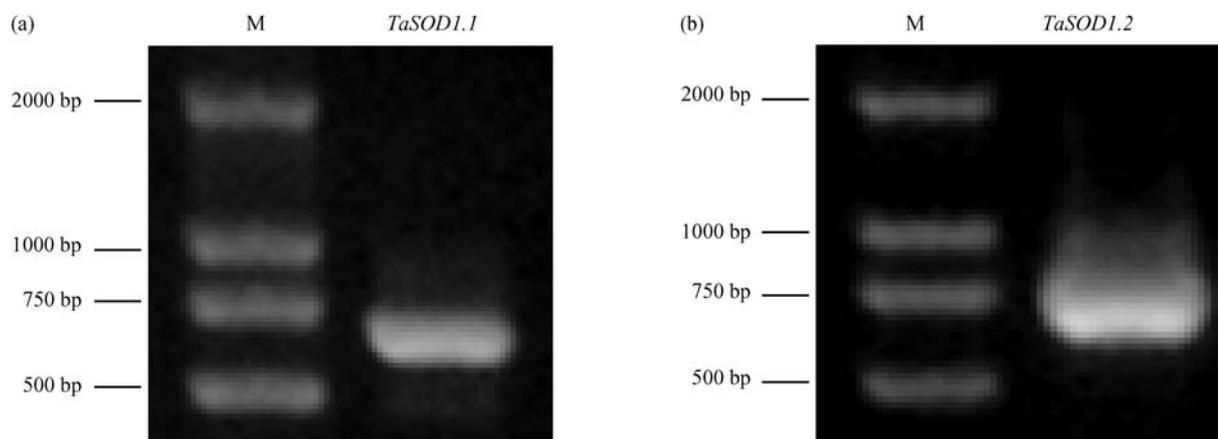## 3.1 Identification and cloning of two novel Cu/Zn SOD genes in wheat

In the international bioinformatics website (www.tigr.org), two uncharacterized wheat CuZnSOD tentative consensus (TCs numbers were TC250697 and TC250698, respectively) were identified. Multiple alignment analysis at the nucleic level found that TC250697 and TC250698 were 98.20% and 99.6% similar with *SOD1.1* and *SOD1.2*, the released and characterized CuZnSOD genes in wheat (Wu et al., 1996, 1999), respectively. The similarity between *TaSOD1.1* and *TaSOD1.2* at the nucleic level was 98.5%. No other SOD genes with the same sequence with TC250697 or TC250698 were reported. Therefore, TC250697 and TC250698 in this study were referred to *TaSOD1.1* and *TaSOD1.2*, respectively.

Based on the RT-PCR approach, the open reading frame (ORF) of *TaSOD1.1* and *TaSOD1.2* were amplified using the transcribed single strand cDNAs from the 36 h drought-treated leaves as templates and specific primers. The RT-PCR results of *TaSOD1.1* and *TaSOD1.2* were listed in Fig. 1(a) and 1(b). Sequencing results showed that the products were the same as the TCs corresponding to *TaSOD1.1* and *TaSOD1.2* released in TIGR.

## 3.2 Characterization analysis of TaSOD1.1 and TaSOD1.2

The cDNA full length of *TaSOD1.1* and *TaSOD1.2* was 980 bp and 1120 bp, respectively, predicting that all were translated into 201 amino acids. TaSOD1.1 had 98.5% similarity with TaSOD1.2 at the amino acid level. Target P analysis detected a 45-aa length of transit peptide located at the N-terminal of TaSOD1.1 and TaSOD1.2 (chloroplast target peptide (cTP) value, 0.754) (Fig. 2(a) and 2(b)), indicating that TaSOD1.1 and TaSOD1.2 targeted the chloroplast after posttranslational modifications by the guidance of the transit peptide. Similar to SOD1.1 and SOD1.2 (Wu et al., 1996; 1999), the conserved CuZn-SOD domain consisting of 79-aa (48aa-126aa) was explored in TaSOD1.1 and TaSOD1.2. TaSOD1.1 and TaSOD1.2 together should be classified into subclasses of CuZnSOD, and be functional in the chloroplast.

Multiple alignments show that the dramatic genetic diversities existed for transit peptide sequences among the plant species. Figure 3 indicates that there were low similarities in the transit peptides between wheat (TaSOD1.1, TaSOD1.2, SOD1.1, and SOD1.2), Arabidopsis (NP_565666, and AAK60277), and rice (BAD09607). This result implicates that different mechanisms were used for the CuZnSODs to target the

**Fig. 1** Agarose electrophoresis of reverse transcriptase-polymerase chain reaction (RT-PCR) products of *TaSOD1.1* (a) and *TaSOD1.2* (b)

chloroplast in the plant species. However, the CuZn-SOD domains among the plant species had very high similarities, indicating that this domain was much conserved, independent of the genetic differences in the plant species. A CuZnSOD from *N. nucifera* (AAW80436) did not have a transit peptide at the N-terminal, indicating that it targeted the cytosol instead of the chloroplast (Fig. 3).

The phylogenetic tree was drawn based on the total forty-two SODs from different plant species (Fig. 4). In this tree, the similarities at the amino acid level between TaSOD1.1 and other SODs from wheat and other plant species changed from 64.5% to 98.5%. Based on the unrooted branches in the tree, all the query SODs could be clustered into four subgroups, ranging from Subgroup I to Subgroup IV. The numbers of SODs in Subgroups I, II, III, and IV were 15, 10, 4, and 13, respectively. Among them, TaSOD1.1 and TaSOD1.2 were classified into Subgroup I, having much higher similarities with rice CuZnSOD (GenBank accession number BAD09607), *N. nucifera* CuZnSOD (GenBnak accession number AAW80436), and *D. lanuginosum* CuZnSOD (GenBank accession number AAK60277). This result, though, implied that the CuZn-SOD domains were much conserved, and that the evolution of this type of SODs in plant species was largely divergent.

### 3.3 Expression patterns of *TaSOD1.1* and *TaSOD1.2* under drought, salt, low and high temperature conditions

The abundances of *TaSOD1.1* and *TaSOD1.2* transcripts under the drought, salt, control (CK), low and high temperature conditions are listed in Figs. 5(a) and 5(b). Compared with CK, 36-h treatments of 10% PEG, 100 mmol·L$^{-1}$ NaCl, at 4°C and 35°C did not cause the changes of *TaSOD1.1* transcripts (Fig. 5(a)). However, 36-h treatments of 10% PEG, 100 mmol·L$^{-1}$ NaCl, at 4°C and 35°C all strongly induced the *TaSOD1.2*

expression. The abundances of *TaSOD1.2* transcripts under PEG, NaCl, and low and high temperatures were all significantly higher than that of CK (Fig. 5(b)), though similar expression patterns were found among the treatments of PEG, NaCl, at 4°C and 35°C, indicating that *TaSOD1.2* was involved in mediating the signal transductions initiated by abiotic stresses, such as drought, salt, low and high temperature. These results showed that the expression of *TaSOD1.1* was not modulated by the drought, salt, low and high temperature stresses, whereas the expression of *TaSOD1.2* was regulated by the above environmental stimuli.

### 3.4 SOD activities in leaves under drought, salt, low and high temperature conditions

The SOD activities in leaves at 6 h, 12 h, and 36 h of the drought, salt, low and high temperature treatments were assayed. Compared with that of CK, the SOD activities at the indicated time points in treatments of drought, salt, low and high temperature were all elevated. Comparisons of the SOD activities among the time points indicated that the SOD activities were declined with the prolonged treatments, especially in high temperature treatments. The SOD activities in the treatments of drought, salt, low and high temperature, however, always kept higher levels than those in CK. This result implied that the stress conditions, such as drought, salt, low and high temperature could elevate the SOD activities, by which to alleviate the cellular damage from the ROS generated by the abiotic stresses. Thus, enhancements of SOD activities could be one of the important pathways for plants to acclimate the adverse environments, such as those abiotic stresses mentioned above. The strong inducement of *TaSOD1.2* under the drought, salt, low and high temperature stresses was in accordance with the increase of SOD activities under the above stress conditions, suggesting that *TaSOD1.2* has

(a)   1    CCACGCGTCCGCACCTCCACCACCAACCCCCAAAAGTTCTTCCGCTCCCGACGACCGCCATGGCCGCTCAGAGC
                                                                           M  A  A  Q  S
     75    CTCCTCTTTGCCGCCGCCGCGCCTCTCTTCCAGGCTCCTGCCTCTGCCCGCCCTTTCCAGTCGCTCCGAATTGTC
            L  L  F  A  A  A  A  P  L  F  Q  A  P  A  S  A  R  P  F  Q  S  L  R  I  V
    150    TGCACCCCAGAAGGCGCCACCGCCGCCGCCAGGGCGCTCGTCGTCGCCGACGCCACCAAGAAGGCAGTCGCGGTG
            C  T  P  E  G  A  T  A  A  A  R  A  L  V  V  A  D  A  T  K  K  A  V  A  V
    225    CTCAAGGGCACCTCGCAGGTCGAGGGCGTCGTCACGCTCACCCAGGAAGACGACGGTCCTACGACGGTGAACGTT
            L  K  G  T  S  Q  V  E  G  V  V  T  L  T  Q  E  D  D  G  P  T  T  V  N  V
    300    CGTATCACTGGACTTGCTCCTGGACTTCATGGCTTCCACCTCCATGAGTTTGGTGACACGACTAATGGATGCATA
            R  I  T  G  L  A  P  G  L  H  G  F  H  L  H  E  F  G  D  T  T  N  G  C  I
    375    TCAACAGGTCCACATTTTAACCCAAACGGCCTGACACATGGTGCACCAGAAGATGAAGTCCGTCATGCGGGTGAC
            S  T  G  P  H  F  N  P  N  G  L  T  H  G  A  P  E  D  E  V  R  H  A  G  D
    450    CTGGGAAACATTGTTGCCAATGCTGAAGGTGTGGCCGGAGACAACCATTGTCGATAGCCAGATTCCTTTGACTGGC
            L  G  N  I  V  A  N  A  E  G  V  A  E  T  T  I  V  D  S  Q  I  P  L  T  G
    525    CCTAATGCAGTTGTTGGGAGAGCGTTTGTTGTTCATGAGCTTGAAGATGACTTGGGAAAAGGTGGGCATGAGCTC
            P  N  A  V  V  G  R  A  F  V  V  H  E  L  E  D  D  L  G  K  G  G  H  E  L
    600    AGCCTCAGTACTGGAAATGCTGGTGGAAGACTTGCATGTGGTGTTGTTGGCCTGACCCCGTTGTAGGTCGCTGGT
            S  L  S  T  G  N  A  G  G  R  L  A  C  G  V  V  G  L  T  P  L
    675    TTCCATCTCTGGTTTTGCATCTCCAGTCATACTGGTTTCCATCTCTGGTTTTGCATCTCCAGTCATACTTGTAAC
    750    GACGCCATTCTCGTTTTACCTGGATTTCAAATATCGGATGCTTAATAGTTTCTGTTGATCGTTTGTATCAGTGTA
    825    AAGTTGGGTTTATCAAATAAATGTTTGCACACTTTCGTCTTGTTGAGCTATGAAGCGTGAACTCGGGAATTTTGG
    900    TGTGCGTAAGCTATAAAATGCTCAAAGAATTATATTTGTGGATGTCCTTATTCAAAGGGGGGCCCGGTACCAATC
    975    GCCTAA


(b)   1    GAACCTCGCGAGCTCGACGCAACGGCCGCGGAACATTTTGGCCCTCGTGAATCAGTCCCAATAAAGCCCGCGCG
     75    GCCCGAGCCCACCTTATCCCCAGTCACCACCTCCACCACCAACCTCCAAAAGTTCTTCCGCTTCCGACGACAGCC
    150    ATGGCCGCTCAGAGCCTCCTCTTTGCCGCCGCCGCGCCTCTCTTCCAGGCTCCTGCCTCTGCCCGCCCTTTCCAG
            M  A  A  Q  S  L  L  F  A  A  A  A  P  L  F  Q  A  P  A  S  A  R  P  F  Q
     225   TCGCTCCGAATTGTCTCCACCCCAGGAGGCGCCACCGCCGCCGCCAGGGCGCTCGTCGTCGCCGACGCCACCAAG
            S  L  R  I  V  S  T  P  G  G  A  T  A  A  A  R  A  L  V  V  A  D  A  T  K
     300   AAGGCAGTCGCGGTGCTCAAGGGCTCCTCCCAGGTCGAGGGCGTCGTCACGCTCACCCAGGAAGACGACGGTCCT
            K  A  V  A  V  L  K  G  S  S  Q  V  E  G  V  V  T  L  T  Q  E  D  D  G  P
     375   ACGACGGTGAACGTTCGTATCACTGGACTTGCTCCTGGACTTCATGGCTTCCACCTCCATGAGTTTGGTGACACG
            T  T  V  N  V  R  I  T  G  L  A  P  G  L  H  G  F  H  L  H  E  F  G  D  T
     450   ACTAATGGATGCATATCAACAGGTCCACATTTTAACCCAAACGGCCTGACACATGGTGCACCAGAAGATGAAGTC
            T  N  G  C  I  S  T  G  P  H  F  N  P  N  G  L  T  H  G  A  P  E  D  E  V
     525   CGTCATGCGGGTGACCTGGGAAACATTGTTGCCAATGCTGAGGGTGTGGCCGGAGACAACCATTGTCGATAGCCAG
            R  H  A  G  D  L  G  N  I  V  A  N  A  E  G  V  A  E  T  T  I  V  D  S  Q
     600   ATTCCTTTGACTGGCCCTAATGCAGTTGTTGGGAGAGCGTTTGTTGTTCATGAGCTTGAAGATGACTTGGGAAAA
            I  P  L  T  G  P  N  A  V  V  G  R  A  F  V  V  H  E  L  E  D  D  L  G  K
     675   GGTGGGCATGAGCTCAGCCTCAGTACTGGAAATGCTGGTGGAAGACTTGCATGTGGTGTTGTTGGCCTGACCCCG
            G  G  H  E  L  S  L  S  T  G  N  A  G  G  R  L  A  C  G  V  V  G  L  T  P
     750   TTGTAGGTCGCTGGTTTCCATCTCTGGTTTTGCATCTCCAGTCATACTTGTAACGACGCCATTCTCGTTTTACCT
            L
     825   GGATTTCAAATACCAGATGCTTAATAGTTTCTGTTGATCGTTTGTATCAGTGTAAAGTTGAGTTAATCAAATAAA
     900   TGTTTGCACACTTTCGTCTTGTTGAGCTATGAAGCGTGAACTTGGGAATTTTGGTGTGCGTAAGCTATAAAATGC
     975   TCAAAGAATAATATTTGTGGATGTCCTTATTTCCGAACTTGGTGTCAATAATATCATTGTTACTCTGCAAAAGA
    1050   AAAACATTGTTTTCTGATTTAGTTGAAAGAGAGTATTCCAGGTCAGCCTTAAAACAAAAGCGAGAGGGCTT
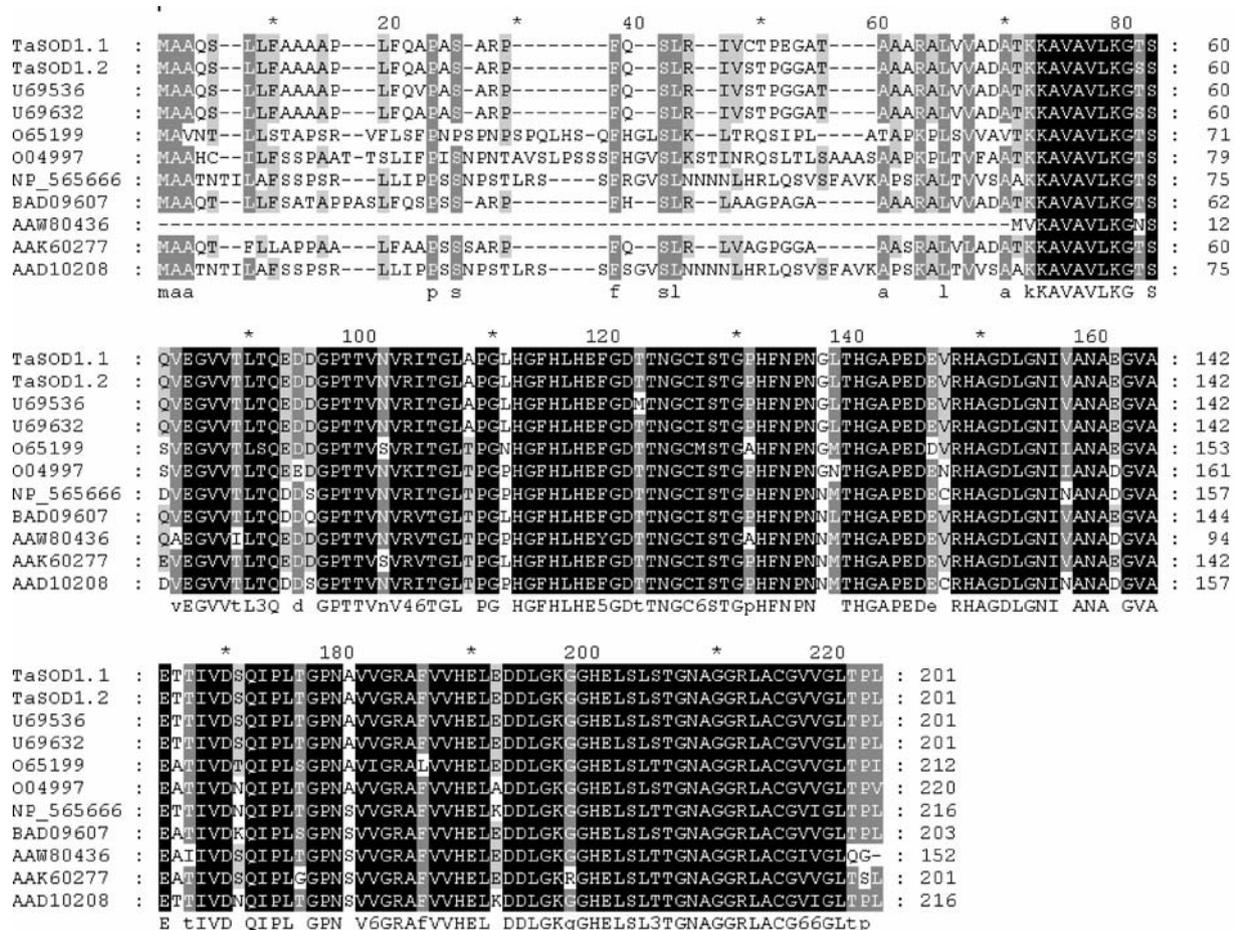
**Fig. 2**   cDNA full lengths and deduced amino acids of *TaSOD1.1* (a) and *TaSOD1.2* (b)

played an important role for the enhancement of SOD activities in leaves.

## 4   Discussion

When plants are exposed to photoinhibitory light, ozone, or other environmental conditions such as drought, salt, low and high temperature, the oxidative stress in plants will be brought about and the $O_2^-$ levels may increase (Yruela et al., 1996; Runeckles and Vaartnou, 1997; Du et al., 2001). It is found that abiotic stresses such as drought, salt, low and high temperature affecting the oxidation metabolism in plant cells may be due, at least in part, to the inhibition of superoxide dismutase (SOD) and other endogenous active oxygen elimination system. Accumulation of active oxygen such as superoxide anion ($O_2^-$), hydroxyl ($OH^-$), hydroxyl radical (OH), and hydrogen peroxide ($H_2O_2$) is harmful to proteins, membranes lipid, DNA and other cell components. As a result, many physiological and biochemical steps and normal growth and development of plants are heavily

**Fig. 3** Multiple alignments of TaSOD1.1, TaSOD1.2, SOD1.1, SOD1.2 and other related CuZuSODs in plant species with high similarities with TaSOD1.1 and TaSOD1.2
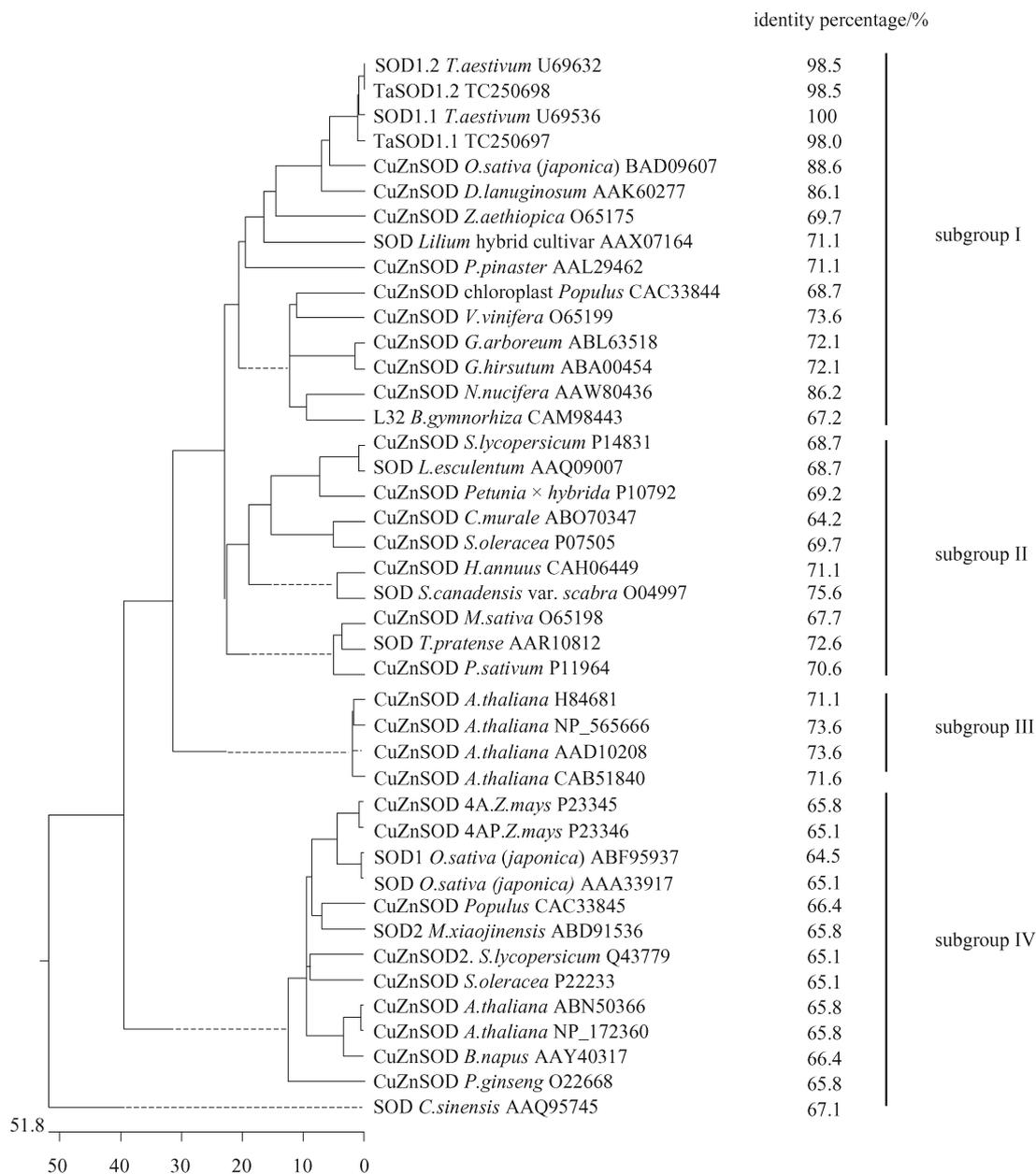
Note: U69536, SOD1.1, *T. aestivum*; U69632, SOD1.2, *T. aestivum*; AAD10208, *A. thaliana* CuZnSOD; NP_565666, *A. thaliana* CuZnSOD; AAK60277, *D. lanuginosum* CuZnSOD; AAW80436, *N. nucifera* CuZnSOD; BAD09607, *O. sativa* (*japonica* cultivar-group) CuZnSOD; O65199, *V. vinifera* CuZnSOD; O04997, *S. canadensis* var. *scabra* SOD

influenced (Du et al., 2001; Pastori et al., 2000; Prasad, 1997; Shen et al., 1995; Zhu and Scandalios, 1994). In maize, it is found that SOD activity correlates positively with the oxidation resistance of plants under drought stress and can be used as an index for the evaluation of drought tolerance (Jiang et al., 1991; Hodges et al., 1997).

Though a lot of SOD genes in the plant species have already been isolated and characterized (Newman et al., 1994; Rounsley et al., 1996; Delseny et al., 1997; Kliebenstein et al., 1998), in wheat, only few SOD genes including three MnSOD genes and two CuZnSOD genes (*SOD1.1* and *SOD1.2*) have been released and reported (Wu et al., 1996; 1997; 1999). In Arabidopsis, the total of seven SOD genes have been characterized (Delseny et al., 1997; Kliebenstein et al., 1998), suggesting that some novel SOD genes, except the released or previously reported, were still unknown in wheat, considering that wheat has a much more complicated genome. In this study, two TCs corresponding to novel CuZnSOD genes, which had high similarities with *SOD1.1* and *SOD1.2*

(Wu et al., 1996; 1999), were identified in the inter-national bioinformatics website, TIGR. It was previously reported that the CuZnSOD can be located at two positions of the cell, cytosol or chloroplast (Bowler et al., 1992). The explored a 45-aa length of transit peptide in N-terminal of TaSOD1.1 and TaSOD1.2, suggesting that their target is at the chloroplast. The identities between TaSOD1.1 and CuZnSODs from other plant species varied a lot, showing the evolutions of CuSODs in plant species were largely divergent, though the CuZnSOD-domains were much conserved.
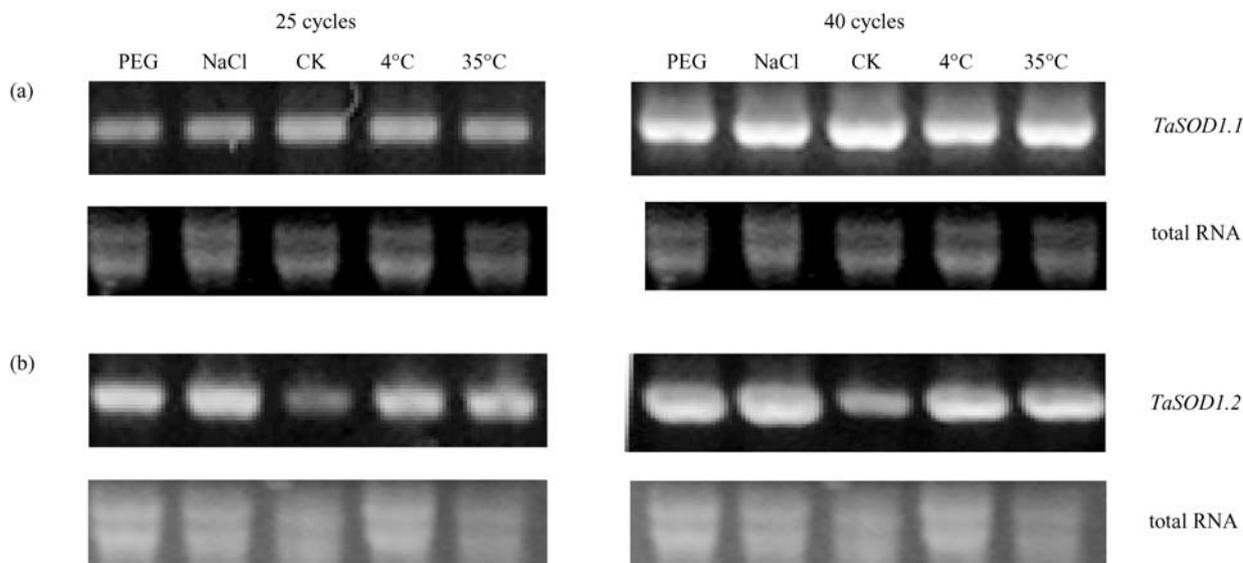
In wheat, expression analysis indicated that MnSOD genes (*SOD3.1* and *SOD3.2*) were drought inducible and decreased after rehydration. In contrast, Cu/ZnSOD (*SOD1.1* and *SOD1.2*) mRNA was not drought inducible but increased after rehydration (Wu et al., 1999). Under a low temperature (2°C) condition, the transcripts of *SOD3.1* and *SOD3.2* in seedlings were induced and attained maximum levels between 7 d and 49 d, depending on the variety types. The transcripts of *SOD1.1* and

**Fig. 4** Phylogenetic tree analysis of TaSOD1.1, TaSOD1.2 with other related superoxide dismutases (SODs) in plant species
Note: For each SOD in the tree, the name was given first, followed by the name of plant species, and then the GenBank accession number.

SOD1.2 were detected sooner, but disappeared after 21 d of acclimation (Wu et al., 1999). In this paper, the transcripts of *TaSOD1.1* under different growth conditions, such as control (CK), 36-h of 10% PEG, 100 mmol·L$^{-1}$ NaCl, at 4°C and 36°C, were similar, indicating the expression of *TaDOD1.1* was not regulated by the drought, salt, low and high temperature stresses, while that of *TaSOD1.2* was strongly induced by them, suggesting the *TaSOD1.2* was involved in mediating signal transductions initiated by the above stresses. Though *TaSOD1.2* had a similarity of 99.6% with *SOD1.2* at the nucleic level, the expression patterns under the drought stress were different. The transcripts of *TaSOD1.2* were dramatically elevated by drought, whereas those of *SOD1.2* were not induced by drought but had an increased expression level after rehydration (Wu et al., 1999). This could result from the differences at the promoter regions in *TaSOD1.2* and *SOD1.2*, which further resulted in the differences on gene transcription regulation in the two homologous genes. We have noticed that the whole ESTs composing the tentative consensus (TC250697, *TaSOD1.1* in this paper) contained one EST expressed in the drought condition, but this gene was expressed with a similar level with that under control, in the condition

**Fig. 5** Expression patterns of *TaSOD1.1* and *TaSOD1.2* in leaves under control (CK) and the treatments of drought (10% PEG), salt (100 mmol·L$^{-1}$ NaCl), low temperature (4°C) and high temperature (35°C)
Note: The treatments of drought, salt, low and high temperature are all 36-h before sampling. (a) *TaSOD1.1* and (b) *TaSOD1.2*. Total RNAs are shown for the loaded control in semi-quantitative RT-PCRs.

without abiotic stimuli, suggesting that *TaSOD1.1* did not respond to the drought nor was involved in mediating the drought signal.
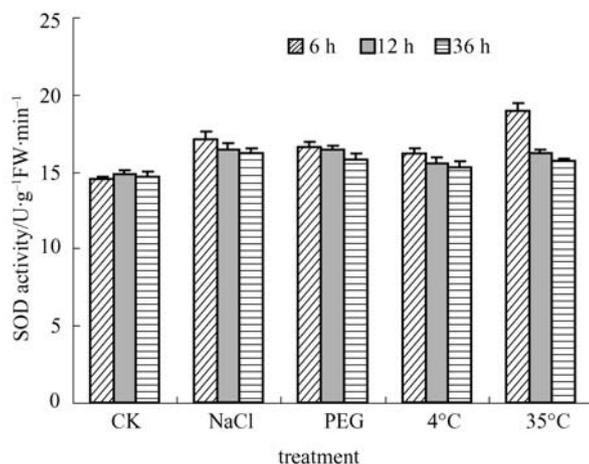
In recent years, superoxide dismutase (SOD) gene was used to transform plants for the improvement of abiotic stresses. van Camp et al. (1994) transferred manganese superoxide dismutase gene (MnSOD) from tobacco into clover. The transgenic plants showed a significant increase in growth vigor and yield under drought conditions. Meanwhile, oxidation resistance under the drought stress was reported to significantly increase within transgenic tobacco, overexpressing a copper/zinc superoxide dismutase gene (Cu/ZnSOD) from tomato and from pea (Simontacchi et al., 1993). Overexpression of tobacco MnSOD gene in maize chloroplast under the control of



**Fig. 6** Superoxide dismutase (SOD) activities in leaves at different time points under control (CK) and the treatments of PEG, NaCl, at 4°C and 35°C

cauliflower mosaic virus promoter CaMV35S was found to be helpful in the decrease in the oxidation of leaves (Frank et al., 1999). Recently, Du et al. (2006) reported that overexpression of exotic superoxide dismutase gene MnSOD could alleviate the oxidative stress in maize. Taken together with it, the enhancement of SODs in plants played an important role on protecting the plant damage from the abiotic stresses and the oxidation stress by scavenging the more generated ROS. In this paper, *TaSOD1.2* was strongly induced under the drought, salt, low and high temperature conditions, suggesting that *TaSOD1.2* could be a useful target gene in alleviating the plant damage which resulted from adverse environments, such as the abiotic stresses mentioned above and other oxidation stresses, which occur frequently in crop production. Thus, a binary vector fused in the open reading frame of *TaSOD1.2* has been constructed, and the transformation of this construct to tobacco, cotton, and wheat is underway. By using transgene approaches, the function of *TaSOD1.2* in the alleviation of plant damages caused by the adverse environments, such as drought, salt, low and high temperature, and oxidation stresses will be evaluated in different plant species in the future.

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