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# Molecular Characterization and Expression of CuZn-superoxide Dismutase *(PSOD1)* from *Populus alba* ×*Populus glandulosa*

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## ABSTRACT

**A cDNA, *PSOD1*, encoding cytosolic copper/zinc superoxide dismutase (CuZn-SOD) was cloned and characterized from a full length cDNA library prepared from *Populus alba* × *Populus glandulosa* cultured in vitro. *A PSOD1*, is 725 nucleotides long and has an open reading frame of 459 bp with 152 amino acid residues (pI 5.43). The deduced amino acid sequence of *PSOD1* perfect matched to the previously reported CuZn-SOD (CAC33845.1). Consensus amino acid residues (His-45, -47, -62, -70, -79, -119) were involved in Cu-, Cu/Zn-, and Zn- binding ligands. The deduced amino acid sequence of *PSOD1* exhibited the high level of similarity from 100 to 85% among previously registered SOD genes. The expression of *PSOD1* in poplar increased at the 1 mM $H_2O_2$ and drought stress during 30 min and 60 min, but the ozone treated poplar increased at 30 min in the early time and then decreased at 60 min.**

***Key words*** : Abiotic stress, *Populus alba* ×*Populus glandulosa*, RT-PCR, superoxide dismutase

## INTRODUCTION

Superoxide dismutase (SOD) is of major importance in protecting living cells against superoxide anion toxicity produced under oxidatively stressed circumstances. SODs are metalloenzymes of 17 to 85 kDa that are ubiquitous in aerobic organisms and function by catalyzing the dismutation of O2- to $H_2O_2$ and $O_2$. This disproportionation reaction is very efficient and is limited only by the rate of diffusion of $O_2^-$ into the active site of the enzyme and the availability of hydrogen ions (Albright *et al.*, 1989).

These proteins are ubiquitous in aerobic organisms, where it plays a major role in defense against reactive oxygen species (ROS)-mediated toxicity. ROS can react very rapidly with DNA, lipids and proteins, which causes severe cellular damage (Van Breusegem *et al.*, 1999). Most plants contain a number of SOD isozymes that are located in various cellular compartments. Three types of SODs have been classified on the basis of the metals present at the active site. These are iron (Fe-SOD), manganese (Mn-SOD), and copper/zinc (CuZn-SOD) (Fridovich 1989).

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Plants generally contain CuZn-SOD in the cytosol and chloroplast, Fe-SOD in chloroplast and Mn-SOD in the mitochondrial matrix and peroxisomes (Palma *et al.*, 1986; Salin, 1988; Asada, 1992; Bowler *et al.*, 1994). Several SOD cDNAs have been cloned from plants (Perl *et al.*, 1993), and transgenic plants have been produced that exhibit enhanced SOD activity.

Poplar plants have been shown to be excellent candidates for phytoremediation purposes. They can be cultivated at high rates of growth and produce a large biomass. But the poplar plants are damaged by the increasing air pollutants, such as sulfurous acid gas, acid rain and ozone. These air pollutants have been associated with the increased production of ROS. To increase the abiotic stress-tolerance by gene transformation in poplar, we analyzed 2,000 ESTs from a full length cDNA library constructed with ozone-treated poplar and isolated superoxide dismutase gene (*PSOD1*). In this present work, we characterized *PSOD1* gene and performed the expression analysis using quantitative RT-PCR.

## MATERIALS AND METHODS

### Plant materials

Poplar (*Populus alba*×*Populus glandulosa*) were grown in vitro on the Mccown woody plant medium (Lloyd and McCown, 1980) supplemented with 3% sucrose under the condition for 16 hr in the light irradiation and 8 hr in the dark. The poplar plants *in vitro* serially subcultured on the same medium with one-node culture attached a leaf per 5 weeks.

### Abiotic stress treatments

To investigate the response of poplar against oxidative stresses, such as hydrogen peroxide ($H_2O_2$), drought and ozone, we used the leaf of poplar cultured in vitro for 4 weeks. Poplar leaves attached in the middle region cutted with a scalpel. Cutted poplar leaves soaked in 1 mM $H_2O_2$ for the oxidative stress and left on the dried filter paper (Whatman, England) for the drought during 30 min and 60 min, respectively. Poplars grown during 4 weeks *in vitro* opened the stopper of glass bottle and then transferred in the incubator. Ozone was fumigated with 300 ppb (Ozonature, Korea) into the incubator during 30 min and 60 min. After the stress treatments, poplar leaves were immediately frozen in liquid nitrogen ($N_2$) and stored at -80℃.

### Purification of RNA and construction of cDNA library

Total RNA was isolated from the stress treated poplar leaves using the method of guanidine isothiocyanate (TRIzol, Gibco BRL). The tissue was frozen with liquid nitrogen and ground to a fine powder with a mortar and pestle. Extraction reagent was added 1 ml and ground more than 3 min. The mixture was centrifuged for 5 min at 4℃. The supernatant was removed to a new tube, added 0.2 ml chloroform, and vigorously mixed. Following centrifugation, total RNA was precipitated with isopropanol. The pellet was washed once with 75% ethanol, dried in vacuum for 3 min and dissolved in DEPC treated DDW. The RNA solution was stored at -80℃ for the RT-PCR analysis.

### Nucleotide sequencing and sequence analysis

pTriplEx phagemids were excised from the Uni-ZAP XR library and used as templates for sequence analysis. The 5' ends of randomly selected cDNA inserts were sequenced using the 5' sequencing primer by an automatic DNA sequencer (ABI prism 3700). Nucleotide and amino acid sequence analyses were performed using DNASIS program (Hitachi). Comparison of sequences to DNA and protein databases at NCBI was performed using the blast algorithm of Altschul *et al.* (1990).

**Quantitative RT-PCR analysis**

For the expression analysis of the *PSOD1* against the oxidative stresses, we employed the quantitative RT-PCR. A pair of gene-specific primers of the *PSOD1* gene was designed and used for RT-PCR analysis. Specific primers included the following: (*PSOD1*-forward) 5'-TAA CAC CAC ATG CTA CTC TGC CAC C-3'; (*PSOD1*-reverse) 5'-GCA GTG AAG GTG TCA AAG GCA CCA T-3'. As a control, we used a pair of the specific primers to poplar actin gene (*PAct*), 5'-CTT TCT GGT GGT GCA ACC ACC TTG A-3' (forward) and 5'-CAC CAT TGG TGC TGA GCG ATT CCG T-3' (reverse). Ten microgram of total RNA was used for the RT-PCR analysis, according to the method of Takakura *et al.* (2000). The PCR cycles for quantitative RT-PCR numbered 30 for the *PSOD1* and the *PAct* genes. RT-PCR products were run on 1% (W/V) agarose gel in 0.5×TAE buffer and then photograped for the expression analysis.

## RESULTS AND DISCUSSION

Poplar plants, excellent candidates for phytoremediation, are damaged by the increasing air pollutants, such as sulfurous acid gas, acid rain and ozone. These air pollutants have been associated with the increased production of ROS. To increase the abiotic stress-tolerance by gene transformation in poplar, we analyzed 2,000 ESTs from a full length cDNA library constructed with ozone-treated poplar plants.

The EST clones homologous to CuZn-siperoxide dismutase genes related in abiotic stress were isolated and the ESTs were named as *PSOD*. One cDNA clone (*PSOD1*) of them was 725 nucleotides long and possess an open reading frame of 459 bp encoding a deduced 152 amino acid polypeptide with pI 5.43 (Fig. 1). The sequence context around the translation start site perfectly matched the consensus sequence reported from various plants (AACAATGG, Kaminaka *et al.*, 1997). The N-terminal regions of the SODs are generally divergent and the conserved regions exist in the C-terminus. The deduced amino acid sequences encoded by *PSOD1* had a strong similarity to the previously described CuZn-SODs of other plants. Consensus amino acid residues (His-45, -47,-62, -70, -79, -119) were involved in Cu-(45, 47, 70, 119), Cu/Zn-(His-62), and Zn- (His-79) binding ligands (Fig. 2). It has been suggested that the functional role of SOD in protecting cells involves several residues including metal binding sites in the C-terminal part of the protein. Comparison of PSOD1 with other cytosolic CuZn-SODs showed higher homology (85% - 100%, Fig. 2) than chloroplastic CuZn-SODs (~60%; data not shown) regardless of species. In the phylogenetic analysis, PSOD1 was closer with CuZn-SODs of *P. tremula*×*P.tremuloides* and *C. limon* (Fig. 3).

We used semi-quantitative RT-PCR (QPCR) analysis to examine wether the expression of *PSOD1* gene related with abiotic stresses. Identity of the PCR bands was confirmed by DNA sequence analysis. Expression of the poplar actin gene, *PAct*, served as an internal control for PCR. The gene expression of *PSOD1* by the drought treatment was increased 30 min and 60 min gradually (Fig. 4). bu the expression of *PSOD1* by the 1 mM $H_2O_2$ and 300 ppb ozone treatment was increased up to 30 min after oxidative stress and then declined gradually (Fig. 4).

It is related that PSOD1 works in the early stage of ROS detoxification. Somehow the PSOD1 should protect the photosynthetic apparatus by efficiently removing the superoxide radicals generated from chloroplasts during the stress. But, the cytosolic form of CuZn-SOD could not scavenge the ROS existed in chloroplast directly because superoxide anion could not transferred through the membrane. Thus, it could be presented that PSOD1 protected chloroplast external membrane against ROS existed in the cytosol. And according to the rescent scavenging system model,

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GACTCTTCACTGCTTCACTCTCTAACACGACGACCAAGCATACTCTCTTTTTCTCTCTCT      60
CTACTCTCGAAGGGGCGCTCTGAGATCACATAGAACAATGGTGAAGGCTGTTGCTGTTCT     120
                                    M  V  K  A  V  A  V  L        8

GAATAGCAGTGAAGGTGTCAAAGGCACCATCAACTTTACCCAAGAAGGAGATGGTCCAAC     180
 N  S  S  E  G  V  K  G  T  I  N  F  T  Q  E  G  D  G  P  T      28

TACTGTAACTGGAAGCCTCTGTGGTCTTAAGCCAGGCCTTCATGGCTTCCATGTTCATGC     240
 T  V  T  G  S  L  C  G  L  K  P  G  L  H  G  F  H  V  H  A      48

CCTTGGAGACACCACAAATGGCTGCATGTCAACTGGCCCGCATTTTAATCCTGTAGGCAA     300
 L  G  D  T  T  N  G  C  M  S  T  G  P  H  F  N  P  V  G  K      68

AGAGCATGGTGCCCCTGAGGATGAGAATCGTCATGCTGGTGATTTGGGAAATGTCACTGT     360
 E  H  G  A  P  E  D  E  N  R  H  A  G  D  L  G  N  V  T  V      88

TGGTGATGATGGCACCGCTACTGTCTCAATCATTGACAACCAGATTCCTCTTACTGGACC     420
 G  D  D  G  T  A  T  V  S  I  I  D  N  Q  I  P  L  T  G  P     108

AAATTCCATTGTTGGAAGGGCAGTTGTTGTTCATGCAGATCCTGATGATCTTGGCAAGGG     480
 N  S  I  V  G  R  A  V  V  V  H  A  D  P  D  D  L  G  K  G     128

AGGACATGAACTTAGCAAAAGCACTGGTAATGCTGGTGGCAGAGTAGCATGTGGTGTTAT     540
 G  H  E  L  S  K  S  T  G  N  A  G  G  R  V  A  C  G  V  I     148

TGGTTTGCAAGGCTGAACTTCCGCACTCCACCATAGTTTGTTAATAAATGCAGTTGCATA     600
 G  L  Q  G  *                                                  152

TGTATCAACCCGCAGCTACAGAGGTTTCTGATAGACTCTGAATAAAAGGATGAAAACCTA     660
CTGTTGTACTTTTTTGGTGGATTGGTTGTGTAATCTAAAAGTGTGTTCTGTTTGACCTCT     720
TACTC                                                            725
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Fig. 1. **Nucleotide and deduced amino acid sequence of *PSOD1*, CuZn-SOD cDNA, from *Populus alba*×*Populus glandulosa*.** The positions of nucleotides are shown on the left and the positions of amino acids under the below. Asterisk shows the termination codon. The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequencing Database under the accession number AB190500.

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P.alba ×glandulosa      MVKAVAVLNSSEGVKGTINFTQEGDGPTTVTGSLCGLKPGLHGFHVHALGDTTNGCMSTG 60
P.tremula ×tremuloides  MVKAVAVLNSSEGVKGTINFTQEGDGPTTVTGSLCGLKPGLHGFHVHALGDTTNGCMSTG 60
P.tremuloides           MVKAVAVLGGTEGVKGTVSFTQEGDGPTTVSGSLSGLKPGPHGFHVHALGDTTNGCMSTG 60
O.europaea              MVKAVTVLNSSEGVTGTVYFTQEGDGPTTVTGNLSGLKPGLHGFHVHALGDTTNGCMSTG 60
C.limon                 MVKAVAVLSSSEGVSGTIFFSQEGDGPTTVTGNVSGLKPGLHGFHVHALGDTTNGCMSTG 60
I.batatas               MVKAVAVLNSSEGVSGTIFFTQEGDGPTTVTGNLSGLKPGLHGFHVHALGDTTNGCMSTG 60
B.juncea                MGKGVRVLNSSEGVKGTIFFTQEGNGTTTVTGTVSGLKPGLHGFHVHALGDTTNGCMSTG 60
                        * * * * **   *** **  * *** * *** *  ***** ********************

P.alba ×glandulosa       PHFNPVGKEHGAPEDENRHAGDLGNVTVGDDGTATVSIIDNQIPLTGPNSIVGRAVVVHA 120
P.tremula ×tremuloides  PHFNPVGKEHGAPEDENRHAGDLGNVTVGDDGTATVSIIDNQIPLTGPNSIVGRAVVVHA 120
P.tremuloides           PHFNPAGKEHGAPEDDNRHAGDLGNVNVSDDGTATFTVVDNQIPLSGPNSIIGRAVVVHA 120
O.europaea              PHFNPVGKEHGAPGDENRHAGDLGNITVGEDGTAAINIVDKQIPLTGPHSIIGRAVVVHS 120
C.limon                 PHFNPAGKEHGAPGDDNRHAGDLGNITVGEDGTASFTITDKQIPLTGANSVIGRAVVVHG 120
I.batatas               PHFNPVGKEHGAPEDENRHAGDLGNVTVGDDGTAAFTIIDFQIPLTGPHSIIGRAVVVHG 120
B.juncea                PHFNPEGKTHGAPEDANRHAGDLGNITVGDDGTATFTITDSQIPLDGPNSIVGRAVVVHA 120
                        ***** ** **** * *********  *  ****    * **** *  *  ******* 

P.alba ×glandulosa       DPDDLGKGGHELSKSTGNAGGRVACGVIGLQG                          152
P.tremula ×tremuloides  DPDDLGKGGHELSKSTGNAGGRVACGVIGLQG                          152
P.tremuloides           DPDDLGKGGHELSKTTGNAGGRVACGIIGLQG                          152
O.europaea              DPDDLGRGGHELSKSTGNAGGRVACGIIGLQG                          152
C.limon                 DPDDLGKGGHELSKSTGNAGGRVACGIIGLQG                          152
I.batatas               DPDDLGKGGHELSKTTGNAGGRVACGIIGLQG                           52
B.juncea                EPDDLGKGGHELSLTTGNAGGRVACGIIGLQG                          152
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Fig. 2. **Multiple alignment of the deduced amino acid sequence of PSOD1 with those of CuZn-SOD genes from other plants;** *P. alba ×glandulosa* (AB190500), *P.tremula ×tremuloides* (CAC33845.1), *Fagus sylvatica* (AJ586519.1), *P. tremuloides* (AF016893.1), *O. europaea* (AJ428575.2), *C. limon* (AF318938.1), *I. batatas* (X73139.1) and *B. juncea* (X95728.1). Consensus amino acid residues (His-45, -47, -62, -70, -79, -119) show in bold. Sequence data was obtained from GeneBank listed and aligned using DDBJ ClustalW (Thompson *et al.*, 1994 and 1997) and GeneDoc (Nicholas *et al.*, 1997).

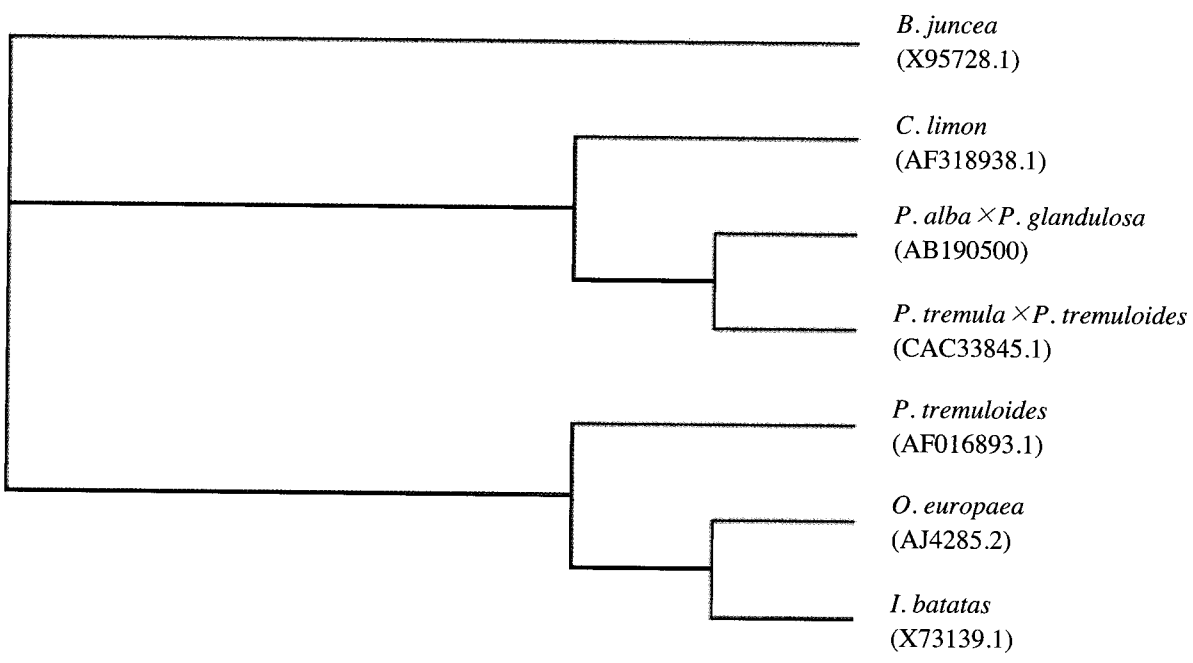


Fig. 3. **Phylogeny of the CuZn-superoxide dismutase protein family from *P. alba*×*glandulosa* and other plants.** Phylogenetic analysis is based on the deduced amino acid sequences of CuZn-SOD genes from various plant species. The branch lengths are proportional to divergence, with the scale of 0.1 representing 10% change.

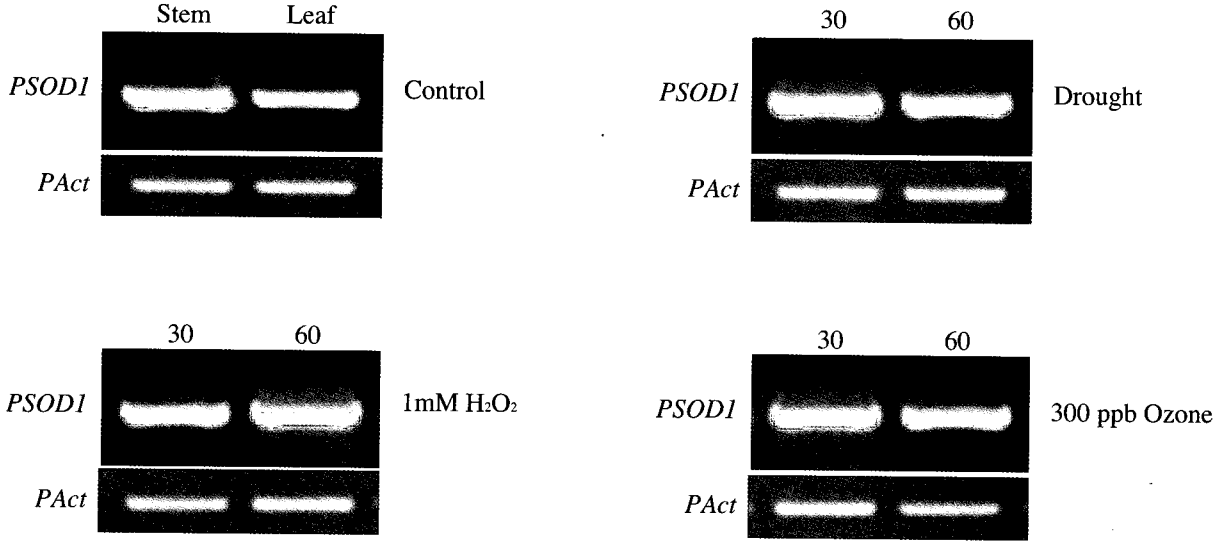


Fig. 4. Expression pattern of *PSOD1* by various stress. Total RNA from stress-treated samples served as templates for quantitative RT-PCR with gene-specific primers. *PAct* gene was used for PCR control.

cytosolic SOD changed superoxide anion (although it was not clear that produced from anywhere) into hydrogen peroxide. And then, the hydrogen peroxide was considered as possible signaling molecule in the signaling pathways (Shigeoka *et al.*, 2002). Therefore, we will study the relations between PSOD1 and abiotic stress and then produce the abiotic stress-tolerant transformants by re-introduction of *PSOD1* into poplar tree.

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## LITERATURE CITED

Albright L.M., E. Huala and F.M. Ausubel. 1989. Prokaryotic signal transduction mediated by sensor and regulator protein pairs. Annu. Rev. Genet. 23 : 311-336.

Altschul S.F., W. Gish , W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215 : 403-410.

Asada K. 1992. Production and scavenging of active oxygen in chloroplast. In: Scandalios JG (eds) Current commucations in cell and molecular biology, vol 5. Molecular biology of free radical research scavenging systems. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, PP. 173-192.

Bowler C., W. Van Camp, M. Van Montagu and D. Inze. 1994. Superoxide dismutase in plants. Crit. Rev. Plant Sci. 13 : 199-218.

Fridovich I. 1989. Superoxide dismutase. an adaptation to a paramagnetic gas. J. Biol. Chem. 264 : 7761-7764.

Kaminaka H., S. Morita, H. Yohoi, T. Masumura and H. Tanaka. 1997. Molecular cloning and characterization of cDNA for chloroplastic copper/zinc-superoxide dismutase in rice (*Oryza sativa* L.). Plant Cell Physiol. 38 : 65-69.

Lloyd G. and B. McCown. 1980. Commercially-feasible micropropagation of moutain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Int. Plant Prop. Soc. Proc. 30 : 421.

Nicholas K.B. and H.B. Nicholas. 1997. GeneDoc: analysis and visualization of genetic variation. http://www.cris.com/~Ketchup/ genedoc.html.

Palma J.M., L.M. Sandalio and L.A. del Rio. 1986. Manganese superoxide dismutase and higher plant chloroplasts: a reappraisal of a controverted cellular localisation. J. Plant Physiol. 125 : 427-439.

Perl A., R. Perl-Trevs, S. Galili, D. Aviv, E. Shalgi, S. Malkin and E. Galun. 1993. Enhanced oxidative stress defense in transgenic potato expressing tomato Cu, Zn superoxide dismutases. Theor. Appl. Genet. 85 : 568-576.

Salin M.L. 1988. Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant 72 : 681-689.

Shigeoka S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta and K. Yoshimura. 2002. Regulation and function of ascorbate peroxidase isoenzymes. J. Exp. Bot. 53 : 1305-1319.

Takakura Y., T. Ito, H. Saito, T. Inoue, T. Komari and S. Kuwata. 2000. Flower-predominant expression of a gene encoding a novel class I chitinase in rice (*Oryza sativa* L.). Plant Mol. Biol. 42 : 883-897.

Thompson J.D., D.G. Higgins and T.J. Gilbson. 1994. CLUSTAL W : improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-secific gap penalties and weight matrixchoice. Nucleic Acids Res. 22 : 4673-4680.

Thompson J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality

analysis tools. Nucleic Acids Res. 25 : 4876-4882.

Van Breusegem F., L. Slootan, J.M. Stassart, T. Moens, J. Botterman, M. Van Montagu and D. Inze. 1999. Overexpression of *Arabidopsis thaliana* FeSOD confers oxidative stress tolerance to transgenic maize. Plant Cell Physiol. 40 : 515-523.

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