SHORT COMMUNICATION

Cloning and DNA Sequencing of Cytosolic Cu/Zn Superoxide Dismutase Gene from Chinese Cabbage

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A cDNA clone for the cytosolic Cu/Zn superoxide dismutase (Cu/Zn SOD) from Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) was isolated and its DNA sequence was determined. The cDNA clone contains a complete coding sequence which encodes a protein of 152 amino acids and a 3-untranslated region including a poly A signal. The deduced amino acid sequence shows that it is highly homologous to the Cu/Zn SODs from other plants (60~90%). The lack of a putative chloroplast targeting transit peptide indicates that the clone represents a cytosolic form of Cu/Zn SOD. Genomic Southern hybridization suggests that cytosolic Cu/ Zn SOD genes are present in 1 or 2 copies per genome.

Keywords: cytosolic Cu/Zn SOD sequence, chinese cabbage

Superoxide dismutase (SOD: EC 1.15.1.1) plays an important role in removing superoxide which is generated during normal aerobic metabolic processes as well as stress conditions (Scandalios, 1993: Bowler et al., 1992). Superoxide itself and superoxidederived hydroxyl radicals (OH-) are known to initiate various cellular damages, such as DNA breakage or membrane degradation (Bowler et al., 1992). Particularly, hydroxyl radicals are considered to be very potent and are mainly responsible for cellular damages. It is well established that SOD is a major defensive mean to protect cells from many environmental stresses. SOD catalyses the dismutation of superoxide (O^2) , producing hydrogen peroxide and dioxygen. The reaction requires metal cofactors, and SODs are classified by the metal cofactor requirements: Mn SOD, Fe SOD and Cu/Zn SOD. In plants, Mn SOD is primarily located in mitochondria and Cu/Zn SOD in cytosol and chloroplast. Fe SOD is also present in certain plants, unlike Mn SOD and Cu/Zn SOD which are present in all plants (Duke et al., 1985). In general, the chloroplastic Cu/Zn SOD protects photosynthesis-related damages, and Mn SOD is mainly involved in removing superoxides produced during the aerobic respiration. Cytosolic Cu/Zn SOD is suggested to play a role in many cytosolic processes which have not been clearly characterized (Mittler et al., 1994).

Recently several experiments have been directed to improve stress-resistance via the transgenic plant system overexpressing SODs in chloroplasts (Bowler et al., 1991: Sen Gupta et al., 1993: Van Camp et al., 1994). Transgenic tobacco carrying overexpressed Mn SOD in chloroplasts became more resistant to photoinhibition and paraguat, a herbicide which artificially generates superoxide through the photosystem I (Bowler et al., 1991). These results suggest that SOD can be effectively used to scarvenge superoxides and protect plants from the various environmental adversities. However, there have been no attempts to overexpress SODs in cytosol where many non-photosynthetic reactions occur. It is reasonable to expect that transgenic plants with overexpressed SODs in cytosol would provide them better resistance during the non-photosynthetic environmental stresses, such as prolonged darkness or infection. Toward this objective, we isolated a cDNA clone which encodes a cytosolic Cu/Zn SOD in Chinese cabbage and the entire 0.8 kb cDNA was sequenced. The coding sequence will be used tobacco plants.

MATERIALS AND METHODS

DNA Sequencing

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A positive clone from the Chinese Cabbage cDNA library (λ ZAP, Strategene) containing the suspected cytosolic Cu/Zn SOD sequence (outcome of the random cDNA sequencing programme of Chinese cabbage, the Korean Agricultural Science-Technology Institute, Suwon, Korea) was used as a starting material. Partial DNA sequencing of the cDNA clone and its homology search indicated that this clone was very homologous to the Arabidopsis Cu/Zn SOD (Hindges et al., 1992). To confirm the clone, the 0.8 kb cDNA insert (EcoRI/Xhol, Fig. 1) was subcloned into pGem-5zf(+) (Strategene) for DNA sequencing. A properly located Ncol site within the insert was used to generate 0.24 kb EcoRI/ Ncol and 0.56 kb Ncol/Xhol fragments, fragments were used for DNA sequencing (Fig. 1). DNA sequencing was done by the dideoxy-chain termination method using the SequenaseII kit (USB).

Plant DNA Isolation and Southern Hybridization

Fresh leaves (2g) were ground to powder using a prechilled mortar and pestle. The extraction buffer contained 1% cetyltrimethylammonium bromide, 0.1 M Tris-HCl (pH 7.5), 10 mM EDTA, 0.7 M NaCl and 1% β -mercaptoethanol. Total plant DNA extraction was essentially done according to Naga, *et al.* (Nagao *et al.*, 1981). Two μ g of the extracted DNA were digested with 10 units of *Bam*HI, *Hind*III and *PstI*. Digested DNA was subjected to agarose gel electrophoresis (0.8%). The separated DNA fragments were transferred into a nylon membrane (BM), and hybridized with the DNA probe. The 0.8 kb *EcoRI/XhoI* fragment was labelled with the DIG-labelling system (BM) and the hybridization process were carried out in a stringent condition (60°C).

RESULTS AND DISCUSSION

The DNA sequence of the cytosolic Cu/Zn SOD



Fig. 1. Restriction map of the cDNA encoding the cytosolic Cu/Zn SOD from *B. campestris* ssp. *Pekinensis* and DNA sequencing strategy. Open box represents the coding sequence of the clone. clone from Chinese cabbage and derived amino acid sequence were determined (Fig. 2). It contains an open reading frame beginning with the ATG initiation codon and ending with the TAA termination codon. It encodes a protein of 152 amino acids, and its calculated molecular mass is approximately 15 kDa. There is the consensus sequence of the translation initiation site (AACTATGG) (Heidecker et al., 1986), well conserved in most dicot plants, and the polyA tail with a preceding AATAA polyadenylation signal. The amino acid sequence was compared to both cytosolic and chloroplastic Cu/Zn SOD from Arabidopsis thaliana (Ahindges et al., 1992). It also showed significant homologies to those from maize (78%) (Cannon et al., 1987), cabbage (86%) (Steffens et al., 1986), tobacco (80%) (Tsang et al., 1991). It is known that cytosolic Cu/ Zn SOD also has high homology to chloroplastic Cu/ Zn SOD. Our cytosolic Cu/Zn SOD showed 55% homology to the chloroplastic Cu/Zn SOD from tomato in which only the sequence from mature Cu/ Zn SOD was used for the comparison (Kardish et al., 1994). This Chinese cabbage clone apparently does not have a transit peptide sequence which is essential for targeting to chloroplast. The transit peptide usually contains many basic, hydroxylated, and hydrophobic amino acids. Taken together, the clone we sequenced is apparently a cytosolic Cu/Zn SOD.

Boxed regions in Fig. 3 represent the conserved sequences throughout the Cu/Zn SODs from different plants, most of them are suggested to be crit-

Fig. 2. The nucleotide and deduced amino acid sequences of the cDNA encoding the cytosolic Cu/Zn SOD from *B. cmpestris* ssp. *Pekinensis*.

¹ CCCCACGAGA TTAAATTCAC TTCAGACATT TGATACCTAC CATCTTACTC ATTTCAAAAG 61 COSTACCETG AGATCACAAA GGATATACAA AACT ATG GCC AAG GCA GTT GCA GTT MAKGVAV 116 TTG AAC AGE GAG GGT GTT AAG GGG ACT ATC TTC TTC ACC CAG GAA GGA SSEGVKGTIFFFQEG 167 GAT GGT CCC ACA ACT GTG ACT GGA ACT GTT TCT GGT CTT AAA CCT GGT CCC (5) D G A T T V T G T V S G L K P G P 18 CAT GGT ITTC CAT GTE CAT GET CTT GGT GAU ACC ACC AAC GGT TGC ATG TCT (2) H G F H V H A L G J T T N G C M S 90 ACC. GET CCA CAT TTU AAC CCT GAT GGT AAA ACC CAC GGT GCA CCC GAG GAT (18) GPHFNPDGKTHGSPED 30 GCT AAT CGT CAT GCT GGA GAT CTA GGA AAC ATC ATT GTT GGG GAT GAT GGA (.35) ANRHAGDLGNIIVGDDG 371 ACT GCC ACC TTC ACA ATC ACT GAC AGC CAG ATT CCT CTT ACT GGA CCA AAC (6) TAFFTITDS Q [PL T G P N 42 TET ATT GTA GGA AGG GET GTT GTT GTT GAT GGA GAC OGT GAT GAC CTT GGA (-10) A G R A V V v A D R 473 AAG GGA GGE CAT GAA CTC AGE TTG TCT ACT GGA AAT GCA GGG GGC CGT GTT SZDKGGHSLSLS GNAGGR 24 GCT TGT GGT ATT ATT GGT CTT CAG GGC TAA CGTGAAGETG TTACTTITCG 144) A (G I I G L Q G + 24 ACCANTUGAN GAGAGAGAGA GAGAGAGATG GAATAACGAG GTCCTACATG ACCTOCCTAC 534 TITGEGETG GEGETGETGEGETATATICA TOGACETTAG TOXECOAATG CATTEGETTE 194 AAGACAAAAA AAAACAGGAG AAAACCTITT TCTATTTCAT GAATAACACA GATCGTTGAA SA AACA

B. campestris ssp. pokinonsis(cyt)	M A K G V A V L N S S E G V K G T I F F T Q E G D G A T T V	тоттяс
A, thaliana(cyt)	NAKGVAV	SGTVSG
Maize(cyt)	MV KAV · AVLAGTD - VKGTIFFSQEGDGPTTV	TCSISC
Cabbegge(cyt)	A K A V N S S A V L I G V K G T I F F T H E G N G A T T V	тстъзс
Tobacco(cyr.)	M V K A V A V L S S S E G V S G T L F F T Q D G D A P T T L	TONVSO
Pea(chI)	AAKKAV - AVLKGTSAVEGTVTLIGEDEGPTTV	SVRITG
B. camapustri ssp. pekinensis(cyt)	L K P G P R G F H V H A L G D T T N G C M S T G P H F N P D G K T N	GSPED
A, the liana(cyt)	LKPGLHGFHVHALGDTTNGCNSTGPHFNPDG-KTH	CAPED
Maize(cyt)	L K P G L H G F H V H A L G D T T N G C M S T G P H F N P V G - K E H	GAPED
Cabbage(cyt)	L B P G L H G F H V H A L G D N T N G C N S T G P H F N P D G - K T H	GAPED
Tohecco(cyt)	L K P G L H G F H V H A L C D T T N G C M S T G P H Y N P A G K E H	GAPED
Pes(chl)	LTPGTHGFHLHEYGDTTNGCISTGPHFNPNKALTH	GAPED
B. campestris sep. pekinensis(cyt)	ANRHAGDLGNIIVGDDGTATFTITDSQIPLTGPNS	GRA
8, campestris sep, pakinensis(cyt) A, theliane(cyt)	ANRHAGDLGNIIVGDDGTATFTITDSOIPLTGPNS ANRHAGDLGNIIVGDDGTATFTITDCQIPLTGPNS	1 A G R A 1 V G R A
<pre>8. compostris sep. pokinensis(cyt) A.theliane(cyt) Haize(cyt)</pre>	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D C Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P H S	1 A G R A 1 V G R A 1 I G R A
8. campestris sep. pokinensis(cyt) A. theliane(cyt) Maize(cyt) Cabbage(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q : P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D C Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P H S A N R H A G D L G N J I V G D D G T A T F T I T O S Q I P L S G P N S	[A G R A [V G R A [] G R A [] G R A [V G R A
8, campestris sep. pokinensis(cyt) A.theliane(cyt) Maize(cyt) Cabbage(cyt) Tobacco(cyt)	A N R H A G D L G N I I V G D D G T A T FT I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T FT I T D C Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P N S A N R H A G D L G N I T V G D D G T A T FT I T D S Q I P L S G P N S E V R H A G D L G N I T V G E D G T A S FT L T D X Q I P L A G P Q C	[A G R A [V G R A [] G R A [V G R A [V G R A [] G R A
8, campestris sup. pokinensis(cyt) A.thuliane(cyt) Haize(cyt) Cubbage(cyt) Tobacco(cyt) Pea(chl)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D C Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P N S A N R H A G D L G N I T V G D D G T A T F T I T D S Q I P L S G P N S E V R H A G D L G N I T V G D D G T A S F T L T D X Q I P L A G P Q C E N R H A G D L G N I V V N A E G V A E A T I V D N Q I P L T G P N S	I A G R A I V G R A I I G R A I V G R A I V G R A V V G R A
8. campestris sep. polimensis(cyt) A. Uniliane(cyt) Haize(cyt) Cabbage(cyt) Tobacco(cyt) Pes(chi)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N J T V G D D G T A T F T I T D C Q I P L T G P N S E D R H A G D L G N Y T A G E D G V N V N I T D S Q I P L A G P H S A N R N A G D L G N J I V G D D G T A T F T I T D S Q I P L S G P N S E V R H A G D L G N I I V G D D G T A S F T L T D S Q I P L S G P N S E N R H A G D L G N I V A N A E G V A E A T I V D N Q I P L T G P N S	[A G R A [V G R A [] G R A [] G R A [] V G R A [] I G R 4 V V G R A
8. campestris sep. pelinerais(cyt) A. theliane(cyt) Heize(cyt) Cabbage(cyt) Tobecos(cyt) Pes(ch) 8. campestris sep. pelinerais(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D C O I P L T G P N S E D R H A G D L G N Y T A G E D G Y V N V N I T D S Q I P L A G P N S A N R H A G D L G N I T V G D D G T A T F T I T D S Q I P L A G P N S E V R H A G D L G N I T V G E D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I T V G E D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I T V G E D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I T V G E D G T A S F T L T D S Q I P L T G P N S V V V L A D R V D L G K G G H E L S L S T G N A C G R V A C G I I G L I	I A G R A I V G R A I J G R A I V G R A I V G R A V V G R A V V G R A
8. compositions sep. polinensis(cyt) A. theliane(cyt) Heliate(cyt) Cabbage(cyt) Tobacco(cyt) Pes(ch1) B. compositions sep. polinensis(cyt) A. theliane(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D C Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P N S A N R H A G D L G N I T V G D D G T A T F T I T D S Q I P L A G P N S E V R H A G D L G N I T V G E D G T A S F T L T D X Q I P L A G P N S E N R H A G D L G N I T V G E D G T A S F T L T D X Q I P L A G P N S E N R H A G D L G N I T V G E D G T A S F T L T D X Q I P L A G P N S E N R H A G D L G N I T V G E D G T A S F T L T D X Q I P L T G P N S Y V V L A D R V D L G N G G H E L S L S T G N A C G R V A C G I I G L V V V H A D P D D L G N G G H E L S L A T G N A G G R V A C G I I G L	I A G R A I V G R A I U G R A I V G R A I U G R A V V G R A V V G R A
8. campostris sap. polimensis(cyt) A. theliane(cyt) Maize(cyt) Cabhage(cyt) Tobacos(cyt) Reach) B. campostris sap. polimensis(cyt) A. thaliana(cyt) Maize(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N I T V G D D G T A T F T I T D S Q I P L T G P N S E D R H A G D L G N V T A G E D G V V N V N I T D S Q I P L A G P H S A N R H A G D L G N I T V G D D G T A T F T I T D S Q I P L S G P N S E V R H A G D L G N I T V G E D G T A S F T L T D S Q I P L S G P N S E V R H A G D L G N I T V G E D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I T V G E D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N L V A N A E G V A E A T I V D N Q I P L T G P N S V V V L A D R V D L G K G G H E L S L S T G N A G G R V A C G I I G L V V V A A D P D D L G K G G H E L S L A T G N A G G R V A C G I I G L V V N A A A D D L G K G G H E L S K S I G N A G G R V A C G I I G L	[A G R A [V G R A [] G R A [] G R A [] C R A [] C R A V V G R A V V G R A Q G Q G Q G
8. campostris sop. polinerais(cyt) 4. thelian(cyt) Heize(cyt) Cobhage(cyt) Tobacco(cyt) Pea(ch) 8. campostris sop. polinerais(cyt) 4. theliana(cyt) Heize(cyt) Cabhage(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N J T V G D D G T A T F T I T D S Q I P L T G P N S E D R H A G D L G N Y T A G E D G V N V N I T D S Q I P L A G P H S A N R H A G D L G N J I V G D D G T A T F T I T D S Q I P L A G P H S E V R H A G D L G N I I V G D D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I V Y A H A E G V A E A T I V D N Q I P L T G P N S V V V L A D R V D L G N G G H E L S L S T G N A G G R V A C G I I G L V V V L A D R V D L G N G G H E L S L A T G N A G G R V A C G I I G L V V N A D P D D L G N G G H E L S K S I G N A G G R V A C G I I G L V V N A D D D L G N G G H E L S K S I G N A G G R V A C G I I G L V V N A D D D L G N G G H E L S K S I G N A G G R V A C G I I G L V V N A D D D L C N G G H E L S K S I G N A G G R V A C G I I G L	I A G R A I V G R A I I G R A I V G R A I I G R A I I G R A V V G R A V V G R A V V G R A
8. campestris sep. polinerais(cyt) 4. thelian(cyt) Heize(cyt) Tobacco(cyt) Pea(ch) 8. campestris sep. polinerais(cyt) 4. thaliana(cyt) Meize(cyt) Cabhage(cyt) Tobacco(cyt)	A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L T G P N S A N R H A G D L G N J T V G D D G T A T F T I T D S Q I P L T G P N S E D R H A G D L G N Y T A G E D G V N V N I T D S Q I P L A G P H S A N R H A G D L G N I I V G D D G T A T F T I T D S Q I P L A G P H S E V R H A G D L G N I I V G D D G T A T F T I T D S Q I P L A G P Q C E N R H A G D L G N I I V G D D G T A S F T L T D S Q I P L A G P Q C E N R H A G D L G N I I V A N A E G V A E A T I V D N Q I P L T G P N S V V V L A D R V D L G K G G H E L S L S T G N A G G R V A C G I I G L V V V A A D P D D L G K G G H E L S L S T G N A G G R V A C G I I G L V V N A A D P D D L G K G G H E L S K S I G N A G G R V A C G I I G L V V N A A D P D D L G K G G H E L S K S I G N A G G R V A C G I I G L V V N A A D P D D L G K G G H E L S K S I G N A G G R V A C G I I G L V V N A A D P D D L G K G G H E L S K S I G N A G G R V A C G I I G L V V N A A D P D D L G K G G H E L S K T G N A G G R V A C G I I G L	I A G R A I V G R A I I G R A I V G R A I I G R A V V G R A V V G R A G G G G G G G G G G

Fig. 3. Comparisons of the deduced amino acid sequences of the *B. cmpestris* ssp. *Pekinensis* Cu/Zn SOD with the sequences from other species (References in the text).

ical for the catalysis. The histidines (positions 45, 47, 62, 70, 79) and aspartic acid (position 82) residues in these conserved regions are suggested to be particularly important for the interactions with Cu and Zn atoms, and they are well conserved in this clone (Scioli et al., 1988). The two cysteine residues (positions 56 and 145) are suggested to form the only disulfide bond which is important for the overall Cu/Zn SOD polypeptide structure, since other Cu/Zn SODs also contain these cysteine residues at the equivalent locations. Chloroplastic Cu/Zn SOD gene from Arabidopsis was suggested to present in one or two copies in the nuclear genome. In order to determine the copy number of the Chinese cabbage clone, a genomic Southern hybridization was carried out in high stringency condition (Fig. 4). BamHI digestion of the total leaf DNA produced a single strong band, whereas EcoRl and Pstl digestions produced two major bands with one or two minor bands. Since these restriction enzyme sites were not present in the coding sequences, it would be expected that there is only one single major band if there is only one copy per genome. The discrepancies may explain that there are EcoRI and PstI sites in the putative intron regions, generation the



Fig. 4. Southern hybridization of total leaf DNA probed with the 0.8 kb Cu/Zn SOD cDNA. Lanes a: digested with BamHI, b: HindIII, c : PstI, d: undigested, m: size markers.

two major bands. With the uncertainty described, it appears that there is likely one or two cytosolic Cu/ Zn SOD copies per genome of Chinese cabbage.

Superoxide generated in cytosol would react with hydrogen peroxide to produce hydroxyl radicals which could damage many cytosolic enzymes or organelle membranes. Therefore presence of SOD in cytosol must be important for various cytosolic functions. Recently drought stress induced the cytosolic SOD activities as well as other reactive scarvenge enzymes, such as ascorbate peroxidase and catalase (Mittler *et al.*, 1994). Currently, the Cu/Zn SOD gene from Chinese cabbage is being transformed into tobacco to observe enhanced stress-resistance.

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LITERATURE CITED

- Bowler, C., L. Slooten, S. Vandenbranden, R. De Rycke, J. Botterman, C. Sybesma, M. Van Montague and M. Inze. 1991. Manganase superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *EMBO J.* 10, 1723.
- Bowler, C., M. Van Montague and D. Inze. 1992. Superoxide dismutase and Stress tolerance. Ann. Rev.

Plant Physiol. Plant Mol. Biol. 43: 83-116.

- Cannon, R.E., J.A. White and J.G. Scandalios. 1987. Cloning of cDNA for maize superoxide dismutase 2 (SOD2). P.N.A.S. 84: 179-183.
- Duke, M.M. and M.L. Salin. 1985. Purification and characterization of an iron-containing superoxide dismutase from an eukaryote. *Ginko bilova. Arch Biochem. Biophys.* 243: 305-314.
- Heidecker, G. and J. Messing. 1986. Structural analysis of plant genes. Ann. Rev. Plant Physiol. 37: 439-466.
- Hindges, R. and A. Slusarenko. 1992. cDNA and derived amino acid sequence of a cytosolic Cu. Zn superoxide dismutase from Arabidopsis thaliana. *Plant Mol. Biol.* 18: 123-125.
- Kardish, N., N. Magal, D. Aviiv and E. Galun, 1994. The tomato gene for the chloroplastic Cu, Zn superoxide dismutase: regulation of expression imposed in transgenic tobacco plants by a short promoter. *Plant Mol. Biol.* 25: 887-897.
- Mittler, R. and B. Zilinskas. 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant J.* 5: 397-405.
- Nagao, R.T., D.M. Shah, V.K. Eckenrode and R.B. Meagher. 1981. Multigene family of actin-related sequences isolated from a soybean genomic library. DNA 1: 1-9.
- Scandalios, J.G. 1993. Oxygen stress and Superoxide dismutase. *Plant Physiol.* 101: 7-12.

- Scioli, J.R. and B.A. Zilinska. 1988. Cloning and characterization of a cDNA encoding the chloroplastic copper/zinc-superoxide dismutase from pea. P.N.A.S. 88: 7661-7665.
- Sen Gupta, A., J.L. Heinen, AS. S. Holaday, J.J. Burke and R.D. Allen. 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn-superoxide dismutase. *P.N.A.S.* 90: 1629-1633.
- Steffens, J.J., A.M. Michelson, F. Otting, K. Puget, W. Strassburger and L. Flohe. 1986. Primary structure of Cu, Zn superoxide dismutase of Brassica oleracea proves homology with corresponding enzymes of animals, fungi, and prokaryotes. *Biol. Chem.-Hoppe-Seyler* 367: 1007-1016.
- Tsang, E.W.T., C. Bowler, D. Heroaut, W. Van camp, R. Villaroel, C. Genetello, M. Van Montague and D. Inze. 1991 Differential regulation of superoxide dismutases in plants exposed to environmental stresses. *Plant Cell* 3: 783-792.
- Van Camp, W., H. Willekens, C. Bowler, M. Can Montague, D. Inze, R. Reupold-Popp, H. Sandman and C. Langebartels. 1994. Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. *Biotechnology* 12: 165-168.

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