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ISOLATION AND CHARACTERIZATION OF A cDNA FOR CuZn-SUPEROXIDE DISMUTASE OF RICE

Shu-Mei Pan[1,2], Guan-Bor Hwang[1], Daw-Shyng Wei[1] and Kai-Wun Yeh[1]

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ABSTRACT: RT-PCR was used for the SOD-DNA amplification from rice total RNA in a two-step reaction. Using this PCR-made SOD-DNA as the probe to screen a rice λgt11 cDNA library, four putative clones of CuZnSOD were isolated. The clone having the largest insert was subcloned and sequenced. This cDNA clone had a full-length open reading frame of 459bp corresponding to 152 amino acid residues and the derived amino acid sequence from this clone matched the cytosolic CuZnSOD isozyme. Its amino acid sequence showed approximately 99 and 86% identity to rice and corn CuZnSOD sequences. Northern blot hybridization showed that the heat-shock, drought-treated rice seedlings had the enhanced transcripts of CuZnSOD. Genomic Southern analysis indicated that CuZnSODs in rice was encoded by multigenes.

KEYWORDS: Superoxide dismutase, rice, CuZnSOD cDNA.

INTRODUCTION

Superoxide dismutases (SOD; superoxide: superoxide oxidoreductase, EC 1.15.1.1) are a group of metal-containing enzymes and catalyze the dismutation of superoxide radical to molecular oxygen and hydrogen peroxide. SODs have been found in all aerobic organisms and are considered as the major enzymatic defense against active oxygen free radicals (Bowler *et al.* 1992). The unstable superoxide anions are formed in the biological systems through the autoxidations, enzymatic reactions, and leakage from membrane electron transport chains (Elstner, 1987; Fridovich, 1986; Halliwell, 1987), and can cause deleterious oxidations of lipids, proteins and nucleic acids. Therefore, it can seriously disturb normal cell metabolism. In plants, three types of SOD exist, classified by their metal cofactor: copper/zinc (CuZnSOD), manganese (MnSOD), and iron (FeSOD) forms.

1. Department of Botany, National Taiwan University, Taipei, Taiwan, Republic of China.
2. Corresponding author.

The nucleotide sequence data reported will appear in the EMBL, Gen Bank and DDBJ Nucleotide Sequence Databases under the accession number L36320.

Abbreviations: SOD, superoxide dismutase; PCR, polymerase chain reaction; SSC, 150mM NaCl/15mM trisodium citrate; SDS, sodium dodecyl sulfate; TE, 10 mM Tris, pH 8.0/1 mM EDTA; MOPS, 3-(N-morpholino) propanesulfonic acid buffer; DIG, digoxigenin; AMPPD, 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane; NBT, 4-nitro blue tetrazolium chloride.

Stresses can cause enhanced levels of antioxidants in various plants, which are reported to defense the production of oxygen free radicals (Bowler *et al.* 1989; Bowler *et al.*, 1992). The important role of -SOD in the stress tolerance was evidenced by the transgenic plants, which having the high-SOD activity can enhance tolerance towards freezing, and oxidative stress (Mckersie *et al.* 1993; Gupta *et al.* 1993; Perl *et al.*, 1993).

The most abundant SOD activity in higher plants from CuZnSODs is located mainly in cytosol, also some minor activity located in chloroplast (Kanematsu and Asada, 1989). The cDNA encoding cytosolic CuZnSODs from maize (Cannon *et al.*, 1987; Cannon and Scandalios, 1989), tomato (Perl-Treves *et al.*, 1988), spinach (Sakamoto *et al*, 1990), pea (White and Zilinskas,1990), *N. plumbaginifolia* (Tsang *et al.*, 1991), *A. thaliana* (Hindges and Slusarenko,1992), rice (Sakamoto *et al.*, 1992a), and Scots pine (Karpinski *et al.*, 1992) have been reported. In our laboratory, we have studied the isozymes of superoxide dismutase in rice (Pan and Yau, 1991), in *Ganoderma tsugae* (Pan *et al*, 1992) , in *Arabidopsis* (Pan and Yau, 1992), and the SOD of *Miscanthus* as a marker enzyme of the environmental pollution was assessed (Wei, 1991). Also, We observed the enhanced SOD activity in the rice seedlings subjected to various stresses (Wei, 1991; Wei *et al*, 1995). In order to study the regulation of SOD and the molecular structure of SOD in the stressed rice. We decided to isolate cDNA clones from a rice cDNA library.

In the present report, the RT-PCR was employed to make the CuZnSOD-DNA from rice total RNA. This SOD-DNA was used as a probe to isolate the CuZnSOD cDNA clones from a rice cDNA library. We found an increased expression of this CuZnSOD in heat-shock, or drought-treated rice seedlings and genomic Southern analysis were also discussed.

MATERIALS AND METHODS

Plant material and RNA preparation

Rice seeds (*Oryza sativa* L. cv. *Tainung 67*) were germinated in a growth chamber at 28°C. Total RNA was isolated from a-week-old, etiolated, germinating seedlings using the SDS/Phenol method (Sacco de vries *et al.*, 1988).

cDNA library construction

A λgt11 cDNA library was constructed from poly(A) RNA using the standard procedures for recombinant DNA manipulations (Maniatis *et al.*, 1989).

PCR

The primers used for the PCR were oligos complementary to sequences that flank a 0.3 Kb region in the corn SOD cDNA. The primers were added with the *Eco*RI recognition sequences at the 5' as adaptor, P1: 5'TCGAATTCCTCCATGGATTCCA-TGTGCAC 3'; P2: 5'TCGAATTCCCCAGCATTTCCAGTGGTCTT3'. The SuperScript Pre-amplification System was purchased to synthesize the first strand cDNAs from the rice total RNA. The target cDNA was then amplified with gene-specific primers by the PCR method. 7 ug of rice total RNA was used to make cDNA using oligo$(dT)_{12\text{-}18}$ or P2 as the

primer. The corresponding cDNAs used as the template and P1 as the primer were subjected to 30 cycles of PCR amplification by Tag polymerases (Erlich, 1989).

DNA probes preparation

P32-labeling DNA probes: The DNA probes were labeled with P32-dCTP according to random primer labeling kit protocol of the supplier (Amersham). DIG-labeled probes: Digoxigenin-11-dUTP was incorporated into DNA by PCR using the SOD clone as DNA template according to DIG DNA labeling Kit protocol of the supplier (Bochringer Mannheim).

Agarose gel electrophoresis

1.2% agarose gel was made by a mini-gel electrophoretic unit (Mupid-2). Ten ul of PCR products and DNA size markers were applied to the wells, then run in the Tris-borate buffer at 50 V for 60 min. After electrophoresis, the ethidium bromide-stained gel was viewed by the UV transilluminator, and photography was taken by a Polaroid DS-34.

Hybridization

After hybridization, the following three washes were done: two times for 15 min in 2x SSC, 0.2% SDS at room temperature and one time for 1 hr in 0.1x SSC, 0.2% SDS at 42 ℃. When screening the library, positive clones were detected and isolated after autoradiography. Isolation of the positive clones was done after dilution, plating and rehybridization under the same conditions.

Subcloning

Lamda phages were purified by a standard polyethylene glycol precipitation procedure (Maniatis *et al.*, 1989). Phage DNA was obtained by phenol/chloroform extraction, then followed by ethanol precipitation. Insert size determination and its DNA preparation were done by the use of PCR process and Clontech insert screening Amplimers. The DNA made by PCR was end blunted with the Klenow fragment and ligated to pUC19 DNA treated with *Sma*I and calf intestinal alkaline phosphatase. The chimeric DNA was used to transform competent *E. coli* JM109 cells by the $CaCl_2$ method (Sambrook *et al.* 1989).

Sequence analysis

The DNA sequences were determined on both strands by dideoxy chain-termination method (Sanger *et al.*, 1977). Sequences were analyzed by GCG program.

RNA gel and northern blots

Rice seedlings were treated with heat-shock at 42℃ or drought for different period. RNA was extracted from these treated tissues. 30 μg of each RNA sample were fractionated on 1% formaldehyde/agarose gel in MOPS buffer. Blotting to Hybond N membrane was performed according to the protocol of the supplier (Amersham). RNA was fixed on the membrane by XL-1000 UV crosslinker (Spectronicos corporation). Filters were prehybridized at 42℃ for 2 h in 5x SSC, 0.1% SDS, 20 mM Na-phosphate, 0.1% Ficoll, 0.1% PVP, 1% glycine and 50% formamide. The filters were hybridized in the same solution plus radioactive probe ($1x10^6$ cpm/ml) at 42℃ for 12-18 h. The filters

were sequentially washed in 2x SSC, 0.1% SDS at room temperature for 30 min, 0.1x SSC, 0.1% SDS at 42℃ for 60 min. The filters were air-dried and exposed to X-ray film.

DNA isolation and Southern blot analysis

Total DNA from young rice seedlings was extracted with urea extraction buffer (7 M urea, 0.3 M NaCl, 50 mM Tris-HCl, pH 8.0, 20 mM EDTA and 1% sarkosine). After adding equal volume of the mixture of phenol, chloroform and isoamylalcohol (25:24:1), the solution was then incubated at room temperature for 15 min, centrifuged at 8,000 rpm, 4℃ for 10 min. The supernatant was added appropriate amount of 3 M sodium acetate (pH 5.2) and isopropanol to precipitate the DNA. The DNA was washed with 70% alcohol twice and 100% alcohol once, then dissolved with TE buffer. Aliquots of each 50 μg DNA were digested with different restriction endonucleases (*Eco*RI, *Bam*HI, or *Hind*III), then fractionated on 0.8% agarose gel and transfered to a nylon membrane. The prehybridization was carried for at least two hrs. Hybridization was performed using DIG-labled CuZnSOD as probe. Probes were added to prehybridization solution 5x SSC, 0.5% blocking reagent (Boehringer Mannheim), 0.1% N-lauroylsarcosine, Na-salt, and 0.02% SDS to make hybridization solution and hybridized overnight at 68 ℃. Following twice 5 min wash with 2x SSC, 0.1% SDS at room temperature; the blot was washed at 68℃ with 0.1% SSC and 0.1% SDS for 15 min twice. After blocking of the membrane in 0.5% blocking reagent, the membrane was hybridized with DIG-labeled probe, then its binding to antibody-conjugated alkaline phosphatase was carried and visualized with the chemi-luminescent substrate AMPPD or with colorimetric substrates X-phosphate and NBT.

RESULTS

PCR

PCR using the primers, P1 and P2, were complementary to sequences that flank a 0.3-Kb region of the corn CuZnSOD cDNA (Cannon and Scandalios, 1989), which was the conserved region of amino acid sequence of CuZnSOD from various plants (Kitagawa *et al.*, 1986; Masumura *et al.*, 1990). After the first-stranded cDNA was made from rice total RNA using oligo$(dT)_{12\text{-}18}$ or P2 as the primer, the corresponding cDNA were amplified using P1 as the primer to generate a 0.3-Kb DNA fragment. This DNA fragment was subcloned into pGEM-3Z, then sequenced and was proved to be the SOD gene (Hwang, 1993).

Screening of a cDNA library

The PCR-made SOD-DNA was used as the probe to screen a 2 X 10^5 cDNA original library, which was made from one-week-old, etiolated rice seedlings. Four putative clones were obtained from the primary screening. However, the insert DNA cannot be cleaved from the purified λDNA from these four clones. By PCR, we obtained the insert DNA from these phage clones. The largest insert (0.8 kb) was subcloned into pUC19 vector, and designed as pcu101, and sequenced. The nucleic acid sequence and its derived amino acid of the clone were shown in Fig. 1. The cDNA contains a 5' end of 87 bp, an open reading frame was 459 bp corresponding to 152 amino acid residues and a 3' uncoding

```
        10          20          30          40          50          60
CGAGATTCCA AACCAGCAGG AGTCGCCTCG CCTCCTCCTT CATCCTCCTC GTCGTCGCCG

        70          80          90         100         110         120
CGGGGGTCGC CTGAGATCAC ATTAACAATG GTGAAGGCTG TTGTTGTGCT TGGTAGCAGT
                                M   V   K   A   V   V   V   L   G   S   S

       130         140         150         160         170         180
GAGATTGTTA AGGGCACTAT CCACTTTGTC CAAGAGGGAG ATGGTCCCAC CACTGTGACT
 E   I   V   K   G   T   I   H   F   V   Q   E   G   D   G   P   T   T   V   T

       190         200         210         220         230         240
GGAAGTGTCT CTGGCCTCAA GCCTGGTCTC CATGGGTTCC ATATTCATGC ACTTGGTGAC
 G   S   V   S   G   L   K   P   G   L   H   G   F   H   I   H   A   L   G   D

       250         260         270         280         290         300
ACCACCAATG GTTGCATCTC AACTGGGCCA CACTACAATC CTGCCGGAAA GGAGCATGGA
 T   T   N   G   C   I   S   T   G   P   H   Y   N   P   A   G   K   E   H   G

       310         320         330         340         350         360
GCACCAGAAG ATGAGACCCG CCATGCTGGT GATCTTGGAA ATGTCACCGC TGGAGAAGAT
 A   P   E   D   E   T   R   H   A   G   D   L   G   N   V   T   A   G   E   D

       370         380         390         400         410         420
GGTGTTGCTA ATATCCATGT TGTTGACAGT CAGATTCCAC TTACTGGACC AAATTCAATC
 G   V   A   N   I   H   V   V   D   S   Q   I   P   L   T   G   P   N   S   I

       430         440         450         460         470         480
ATTGGCAGAG CCGTCGTTGT GCATGCCGAT CCTGATGATC TTGGAAAGGG TGGGCACGAG
 I   G   R   A   V   V   V   H   A   D   P   D   D   L   G   K   G   G   H   E

       490         500         510         520         530         540
CTGAGCAAGA CCACCGGAAA CGCTGGTGGC CGTGTTGCTT GCGGGATCAT CGGACTTCAA
 L   S   K   T   T   G   N   A   G   G   R   V   A   C   G   I   I   G   L   Q

       550         560         570         580         590         600
GGCTGAAACC TGGAGGTGTG AACTCACCTT CCATCTCCCA GCACCAGAAG CCTGAAACTC
 G   *

       610         620         630         640         650         660
TACGAGCTCT TAGCCCTTTC GTCTTTACCT GAGTGGCTAC TCTAGATTCT ACAATAAGCA

       670         680         690         700         710         720
CCTGATCTCT GCGCATGGTT TTTGGTGTAC CATTCTGTCG CCCGCATCGT TGGCGCCCAA

       730         740         750         760
TGAACTATGT GTTTTGTGTT AAACCTTAAG CTGAAGGGTA CCATTTGT
```

Fig. 1. Nucleotide sequence and its derived amino acid sequence of the cDNA of rice clone pcu101 encoding cytosolic CuZnSOD.
Stop codon is indicated by the asterisk. Polyadenylation signal is underlined.

region of 253bp. A consensus signal for polyadenylation AATAAG was found between nucleotide 653 and 658 (Fig. 1). This clone cDNA has 14-, and 72-bp longer 5' untranslated region than those of RSODA and RSODB, respectively (Fig. 2), which isolated from the different rice cultivars (Sakamoto *et al.*, 1992a). The derived amino acid sequence from clone pcu101 matched the rice cytosolic CuZnSOD. They show 99% identity of amino acid sequence and has only different at the 57th amino acid residue (isoleucine instead of methionine at RSODA) (Fig. 1, 3). Comparison of the amino acid sequence of this rice CuZnSOD with those of other plants (Fig. 3; Table 1) also shows very high identity.

```
          1                                                    50
D00999    .......... ....TTCTGG AGTCGCCTCG CCTCCTCCTT CATCCTCCTC
L36320    cgagattcca aaccagcagg agtcgcctcg cctcctcctt catcctcctc
D01000    .......... .......... .......... .......... ..........
M54936    .......... .......... .......... .......... ..........
X53872    .......... .......CTC GAATCTTCAA CTCCTCTCTC TTTCTCTCTC
M63003    .......... .......... .......... .......... ..........
X14040    .......... .......... .......... .......... ..........

          51
D00999    GTCGTCGCCG CGGGGGTCGC CTGAGATCAC ATTAACA
L36320    gtcgtcgccg cgggggtcgc ctgagatcac attaaca
D01000    .......... .......... ..GAGAACAC ATAGACA
M54936    .CTCGCGCAG GGGGGGTCGC CTGAGATCAC AGAGACA
X53872    CTCCAAATTG CAAGGGTGAT CTGAGAATAC ACACAAC
M63003    .......... .......... ..GGATCACA TTGAACA
X14040    .......... .......... .......... ..CAAAA
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Fig. 2. Comparison of nucleotide sequences of CuZnSOD of rice (D00999, D01000, L36320), corn (M54936), spinach (X53872), pea (M63003) and tomato (X14040).

Northern blot hybridization

Northern hybridization was performed at high stringency to eliminate cross hybridization. The transcripts of CuZnSOD, from both heat-shock, or drought-treated rice seedlings, were enhanced (Fig. 4), but no apparent change in the cadmiun-treated tissues was found (Hwang, 1993).

Southern blot hybridization

CuZnSOD gene organization was analyzed by Southern blot experiment. Rice DNA was cleaved with *Eco*RI, *Bam*HI or *Hind*III (none of which digests within CuZnSOD cDNA sequence) and hybridized with rice CuZnSOD cDNA probe. A single signal was detected in *Hind*III-digested genomic DNA, whearas two bands, with different intensity,

appeared in the DNA treated with *Bam*HI or *Eco*RI (Fig. 5). It seems likely that rice genome contains more than one copy for cytosolic CuZnSOD.

```
        1                                                   50
D00999  MVKAVVVLGS SEIVKGTIHF VQEGDGPTTV TGSVSGLKPG LHGFHIHALG
L36320  MVKAVVVLGS SEIVKGTIHF VQEGDGPTTV TGSVSGLKPG LHGFHIHALG
D01000  MVKAVAVLAS SEGVKGTIFF SQEGDGPTSV TGSVSGLKPG LHGFHVHALG
M54936  MVKAVAVLAG TD.VKGTIFF SQEGDGPTTV TGSISGLKPG LHGFHVHALG
X14040  MVKAVAVLNS SEGVSGTYLF TQVGVAPTTV NGNISGLKPG LHGFHVHALG
X53872  MGKAVVVLSS SEGVSGTVYF AQEGDGPTTV TGNVSGLKPG LHGFHVHALG
M63003  MVKAVAVLSN SNEVSGTINF SQEGNGPTTV TGTLAGLKPG LHGFHIHALG

        51                                                  100
D00999  DTTNGCMSTG PHYNPAGKEH GAPEDETRHA GDLGNVTAGE DGVANIHVVD
L36320  DTTNGCISTG PHYNPAGKEH GAPEDETRHA GDLGNVTAGE DGVANIHVVD
D01000  DTTNGCMSTG PHFNPTGKEH GAPQDENRHA GDLGNITAGA DGVANVNVSD
M54936  DTTNGCMSTG PHFNPVGKEH GAPEDEDRHA GDLGNVTAGE DGVVNVNITD
X14040  DTTNGCMSTG PHYNPAGKEH GAPEDEVRHA GDLGNITVGE DGTASFTITD
X53872  DTTNGCMSTG PHYNPNGKEH GAPEDDVRHA GDLGNITVGD DGTATFTIID
M63003  DTTNGCISTG PHFNPNGKEH GAPEDETRHA GDLGNINVGD DGTVSFTITD

        101                                                 150
D00999  SQIPLTGPNS IIGRAVVVHA DPDDLGKGGH ELSKTTGNAG GRVACGIIGL
L36320  SQIPLTGPNS IIGRAVVVHA DPDDLGKGGH ELSKTTGNAG GRVACGIIGL
D01000  SQIPLTGAHS IIGRAVVVHA DPDDLGKGGH ELSKTTGNAG GRVACGIIGL
M54936  SQIPLAGPHS IIGRAVVVHA DPDDLGKGGH ELSKSTGNAG GRVACGIIGL
X14040  KQIPLTGPQS IIGRAVVVHA DPDDLGKGGH ELSKSTGNAG GRIACGIIGL
X53872  SQIPLSGPNS IVGRAVVVHA EPDDLGRGGH ELSKTTGNAG GRVACGIIGL
M63003  NHIPLTGTNS IIGRAVVVHA DPDDLGKGGH ELSKTTGNAG GRVACGIIGL

        151
D00999  QG
L36320  QG
D01000  QG
M54936  QG
X14040  QG
X53872  QG
M63003  QG
```

Fig. 3. Comparison of amino acid sequences of CuZnSOD of rice (D00999, D01000, L36320), corn (M54936), tomato (X14040), spinach (X53872) and pea (M63003).

Table 1. Amino acid identities of rice cytosolic CuZnSODs with those of other plant species.

Species	Accession number	DNA sequence	Polypeptide		Reference
		Residue (bp)	Residue	Identity (%)	
Rice	L36320	793	152	100.0	This article
Rice	D00999	760	152	99.3	Sakamoto *et al.*
Rice	D01000	667	152	87.5	Sakamoto *et al.*
Corn	M54936	694	151	86.0	Cannon & Scandalios
Spinach	X53872	795	152	82.9	Sakamoto *et al.*
Pea	M63003	738	152	81.5	White & Zilinskas
Tomato	X14040	777	152	81.5	Perl-Treves *et al.*

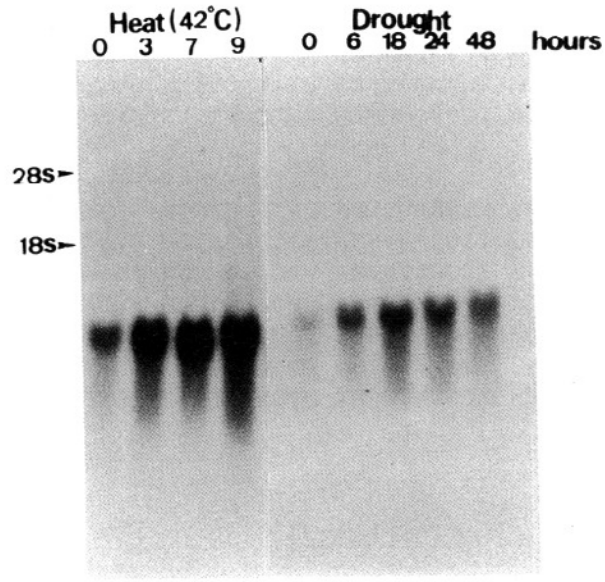

Fig. 4. Expression of CuZnSOD gene in rice seedlings.
Total RNAs were prepared from the seedlings following the heat-shock or drought treatment for the indicated hours. The RNAs (30 μg each) were separated on an 1% agarose gel and transferred onto a nylon membrane. The membrane was hybridized with a P^{32}-labeled cDNA insert of pcu101.

Fig. 5. Genomic Southern analysis of the rice gene encoding cytosolic CuZnSOD.
Total DNA (60 μg) was digested with *Bam*HI (B), *Eco*RI (E), or *Hind*III (H), separated on 0.8% agarose gel and transferred to a nylon membrane. The membrane was hybridized with the DIG-labeled cDNA insert of pcu101.

DISCUSSION

We started the cDNA cloning of rice SOD in 1991 and obtained some positive clones in the early of 1992. However, in the mid of 1992, Japanese scientists reported to isolate one genomic, and two cDNA clones from rice developing seeds (Sakamoto *et al.*, 1992a; Sakamoto *et al.*, 1992b). Our work is on the rice cultivar Tainung 67. We have characterized the pcu101 clone which isolated from the cDNA library of etiolated, 10-day-old seedlings of rice. The cDNA coding regions of this clone was expressed in the bacterium *E. coli* and showed the SOD enzyme activity in the expressed fusion proteins (Hwang, 1993). Comparing with RSODA clone (Sakamoto *et al.*, 1992a), pcu101 has the 99% identity in coding region and 3' end untranslated region, but has 17-bp longer at 5' end untranslated region. The clone having isoleucine at the 57 amino acid residue, instead of methionine in RSODA. The minor difference (polymorphism in the sequences) of pcu101 and RSODA cDNA clone may be due to the different cultivars used (Tainung and Nipponbare) or they are encoded from different genes.

When rice seedlings were imposed to abrupt 1% NaCl stress, we observed a significant increase of MnSOD enzyme activity in the shoot (Wei *et al.*, 1995). However, in the present report, the CuZnSOD transcripts was enhanced in the heat-shock, or drought-treated rice, but the corresponding SOD enzyme activity was not increased (not shown). Because stress is a most unstable factor, its intensity is responsible for eliciting plant responses (Blum, 1994). The various response of -SOD gene expression at the different level in the stressed rice is not unexpected. Although the enhanced CuZnSOD transcripts in the heat-shock, or drought-treated rice was observed in this report, its regulation of CuZnSOD gene expression through the promoted transcription rate or the increased RNA stability needs further study.

Two bands, one major having 90% of total intensity, were detected under high-temperature hybridization and high strigent washings, when rice genomic DNA blots were probed with DIG-labeled CuZnSOD cDNA. Here, the different DNA restriction fragments were found (Fig. 5). Also, we observed the multiple forms of CuZnSOD activities in the various tissues and at different developmental stages of rice (Pan and Yau, 1991). And, two distinct rice CuZnSOD cDNAs have been isolated from developing rice seeds (Sakamoto *et al*, 1992a). Taken together, these results indicate that rice CuZnSODs are encoded by a multigene family. However, on the basis of these data it is impossible to predict the exact number of genes present in the rice CuZnSODs family.

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水稻含銅鋅超氧歧化酶之選殖與性質研究

潘素美[1,2]、黃冠博[1]、魏道行[1]、葉開溫[1]

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摘　　要

以 RT-PCR 合成水稻之含銅鋅超氧歧化酶基因片段（0.3-Kb）。經序列分析證實後，以此 0.3-Kb DNA 做爲探針從水稻 cDNA library 篩選得到 CuZnSOD 之全長 cDNA，其 DNA 及對應之 amino acid 序列與多種植物 CuZnSOD 之序列有很高之同源性。北方轉印結果顯示，熱或乾旱處理水稻幼苗後，其 CuZnSOD 對應之 mRNA 增加。南方轉印顯示水稻 CuZnSOD 之基因多於一個。

關鍵詞：超氧歧化酶，基因選殖，水稻。

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1. 國立台灣大學植物學系。
2. 通信聯絡員。