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Antisense and RNAi expression for a chloroplastic superoxide dismutase gene in transgenic plants

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Abstract. The cDNA of tomato chloroplastic Cu/Zn-superoxide dismutase was used to construct transgenic tobacco (*Nicotiana plumbaginifolia*). It was found that the gene expression of Cu/Zn-superoxide dismutase can constitutively be reduced in the transgenic tobacco plants because of double-stranded (dsRNA) expressed in the form of intron-spliced hairpin structures and antisense suppression. Furthermore, the endogenous chloroplastic Cu/Zn-superoxide dismutase gene in tobacco was established as a target for silencing due to the operation of hpRNA and antisense RNA constructs. However, under salt and PEG-induced stress, the cytosolic Cu/Zn-superoxide dismutase activity in transformed plants obviously increased. At the same time, the PEG pretreatment was able to promote tolerance of the transgenic plants to the salt stress. These results indicated that the use of hpRNA and anti-sense was able to successfully knockout the transcript encoding a chloroplast superoxidase dismutase. The null transformed plants grown under stress produced or retained other superoxide dismutase to compensate for the loss of the chloroplast. Consequently, hpRNA constructs would be helpful in discovering and validating the endogenous chloroplastic Cu/Zn-superoxide dismutase gene, and to prove the cytosolic alternative pathway of plant antioxidation associated with the function of Cu/Zn-superoxide dismutase gene under an unfavorable environment. Meanwhile, the experiment will provide an important technique for the antisensing strategies operating in tobacco.

Keywords: Double-stranded RNA; Salt stress; Superoxide dismutases; Transgenic tobacco.

Introduction

Double-stranded RNA (dsRNA)-mediated interference with expression of specific genes has been observed in a number of organisms including nematodes (Fire et al., 1998), *Drosophila* (Kennerdel and Carthew, 1998) and plants (Chuang and Meyerowitz, 2000). Such self-complementary hairpin RNA (hpRNA, the expression form of dsRNA in plants) can elicit a high degree and frequency of posttranscriptional gene silencing (PTGS) of endogenous genes (Chuang and Meyerowitz, 2000; Wesley et al., 2001) and has great potential as a tool for gene discovery and validation (Wesley et al., 2001).

In natural conditions plants are often exposed to various environmental stresses that can decrease production. Like other biotic, abiotic, and xenobiotic stresses, salt and drought stresses produce many degenerative reactions mediated by toxic reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen, and hydroxyl radical, produced when the H_2O_2 reacts with reduced metal irons such as Fe. These ROS are cytotoxic and can seriously disrupt normal metabolism by way of oxidative damage to lipids (Wise and Naylor, 1987), nucleic acids (Imlay and Linn, 1988), and proteins (Davis, 1987), resulting in mutation, protein destruction, and peroxidation of membrane lipids respectively, which in turn may lead to diseases and degenerative processes (carcinogenesis and immunodeficiency, for example). There is circumstantial evidence that high levels of SOD activity in plants result in tolerance of a variety of environmental and chemical challenges and that overexpression of SOD acts as a safeguard against both drought (Mckersie et al., 1996; Bowler et al., 1992) and salinity (Zhu, 2002; Hasegawa et al., 2000). On the other hand, although transgenic tobacco plants could express high levels of petunia chloroplastic Cu/Zn-SOD (Tepperman and Dunsmuir, 1990), no detectable increase in the protection of photosynthetic activity was detected in these plants after their exposure to MV, nor was there any reduction in symptom development after ozone fumigation (Pitcher et al., 1991).

Although many previous reports have affirmed the protective capabilities of SOD as an antioxidant enzyme and its role in cross tolerance for many stresses, much remains to be clarified, especially the role of SOD isozymes in different cell compartments and in the cross-tolerance mechanism. In this study, we made use of the cDNA of tomato (*Lycopersicon esculentum* cv E.C.) chloroplastic Cu/Zn-SOD to construct transgenic tobacco (*Nicotiana plumbaginifolia*). The result showed that this can constitutively reduce chloroplastic Cu/Zn-SOD gene expression in tobacco through hpRNA and antisense suppression. To the best of our knowledge, this is first report that demonstrates the expression of the Cu/Zn-SOD gene in the chloroplast of transgenic tobacco to be repressed, while it is increased in the cytosol for protection against salinity (NaCl) and polyethylene glycol-induced (PEG) stress. Can

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the constructs of hpRNA and antisense take effect in tobacco, and are the effects between them similar? What are the changes of the phenotype, and what changes will Cu/Zn-SOD activity and the other isozymes of the transgenic plant undergo in comparison with the wild type? What characters have the transformed tobacco in resistance to salt stress? The objective in the present research is to evaluate the possible correlation of the transgenic plant phenotype with Cu/Zn-SOD activity in the presence or absence of the salt stress. This would provide evidence to further explain that endogenous chloroplastic Cu/Zn-superoxide dismutase gene and the increase in the Cu/Zn-superoxide dismutase activity in the cytosol in response to salt stress. It also provides an important technique for the antisensing strategies operating in tobacco.

Materials and Methods

Construction of the Transformation Vector

The vector T_{10}, an honored gift from Dr. Rafeal (Israel) containing the cDNA encoding tomato (*Lycopersicon esculentum* cv E.C.) chloroplastic Cu/Zn-SOD (Perl-Treves et al., 1988), was cut with *Ecol36*II and *Cla*I, and the fragment between them was cloned into *Cla*I and *Sma*I sites of SODINTRON. (It was named cpSOD.) The cDNA encoding tomato chloroplastic Cu/Zn-SOD was cloned into the *Eco*RI and *Xho*I sites of the vector pGEM-7Zf (+) (which was modified by inserting an intron) and was called SODINTRON. The fragment between *Xba*I and *Ecol36*II of cpSOD was transferred into the *Xba*I and *Sac*I sites of the plant expression vector pGA492GI-Bar (+). (The transformation vector harboring Cu/Zn-SOD sense and antisense was obtained.), It was placed upstream of the constitutive cauliflower mosaic virus 35S (CaMV35S) promoter and downstream of the *nos* termination sequence, in place of the *npt*II gene to allow for selection by kanamycin resistance and was then mobilized into *Agrobacterium tumefaciens* EHA105 by triparental mating. The fragment between *Sma*I and *Apa*I of SODINTRON, which harbored Cu/Zn-SOD cDNA, was inserted into the *Sac*I and *Sma*I sites of pGA492GI-Bar(+) and the transformation vector containing Cu/Zn-SOD antisense was acquired.

Plant Transformation

The leaf pieces of *Nicotiana plumbaginifolia* cv (a diploid wild species) were transformed as described by Horsch et al. (1985) and regenerated on a Murashige-Skoog medium solidified with 0.8% agar and containing 3% Suc, 100 μg/mL kanamycin, 500 μg/mL carbenicillin, 2 μg/mL Kinetin, and 1 μg/mL IAA. After about one month's culture, the shoots were regenerated and further rooted on solidified MS root generating medium containing 100 μg/mL kanamycin. The regenerated shoots, which escaped contamination and had a normal phenotype, were transferred to a growth chamber at 25°C, a 12 h light cycle, and 45-55% RH for further observation, evaluation, and analysis. For nontransformed controls, leaf pieces were inoculated with an *Agrobacterium*-free medium but were otherwise carried through identical steps of regeneration and propagation with the exception that they were not exposed to kanamycin.

PCR Screening and Southern Blot Hybridization

Prior to being transferred to the growth chamber, the putatively transgenic plants were screened for the presence of the *npt* II transgene using PCR. Genomic DNA was extracted by Saghai-Maroof et al. (1984). The primers used were 5´-AGCTGTGCTCGACGTTGTCAG-3´ and 5´-GGTGGGCGAAGAACTCCAGCA-3´. The expression of CaMV 35S and Cu/Zn SOD was characterized by using RT-PCR on total RNA prepared from leaf tissues and total RNA was prepared using Trizol (Invitrogen). The primers used were 5´-GCTCCTACAAATGCCATCA-3´ and 5´-GGCTCCTGTAGACATACATCCG-3´.

To verify the presence of tomato Cu/Zn-SOD cDNA in the transgenic plants, Southern blot hybridization was used. Genomic purified DNA from the transgenic and control plants, which was treated with the restriction enzymes *Hind*III and *Eco*RI, was blotted on nitrocellulose membrane (AMRESCO) and hybridized with tomato Cu/Zn-SOD cDNA as a probe labeled with bio-11-dUTP, using a random primer DNA labeling kit (TaKaRa).

Plant Growth and Stress Treatments

Only the PCR-positive plants were transferred to the greenhouse for further study. Regenerated plants were grown to maturity and then self-pollinated; the resulting seeds were germinated on kanamycin-containing medium to both determine the segregation ratios and select the plants for experimentation. The seeds of the selected transgenic progeny and the wild type were grown in solid MS hormone-free media with or without kanamycin (for transgenic and control, respectively). One month and two months after germination, the height and dry weight of the transformed and nontransformed plants were measured.

We selected one transgenic line of each vector for growth analysis and stress-tolerance testing. Eight- to ten-weeks-old transgenic plants, uniform in the number of leaves, were stressed. For the evaluation of salt (NaCl) and PEG-induced stress, three replications from each type of plant (transgenic and control) were used. The transgenic plants that were cultured in Hoagland's solution, were divided into three classes: the first one was treated with Hoagland's solution containing 100 mM NaCl, and the concentration of NaCl was increased 50 mM every day with the result that the final concentration after five days was 300 mM; the second one was pretreated with Hoagland's solution containing 2% PEG for 24 h and then transferred to NaCl stress under the above mentioned conditions; the third one was used as the control.

Enzyme Assays

All assays were carried out spectophotometerically using a Shimadzu, multipurpose recording spectrophotometer (MPS2000). Leaf samples (0.3 g FW) were frozen in

liquid nitrogen and ground to powder in precooled mortars. The powder was homogenized with extraction buffer (3 ml) consisting of 1 mM EDTA and 1 mM ascorbate in 50 mM potassium phosphate buffer (K-P) pH 7.8. The slurry was transferred to the centrifuge tube and centrifuged for 20 min (at 20,000 *g*, 4°C). One part of the supernatant was used directly for ascorbate peroxidase (APX) assays. The other part (1 ml) was transferred to the dialysis membrane (Wako, Japan) to be dialyzed at 4°C for 24 h (dialysis buffer, 10 mM K-P, pH 7.8) and used for a total SOD activity and isoforms assay. Isoforms of SOD were resolved by nondenaturing 15% PAGE and stained for activity basically as described by Donahue et al. (1997). The APX activity in the extract was determined spectrophotometrically by measuring the oxidation of ascorbate at 290 nm (Gossett et al., 1994). Leaf organelles were purified by differential and Percoll density-gradient centrifugation, in accordance with the published protocols (Inaki et al., 1998). The activity of SOD was measured every day starting from zero time (measurement taken immediately before the stress was imposed) and continuing for 5 days. The activities of APX were measured every other day.

Statistical Analysis

All the data points were based on a mean of three or ten replications, and the data were analyzed by Students' *t*-test at 95% confidence limits.

Results

The Identification of Transgenic Tobacco

A transgene consisting of the cDNA encoding chloroplastic Cu/Zn-SOD by different orientations under the control of the CaMV 35S promoter was constructed (Figure 1). For simplicity, the transgenic plants from two vectors containing SOD antisense or double-strands RNA were called antiSOD and dsSOD, respectively. Tobacco plants (*Nicotiana plumbaginifolia*) were used as hosts for the infection of *Agrobacterium* transformation. Twenty-one and thirty-two independent transformed lines for anti-SOD and dsSOD, respectively, were thereby produced. The genomic DNA were isolated from wild-type plants and transgenic lines and analyzed by PCR using synthetic primers specific for nptII. The expected 750 bp fragment was amplified from the DNA of transgenic lines tested, but not from those of wild-type plants (Figure 2A). From the result of RT-PCR, the expected 650 bp fragment was ampli-

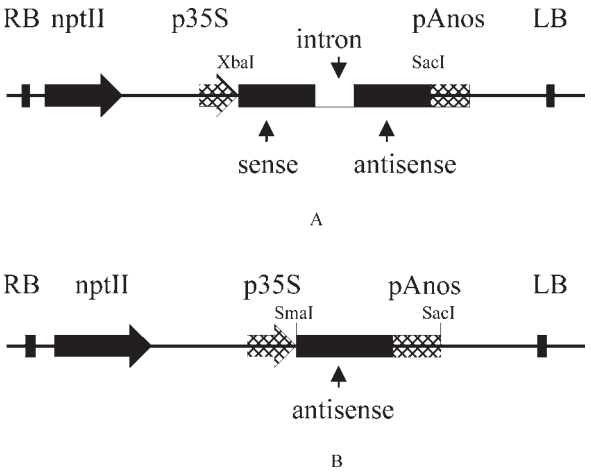

Figure 1. Diagrammatic representation (not to scale) of Cu/Zn SOD silencing constructs used to transform tobacco. A, The SOD-dsRNA construct contains SOD fragment in antisense and sense orientation separated by intron; B, The SOD-antisense construct comprises simply the respective antisense fragment. Both constructs are under control of the CaMV 35S promoter (p35S) and pAnos.

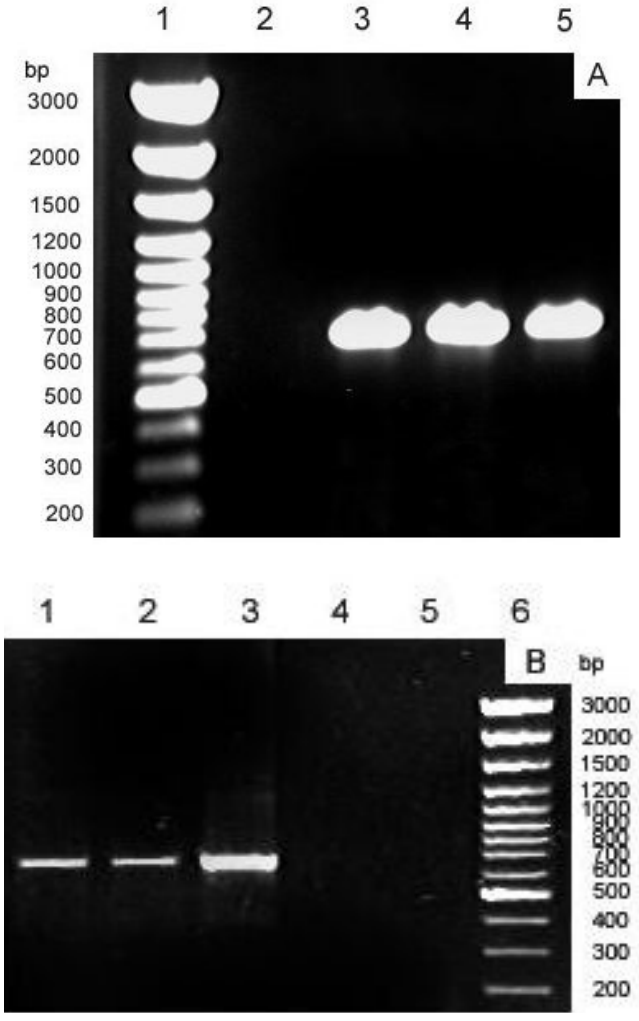

Figure 2. PCR analysis for the detection of the nptII, CaMV35S and Cu/Zn SOD genes in *Nicotiana plumbaginifolia* putative transgenic plants. A, PCR amplification using specific primers for the nptII gene from genome DNA. Lanes: 1 DNA size marker, 2 negative control (untransformed plant), 3 positive control (plasmid DNA), 4-5 putative transgenic plants; B, RT-PCR amplification using specific primers for CaMV35S and Cu/Zn SOD gene. Lanes: 1-3 putative transgenic plants, 4-5 negative control (untransformed plant), 6 DNA size marker.

fied from the transformant, but not from the nontransformed control (Figure 2B). We selected the transgenic plants and confirmed by Southern-blot analysis that each possessed one full insertion of the T-DNA in the chromosomes (data not shown). On the other hand, the number of apparent insertion loci for each transgenic line was determined by kanamycin selection. The tobacco plants sampled were progeny resulting from the self-fertilization of a primary transformant which underwent a significant decline in SOD levels.

The growth of most plants from the two transformed populations did not differ significantly if they were not subjected to stress (Table 1). However, several transformed plants were observed to have delayed flowering, and no seed was found in their fruits. To study the effect of Cu/ZnSOD on salt tolerance, we grew transgenic and control plants under salt stress. Whole plants of the wild type and transgenic lines were exposed to salt stress by treatment with NaCl under normal light intensity. The salt concentration increased gradually with time, so that growth became increasingly inhibited. Phenotypically, the plants subjected to salinity stress for 48 h (both antiSOD and dsSOD plants) became wilted and yellow (after approximately 4 days' recovery from salinity stress), which contrasted with the effects of salt stress in combination with PEG-pretreatment. Nevertheless, the nontransformed plants remained verdant under NaCl stress.

SOD Enzyme Levels in Transgenic Plants

One transformed line of every vector was selected for further detailed evaluation and equal numbers (three) of one transgenic plant type and its nontransformed control were used. Typically, leaf No. 5, the most recent fully expanded one, was sampled. The total SOD activities and SOD activities in different cell compartments were verified in leaf extracts from the two transformant lines and the nontransformed control before exposure to salt stress. A comparison of the leaf extracts from the two transgenic lines and the control indicated that the transgenic plants enjoyed diversity in total SOD activities, which declined by 23% in antiSOD plants compared with the wild type; otherwise, the activities decreased 35.2% in dsSOD plants (Figure 3). In chloroplast, the inhibition of SOD activity was observed in antiSOD plants, but no similar result was detected in mitochondria or cytocol. In dsSOD plants, the SOD activities in three cell compartments decreased dramatically (Figure 4). All these results were consistent with the total SOD activities.

SOD activity in transformant lines was compared with that of the nontransformed controls using activity gel analysis. Five SOD isoforms in the nontransgenic control plant were separated in extracts of leaves by nondenaturing PAGE (Figure 5). These isoforms were identified as Mn (no inhibition by KCN or H_2O_2), Fe-1 and Fe-

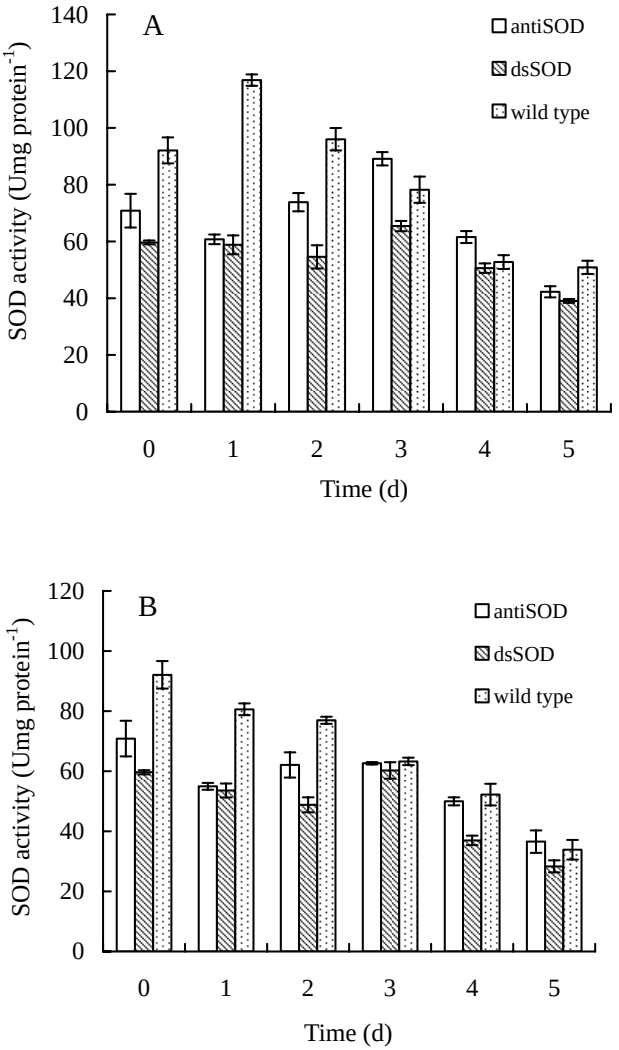

Figure 3. SOD activities in leaves from dsSOD, antiSOD transgenic and control tobacco plants during NaCl stress with PEG pretreatment (A) and single NaCl stress (B). Zero time is measurement from sample taken immediately before transferring the plants to saline solution. Vertical bars represent the SE of the mean for triplicate determination.

Table 1. Comparison of the growth phenotypes of the two transformed plants and the wild type in the absence of stresses.

Plants	One month Height (cm)	One month Dry weight (mg)	Two month Height (cm)	Two month Dry weight (mg)
Wild type	2.8±0.24a	11.5±2.01a	6.8±0.21a	55.1±4.41a
antiSOD	2.5±0.37a	11.0±1.63a	5.8±0.65b	46.9±6.28b
dsSOD	2.7±0.39a	11.8±2.66a	7.4±0.70c	62.5±9.12c

The data are the mean ± SD of ten replicates from each transgenic line.
Values in each column followed by different letters are significantly different at P≤0.05.

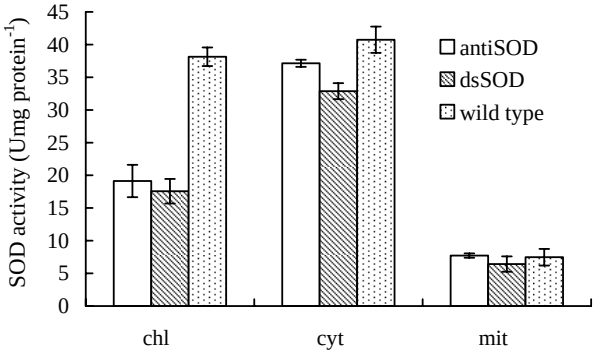

Figure 4. SOD activities in chloroplast, cytosol and mitochondrial in leaves of the control and transformant tobacco. Vertical bars represent the SE of the mean for triplicate determination.

2 (no inhibition by KCN but inhibited by H_2O_2), and CuZn-1, and CuZn-2 (inhibited by both KCN and H_2O_2). Densitometric analysis of the activity of gels showed that the Mn, Fe-1, Fe-2, CuZn-1 and CuZn-2 isoforms in nontransformed plants accounted for approximately 24%, 8%, 10%, 27%, and 28%, respectively, of the total SOD activity. The number and intensity of the SOD bands varied quite dramatically among the transformants. The chloroplastic form of Cu/Zn-SOD (CuZn-2) was not found in antiSOD or dsSOD transformed plants, and the Mn, Fe-1, Fe-2 and CuZn-1 isoforms accounted for 26%, 12%, 21% and 41%, respectively, of the total SOD activity in the former, and 21%, 10%, 24% and 43% in the latter.

To study the effect of PEG-induced stress and salt stress on the induction of Cu/Zn-SOD, we imposed NaCl stress on Cu/Zn-SOD transgenic tobacco and control plants. On the whole, the total SOD activity in all plants increased with the increase of the concentration of NaCl. When NaCl stress continued for 48 h, the antiSOD plants with PEG pretreatment showed a total SOD activity increase, in contrast with the control, but the value was still lower than that of the wide type. A similar increase was found in antiSOD plants under NaCl stress for 72 h. The responses of total SOD activities in dsSOD plants were similar to those in antiSOD plants. The total SOD activity in dsSOD plants began to increase after 72 h NaCl-stress and showed a lower value than that of the wide type. In the wild type, the total SOD activity increased markedly after 24 h PEG treatment, and then decreased continuously in the salt stress. PEG was also able to increase SOD activity and even make it surge higher (Figure 3). The results of a gel analysis of SOD activity, showed Cu/ZnSOD$_{cyt}$ bands noticeably enhanced, after a 3-day salt stress in dsSOD plants (Figure 6).

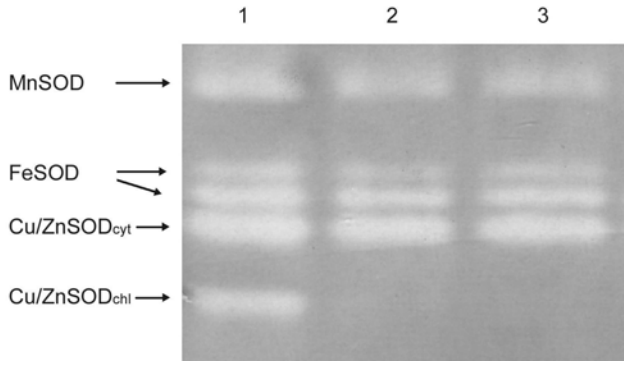

Figure 5. Samples (15 ug) of total protein from leaf extracts of Cu/Zn SOD transgenic tobacco plants. Untransformed tobacco plants were electrophoresed in nondenaturing polyacrylamide SOD activity gels. The result show the condition of isozyme patterns in the absence of stresses. Samples lanes correspond to untransformed control (lane1), antiSOD (lane 2) and dsSOD (lane 3).

Activity of Antioxidant Enzymes During Oxidative Stress

To further characterize the putative role of oxidative stress in salt stress, the activities of APX were measured in extracts of leaf tissues. As ROS scavengers, these enzymes manifested various activities with different levels of ROS to maintain the redox balance in cells. Immediately before the start of the stress, dsSOD transgenic plant showed lower SOD activity compared with the untransformed plants, while the activities APX were almost the same. After 3 days' of salt stress, the activity of SOD was higher in the dsSOD plants than in the unstressed ones. The APX activity was increased to the same level as that of the control. The antiSOD transgenic plants showed a parallel condition (Figure 7).

Discussion

We constructed chimeric genes by fusing the coding region from a cDNA encoding tomato chloroplastic Cu/ZnSOD to CaMV35S through different orientations. When this construction was expressed in transgenic plants, it changed the Cu/ZnSOD activity in them. This study showed that dsRNA-mediated genetic interference could operate in *Nicotiana plumbaginifolia* to efficiently induce sequence-specific inhibition of the gene function.

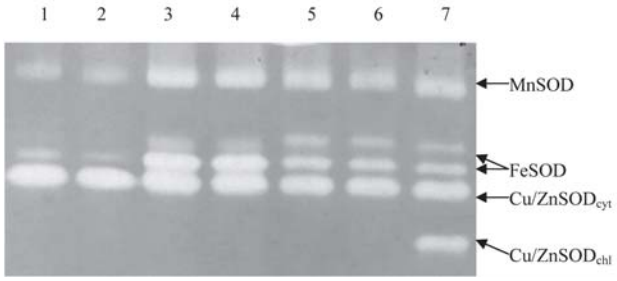

Figure 6. Samples (15 ug) of total protein from leaf extracts from Cu/Zn SOD transgenic tobacco plants. Untransformed tobacco plants were electrophoresed in nondenaturing polyacrylamide SOD activity gels. The result show the condition of isozyme patterns in the presence of stresses. Samples lanes correspond to dsSOD for 5 days' salt stress (lane 1, 2), dsSOD for 3 days' salt stress (lane 3, 4), antiSOD for 3 days' salt stress (lane 5, 6), and untransformed control for 3 days' salt stress (lane 7).

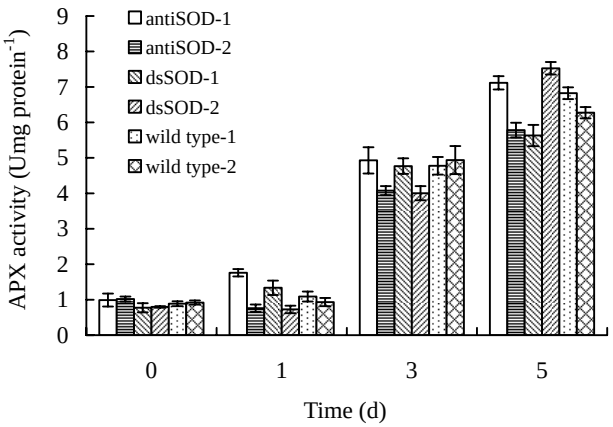

Figure 7. APX activities in leaves of the control and transformant tobacco grown for 5 days in the solution from 100 mM to 300 mM NaCl. Vertical bars represent SE for three samples. antiSOD-1 and antiSOD-2 showed the antiSOD transgenic plants in NaCl stress with PEG pretreatment or in single NaCl stress, respectively. dsSOD-1, dsSOD-2, wild type-1, and wild type-2 correspond similarly.

Agrobacterium-mediated transformation provides a convenient and efficient method for introducing dsRNA-expressing constructs into the plant genome. Double-strands and antisense constructs of Cu/ZnSOD had the ability to induce genetic interference in the background, but no difference in the ability of each to induce it appeared in our results. Waterhouse (1998) suggested that low levels of dsRNA might be produced from transgenes designed to produce only antisense, via the readthrough transcription from transgenes arranged as an inverted repeat, or transcription from transgenes with a 3´ end adjacent to an endogenous promoter. Alternatively, it seems possible that celluar RNA-dependant RNA polymerase could be involved in the conversion of single stranded RNA into dsRNA in a cell-specific manner (Schiebel et al., 1998). In addition, a recent report on the isolation of an RNaseD homolog from *C. elegans* mutants resistant to RNAi suggested that RNAi works by enzymatic RNA degradation (Ketting et al., 1999). Determination of silencing in the difference degree could be achieved by inducible expression of dsRNA constructs on the biochemical level.

The shoot buds from transformed explants were quickly induced, and they were green and thick. They were subcultured every two weeks. After about fifty days some of the shoot buds of dsSOD and antiSOD transformant became grayish white and grew much more slowly. 23.8% and 8.0% of the shoot buds or calli turned brown and necrotic from dsSOD and antiSOD transformants, respectively. SOD activity was measured in the extracts of these tissues, and a very low activity was found (data not shown). Our results indicated that Cu/Zn SOD was suppressed in transgenic tobacco chloroplasts, because the transgenic plants showed a 20% decrease of SOD activity in leaf extracts and a 50% reduction in isolated chloroplastics. Further confirmation came with the staining of the isozyme pattern being observed in SOD activity. From the result of activity gel analysis, the Cu/Zn SOD in chloroplast disappeared in transgenic plants. To study the effect of PEG-induced stress and salt stress on the induction of antioxidant enzymes, we imposed NaCl stress on Cu/ZnSOD transgenic tobacco and control plants and found that osmotic stress could probably influence antiRNA-mediated genetics interference and induce the Cu/Zn SOD expression. Yu and Rengel (1999) demonstrated that salt treatment increased the activity of Cu/Zn SOD on a soluble protein basis, without affecting the activities of the other SOD forms, and the increase in the total SOD activity under salt stress was due to the increase in Cu/Zn SOD activity. However, it is not clear thatwhat kind of signal expression of the Cu/Zn SOD gene responds to. As seen from the experiments, the $Cu/ZnSOD_{chl}$ bands appeared to be the most responsive to osmotic stress, implying that the chloroplastic might be the chief site of oxyradical formation. So it can be used to help us understand both how each stress may affect different subcellular compartments and the possible mechanism of SOD gene induction and direct oxygen radical involvement in these signaling responses. This is not to argue against the possibility that SOD overproduced in other cellular compartments can likewise be effective in preventing salinity injury. As other researchers have concluded (Mckersie et al., 1996), not only does SOD activity need to be enhanced to protect against oxidative stress, the performance of one or more other antioxidant enzymes that scavenge the product of SOD, such as APX or catalase or both, needs also to be enhanced.

Many workers (Tanaka et al., 1999; Yu and Rengel, 1999) have found a positive correlation between salt stress and the abundance of SOD in plants. Major ROS-scavenging mechanisms in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Bowler et al., 1992; Willekens et al., 1997). The balance between SOD and APX or CAT activation in cells is crucial for determining the steady-state level of superoxide radicals and hydrogen peroxide (Bowler et al., 1991). This balance, together with the sequestering of metal ions, is thought to be important in preventing the formation of the highly toxic hydroxyl radical via the metal-dependent Haber-Weiss or the Fenton reactions. Unfortunately, our knowledge of the processes involved is not yet sufficient to explain this observation. Our results demonstrated that the activity of APX in transformed plants differed to a certain extent from that of untransformed plants in the absence of stresses.

In conclusion, our work showed that the use of the Cu/Zn-SOD cDNA to construct transgenic tobacco by hpRNA and antisense suppression could constitutively reduce this gene expression in chloroplast. We discussed the response of Cu/ZnSOD to salt stess in transgenic and control plants. It indicated that an antioxidative alternative pathway, at least, associated Cu/Zn-SOD activity existed in the cytosol of the transgenic plant, the chloroplastic Cu/Zn-SOD gene expression of which was suppressed under an unfavorable environment for acclimation. Further study should help clarify the overall importance of the phenomenon of oxidative stress in plants. In addition, our results can be

used as a basis for elucidating the important role that compartment-specific regulation plays in resistance of the cell to stress.

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RNA干涉技術對植物葉綠體超氧化物歧化酶基因功能的研究

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從番茄中克隆而得的葉綠體銅鋅超氧化物歧化酶（Cu/Zn-SOD）基因通過轉基因技術導入煙草中。雙鏈 RNA（dsRNA）和反義 RNA 結構能特異地抑制轉基因煙草葉綠體 Cu/Zn-SOD 的表達，其機理是內源葉綠體 Cu/ZnSOD 基因成為了髮卡 RNA（hpRNA）和反義 RNA 的靶子而表現為沉默。與野生型煙草相比，不僅 SOD 整體活性下降，而且原應存在於葉綠體中的 Cu/Zn-SOD 同功酶條帶完全消失。在鹽和聚乙二醇（PEG）脅迫中，兩種轉基因煙草胞質 Cu/ZnSOD 活性明顯上升，而 PEG 的預處理使植株的 SOD 活性上升早於未進行預處理的植株。結果說明，通過雙鏈 RNA 和反義 RNA 結構能有效的抑制目的基因的表達，但由於同功酶的存在，轉基因植株在脅迫條件下仍能保持一定的總 SOD 活性，且其他 SOD 同功酶活性能補償葉綠體 Cu/Zn-SOD 的缺失。總而言之，RNAi 技術對於揭示葉綠體 Cu/ZnSOD 的功能有極大的幫助，而且發現在脅迫條件下植物與 Cu/Zn-SOD 相關的抗氧化過程存在胞質同功酶的交替途徑，同時也為在煙草中進行反義抑制的操作提供了技術支援。

關鍵詞：超氧化物歧化酶；雙鏈RNA；轉基因煙草；鹽脅迫。