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Over-expression of Cu/ZnSOD Increases Cadmium Tolerance in *Arabidopsis thaliana*

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ABSTRACT: Over-expression of a copper/zinc superoxide dismutase (Cu/ZnSOD) resulted in substantially increased tolerance to cadmium exposure in *Arabidopsis thaliana*. Lower lipid peroxidation and H_2O_2 accumulation and the higher activities of H_2O_2 scavenging enzymes, including catalase (CAT) and ascorbate peroxidase (APX) in transformants (*CuZnSOD-tr*) compared to untransformed controls (*wt*) indicated that oxidative stress was the key factor in cadmium tolerance. Although progressive reductions in the dark-adapted photochemical efficiency (Fv/Fm) and quantum efficiency yield were observed with increasing cadmium levels, the chlorophyll fluorescence parameters were less marked in *CuZnSOD-tr* than in *wt*. These observations indicate that oxidative stress in the photosynthetic apparatus is a principal cause of Cd-induced phytotoxicity, and that Cu/ZnSOD plays a critical role in protection against Cd-induced oxidative stress.

Key words: *Arabidopsis*, Cadmium tolerance, Oxidative stress, Superoxide dismutase

INTRODUCTION

Reactive oxygen species (ROS) have been implicated in a variety of physiological stresses in plants, and cellular antioxidant systems are considered to constitute a front-line defense. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into O_2 and H_2O_2 and thereby reduces the titer of activated oxygen molecules within a cell. A number of environmental stresses, including ozone (Lee and Bennet 1982), cold (Thomas et al. 1999), drought (Wu et al. 1999) and light (Mishra et al. 1993) have been shown to result in enhanced superoxide production within plant tissues, and plants are believed to rely on the SOD enzyme to detoxify this ROS.

Various SOD isoforms are located in various cellular compartments. Copper/zinc superoxide dismutase (Cu/ZnSOD) is detected in the cytosol and in the chloroplast stroma. An iron superoxide dismutase (FeSOD) can also be detected in the stroma. Mitochondria and peroxisomes were shown to harbor two electrophoretically distinct SODs, a Mn- and a FeSOD (Droillard and Paulin 1990). Seven cDNAs and genes for SOD have been identified in *Arabidopsis* (Kliebenstein et al. 1998). These consist of three Cu/ZnSODs (*CSD1*, *CSD2*, and *CSD3*), three FeSODs (*FSD1*, *FSD2*, and *FSD3*), and one MnSOD (*MSD1*).

Over-expression of SODs is often shown to enhance tolerance to oxidative stress and contribute to stable growth under stress conditions. *Nicotiana tabacum* which overproduces cytosolic Cu/ZnSOD evidenced less profound foliar necrosis than was seen in nontransformed controls when exposed to acute doses of ozone constituents (Pitcher and Zilinskas 1996). This may demonstrate the importance of Cu/ZnSOD in the cytosol as a protector of plasma membrane integrity, and possibly the integrity of other cellular components. However, an increase in Cu/ZnSOD alone is insufficient to reduce oxidative toxicity. Transgenic tobacco plants that overexpress chloroplast Cu/Zn SOD also evidence a 3- to 4-fold increase in ascorbate peroxidase (APX) specific activity as compared to non-transgenic plants (Pitcher et al. 1991). These results show that transgenic plants overexpressing Cu/ZnSOD can compensate for increased SOD levels with increased expression of H_2O_2-scavenging enzymes, including APX. When all of the results are considered, an increase in the SOD alone appears to be insufficient to reduce oxidative toxicity, and the concurrent enhancement of other antioxidant enzymes involved in ROS scavenging may also be required to protect plants against oxidative stresses.

Cadmium (Cd) is one of the most toxic metals in the environment. It has been established that leafy vegetables, roots, and grains can accumulate relatively high levels of Cd from the soil, and that this accumulation can become extremely toxic to humans and plants. Thus, mechanisms must exist to protect plants against such toxic effects. In parallel with metal-induced phytotoxicity, symptoms of oxidative stress including alterations of antioxidant systems and ROS accumulation have been observed (Schützendübel et al. 2001, Yamamoto et al. 2002, Cho and Seo 2005, Chiang et al. 2006). Therefore, metal-induced phytotoxicity may be attributed, at least in part, to oxidative damage. In plant cells exposed to Cd

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at concentrations exceeding their detoxification capacity, the levels of superoxide radicals are elevated and H_2O_2 accumulates due to an imbalance in redox systems, and higher ROS-quenching activities contribute to Cd tolerance via an alleviation of ROS-induced damage (Cho and Seo 2005, Chiang et al. 2006, Maksymiec and Krupa 2006). The increase in ROS levels was partially associated with an increase in SOD activity (Maksymiec and Krupa 2006), and Cd hyperaccumulators displayed significantly higher endogenous SOD activity and glutathione concentrations (Boominathan et al. 2003). Therefore, Cd-induced phytotoxicity may also be induced by oxidative stress, and SOD may contribute to Cd tolerance in plants.

In the present study, we evaluated the hypothesis that plants constitutively over-expressing cytosolic Cu/ZnSOD are more tolerant to Cd exposure than are non-over-expressing wild type plants. The over-expression of Cu/ZnSOD will suppress oxidative stress and enhance Cd-tolerance. In order to assess this hypothesis, *Arabidopsis* plants were transformed with a Cu/ZnSOD gene, and the growth and antioxidative responses of transgenic plants were compared with the non-transgenic wild type plants under Cd stress conditions. The information obtained in this experiment may provide a basis for our understanding of the mechanism(s) underlying Cd detoxification or Cd tolerance in plants, and to develop plants for the phytoremediation of heavy metals.

MATERIALS AND METHODS

Plant Material

Arabidopsis thaliana seeds (Col-0) were purchased from Lehle Seeds (USA) and grown in a controlled environment in a chamber maintained at 23℃ with 16 h of light (250 ($M\ m^{-2}s^{-1}$) and 70~80% humidity. The seeds were plated at 1~2 per 25 cm^2 pot containing a perlite:vermiculite (1:1) mixture. In order to obtain more floral buds per plant, inflorescences were clipped after most of the plants had formed primary bolts (Clough and Bent 1998). Plants were infiltrated or dipped when the majority of secondary inflorescences were approximately 1~10 cm tall (4~8 days after clipping).

For Cd treatments, the seeds were germinated and grown on MS medium containing 0, 200, or 500 μM of $CdCl_2$. 21-day-old seedlings collected from each treatment were dried for 48 h at 80℃ and weighed for biomass and Cd measurement. For H_2O_2 level measurements, lipid peroxidation and enzyme activities, 21-day-old fresh seedlings were weighed and analyzed.

Determination of Cadmium Concentration

Twenty-day-old seedlings were washed twice in deionized water, and the roots were washed with ice-cold 5 mM $CaCl_2$ solution for 10 min to replace extracellular Cd (Rauser 1987). The seedlings were then dried for 48 h at 70℃, weighed, and ground to a fine powder before wet-ashing in a 3:1 HNO_3:$HClO_4$ solution. Cd was detected directly by atomic absorption spectrophotometry (Varian 200AA equipped with SIPS; Australia) using an air-acetylene flame and a Cd hollow-cathode lamp.

Chlorophyll Fluorescence Analyses

Dark-adapted photochemical efficiency (Fv/Fm) and steady state yield of quantum efficiency (Y) parameters were measured using a chlorophyll fluorometer (OS1-FL, Opti-Sciences, USA). Variable fluorescence (Fv) is calculated by the subtraction of non-variable fluorescence (Fo) from maximal fluorescence (Fm), and Y = (Fms−Fs)/Fms, here Fms is the maximal fluorescence under steady-state conditions and Fs is fluorescence under steady-state conditions prior to saturation pulse.

Analysis of H_2O_2 and Lipid Peroxidation

Samples of fresh seedlings (500 mg) were ground with liquid nitrogen and suspended in 1.5 mL of 100 mM potassium phosphate buffer (pH 6.8). Afterward, H_2O_2 contents in the tissues were measured in accordance with the methods described by Gay and Gebicki (2000). The levels of lipid peroxidation in the leaves and roots were assessed by the thiobarbituric acid-reactive substance (TBARS) content resulting from the thiobarbituric acid (TBA) reaction, as described by Dhindsa et al. (1987). The TBARS concentration was calculated in accordance with the following equation: $A_{532}-A_{600}$ (ε = 155 $mM^{-1}cm^{-1}$).

Enzyme Assays

All samples (0.2 to 0.5 g fresh weight), with the exception of those analyzed for APX activity, were ground with liquid nitrogen and homogenized with an extraction buffer containing 10 mM potassium phosphate buffer (pH 7.8), 0.5% Triton X-100, and 1.0% polyvinylpyrolidone. In order to evaluate APX activity, the leaves were homogenized and extracted in accordance with the methods described by Lee and Lee (2000). After the homogenates were centrifuged at 12,000*g* for 20 min at 4℃, the supernatants were immediately evaluated. The protein contents were determined in accordance with the method described by Bradford (1976), using BSA as the standard. Total SOD activity was defined using one unit of SOD as the amount of xanthine-xanthine oxidase required to inhibit the reduction of cytochrome C by 50% (McCord and Fridovich 1969). CAT activity was assayed on the basis of the reduction in absorbance at 240 nm due to the degradation of H_2O_2 (ε= 39.4 $mM^{-1}cm^{-1}$, Chance and Maehly 1955). Here, one unit of CAT represented the amount necessary to decompose 1 mmol of H_2O_2 per minute at 25℃. APX activity was determined as previously

described by Rao et al. (1995). All spectrophotometric analyses were conducted with a Bio-20 spectrophotometer (Perkin Elmer, USA).

Activity Gel Analyses of SODs

Protein samples (50 μg per lane) were separated via native PAGE on a separating gel of 12% (w/v) polyacrylamide with 5% (w/v) stacking gel in a tank buffer containing 25 mM Tris (pH 8.3) and 192 mM glycine. After 30 min of incubation in 50 mM potassium phosphate buffer (pH 7.0) containing 3 mM KCN (inhibitor of the Cu/ZnSOD) or 2 mM H_2O_2 (inhibitor of FeSOD and Cu/ZnSOD) (Lee and Lee 2000), the gels were stained for 30 min in darkness using a 1:1 mixture of (a) 0.06 mM riboflavin and 0.651% (w/v) TEMED, and (b) 2.5 mM nitroblue tetrazolium (NBT), both in 50 mM phosphate buffer at pH 7.8, and then developed for 20 min under light conditions (McKersie et al. 2000). After staining, the gels were photographed with a digital camera.

Plasmid Construction and Plant Transformation

The Cu/ZnSOD open reading frame was amplified using *Arabidopsis* cDNA as a template with the following primers: 5'-atttgtgcctactcatttcc-3' 5'-aagtaaacacatcactgtca-3', which were constructed on the basis of accession number AY064050. PCR conditions were as follows: 94℃ for 3 min, followed by 35 cycles of 94℃ for 45 s, 55℃ for 45 s, 72℃ for 1 min and 30 s, and 72℃ for 10 min. Cu/ZnSOD PCR products were confirmed via sequence analysis on an Applied Biosystem 373 Automated DNA sequencer (ABI/Perkin-Elmer. USA). The open reading frame region of SOD was 939 bps. This SOD PCR product was inserted into the pK2GW7 binary plant vector, between the cauliflower mosaic virus 35S promoter and T35S. The resultant plasmid, designated pK2GW7-Cu/ZnSOD, was mobilized to *Agrobacterium tumefaciens* strain GV3101 and used for plant transformation. Five-week-old *Arabidopsis* plants were infected with *A. tumefaciens* via the floral dipping method (Clough and Bent 1998) and grown in a greenhouse. Seeds collected from these plants were screened in MS medium supplemented with 30 μg mL^{-1} of kanamycin.

Statistical Analysis

Data reported in the figures were all analyzed using the statistical program SYSTAT (Version 9, SPSS Inc). Significance of difference was tested at P = 0.05 using ANOVA and post hoc LSD. The data are means ± SE from four replicates.

RESULTS AND DISCUSSION

Seedling Growth, Cd Accumulation and Photosynthesis

Growth of seedlings grown for 21 d on media containing up to 500 μM Cd was monitored (Fig. 1). As compared with the control seedlings, the biomass of wild type *wt* was significantly lower, by 34% and 61%, in the presence of 300 μM and 500 μM of Cd, respectively. However, biomasses for the transgenic *CuZnSOD-tr* were only 13% and 35% lower than control seedlings in the presence of 300 μM and 500 μM of Cd, respectively, indicating that they were more tolerant to Cd exposure.

The Cd concentration in all seedlings increased with Cd treatment. *Wt* and *CuZnSOD-tr* exposed to 300 μM Cd for 21 d accumulated 65.0 μg g^{-1} dry weight and 81 μg g^{-1} dry weight of Cd, respectively. There was a significant difference in CD levels (P< 0.05) between *Wt* and *CuZnSOD-tr* seedlings; the transformant contained a significantly higher level of Cd than *wt*. The dark- adapted photochemical efficiency (F_v/F_m) values (the ratio of variable F_v to maximal F_m fluorescence) and yields of quantum efficiency obtained in the leaves of seedlings grown without Cd were approximately 0.7~0.8 in both *Wt* and *CuZnSOD-tr* (Figs. 2 and 3). A progressive decline in the F_v/F_m ratios was observed with increasing Cd levels (Figs. 2 and 3), but this was less remarkable in the *CuZnSOD-tr* than in *wt*. These observations suggested that the reduced amount of active chlorophyll in Cd-treated leaves as compared to the control leaves resulted in a reduction in F_0 or F_m values (Piertini et al. 2003). The loss of chlorophyll could have resulted from the effects of strong oxidation on the photochemical apparatus (Somashekaraiah et al. 1992). Further, the reduction of F_v/F_m could be the result of a reduction in chloroplast density and size (Baryla et al. 2001). My results also could not be used to exclude the possibility that Cd replaced Mg in chlorophyll (Kupper et al. 1998). The differences in the F_v/F_m ratios and quantum efficiency yields (Y) between the leaves of *CuZnSOD-tr* and *wt* and the relatively

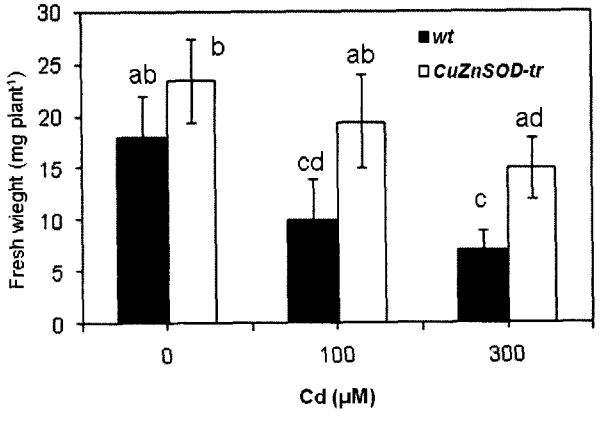

Fig. 1. Fresh weight of *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

higher ratios of these parameters of chlorophyll fluorescence in *CuZnSOD-tr* after Cd exposure indicated that the over- expression of Cu/ZnSOD may contribute to the maintenance of the photosynthetic apparatus and subsequent stable growth.

Oxidative stress

Whereas biomass decreased at higher Cd exposure, the peroxidation of lipids expressed as TBS-RS production was enhanced significantly, and the concentration of H_2O_2 in seedlings more than doubled after exposure to Cd (Figs. 4 and 5). Because increased TBA-RS concentrations are common symptoms of metal stress, the determination of TBA-RS response served as a non-specific index of Cd-phytotoxicity, which is more reliable than total Cd content (Buege and Aust 1978, Pandolfini et al. 1992, De Vos et al. 1993, Lozano-Rodriguez et al. 1997). However, relative to the values seen in the *wt* plants, *CuZnSOD-tr* evidenced significantly lower H_2O_2 concentrations and TBA-RS production levels, results which are indicative of lower oxidative stress. Elstner (1991) has reported that H_2O_2 accumulation itself can contribute to the suppression of seedling growth. Therefore, the reduced levels of H_2O_2, together with reduced TBA-RS formation, could help to explain the more stable growth and Cd tolerance of transgenic plants.

Transition metals, including iron and copper, generate ROS via autoxidation and the Fenton reaction (Haliiwell and Gutteridge 1984, Schutzendubel et al. 2001). The exposure of plants to non-redox reactive metals also resulted in oxidative stress, as indicated by lipid peroxidation, H_2O_2 accumulation, and the oxidative burst (Schutzendubel et al. 2001, Cho and Seo 2005, Chiang et al. 2006).

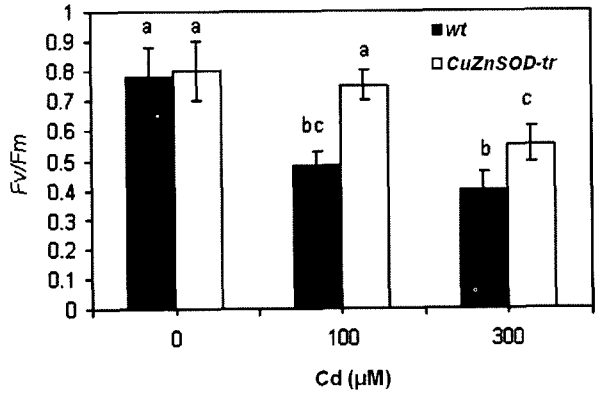

Fig. 2. F_v/F_m ratios of *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

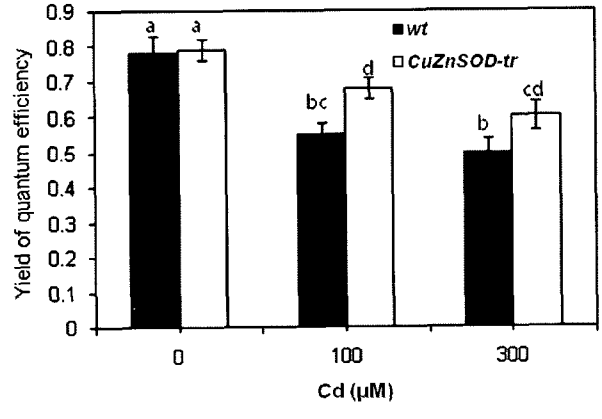

Fig. 3. Photosynthetic yields of *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

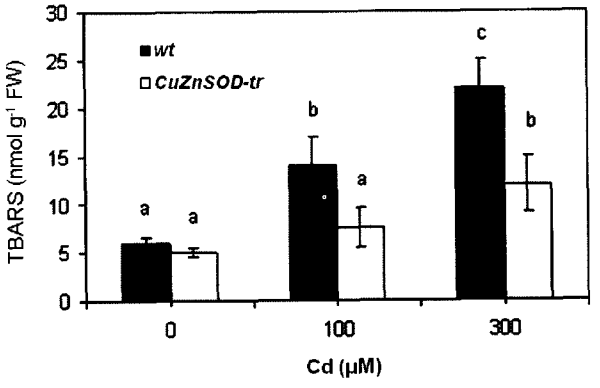

Fig. 4. Lipid peroxidation in *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

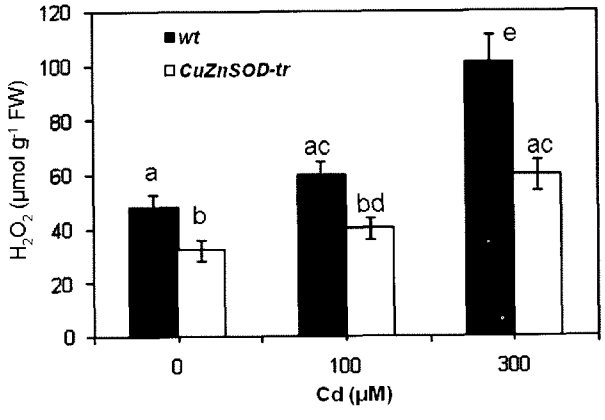

Fig. 5. Hydrogen peroxide contents in *Arabidopsis* seedlings grown on media containing Cd for 21d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

It is conceivable that a reduction in enzymatic and non-enzymatic ROS scavengers, caused by exposure to heavy metals (De Vos et al. 1993), may contribute to a shift in the balance of ROS metabolism towards the accumulation of H_2O_2. Many ROS are themselves reactive and result in further membrane lipid deterioration, which may lead to membrane permeability and subsequent growth inhibition (De Vos et al. 1991). Available data indicate that cadmium, when not detoxified sufficiently rapidly, may trigger, via the disturbance of the redox control of the cell, a sequence of reactions culminating in growth inhibition and, ultimately, cell death.

Activities of SOD

In plants, SOD is a metalloprotein that catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen in the cytosol, mitochondria, and chloroplasts (Fridovich 1986). In our experiment, total SOD activities declined as the result of Cd treatments in both *wt* and *CuZnSOD-tr* (Fig. 6). However, the over-expression of Cu/Zn SOD resulted in higher total SOD activities in transgenic plants as compared to the *wt* plants. The transgenic plants also evidenced increases of other SOD isoforms, including FeSOD and MnSOD (Fig. 6). When KCN was utilized to inhibit Cu/ZnSOD or H_2O_2 for the inactivation of both Cu/ZnSOD and Fe-SOD, three types of SOD isozymes (MnSOD, Fe-SOD and Cu/ZnSOD) were identified (Fig. 7). When the activity of each SOD isozyme was analyzed on native PAGE gels by comparing the intensity of SOD activity in seedlings, the application of Cd caused a decrease of all SOD isoforms. In plants, Cu/ZnSOD is the isoform with the highest induction levels in response to a variety of stimuli (Kaminaka et al. 1999), and chloroplastic Cu/ZnSOD could contribute to the elimination of O_2^- in the chloroplasts (Polle 1997). The Mn-SOD activity observed in this experiment was not greater than the activities of FeSOD and Cu/ZnSOD. Similar results have been observed in tobacco plants (Bowler et al. 1992). The lower activity of MnSOD isoforms in the mitochondria could be related to the fact that O_2^- generation by the electron transport chain (Borsani et al. 2001) was less affected by Cd exposure. Meanwhile, the observed decline in SOD activity might be attributable to damage inflicted on chloroplasts or plastids and metabolic alterations related to oxidative damage, including the lipid peroxidation described above.

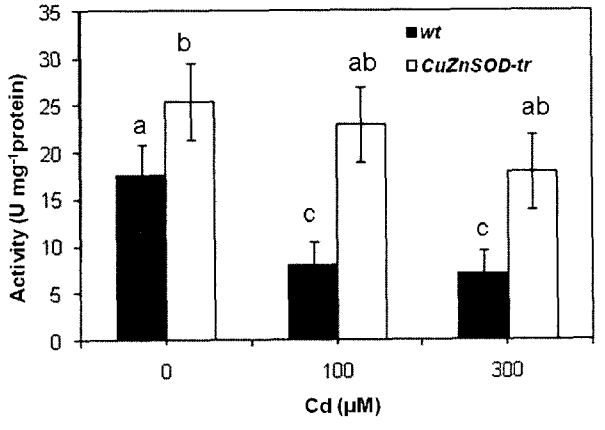

Fig. 6. Total activity of SOD in *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

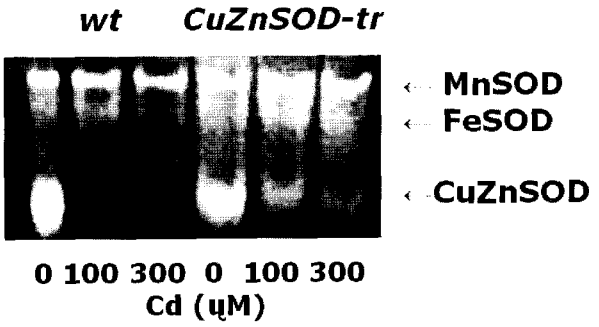

Fig. 7. Activities of SOD isoforms of *Arabidopsis* seedlings grown on media containing Cd for 21 d.

The observed reductions in SOD activity following Cd exposure observed in this study, and the fact that no increases in superoxide radical levels were reported in another study (Cakmak and Horst 1991) indicates that enhanced H_2O_2 generation after exposure to Cd might not be induced entirely by the increased production of superoxide radical and SOD activity. The lower enzymatic activities related to the removal of H_2O_2 might contribute to H_2O_2 accumulation (Asada 1992, Chaoui et al. 1997, Hegedus et al. 2001, Ianneli et al. 2002, Pilon-Smits et al. 2000). Inefficient removal of H_2O_2 and its subsequent accumulation can possibly induce phytotoxicity. Therefore, the stable maintenance of other H_2O_2 removal systems, including the glutathione-ascorbate cycle and the activities of CAT and various peroxidases, may be critical for protection against Cd-induced oxidative stress. Thus, both CAT and APX activities were studied further.

Activities of H_2O_2 Scavenging Enzymes

Cd exposure also resulted in changes in the activities of the H_2O_2 scavenging enzymes, CAT and APX (Figs. 8 and 9). The activities of both enzymes increased as the result of Cd exposure, and *CuZnSOD-tr* maintained higher activity than *wt*. Therefore, the increase in H_2O_2 levels as the result of Cd exposure (Fig. 5) was presumed to contribute to the overall increases of these activities, and the relatively lower H_2O_2 levels observed in *CuZnSOD-tr* as compared to *wt* may be attributable to the higher levels of activities of these enzymes.

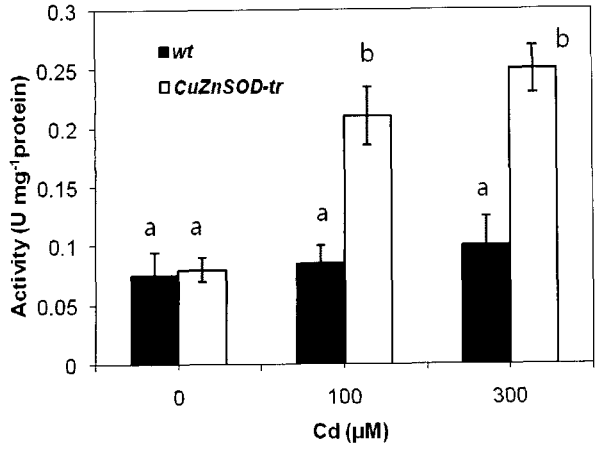

Fig. 8. Activity of ascorbate peroxidase in *Arabidopsis* seedlings grown on media containing CD for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

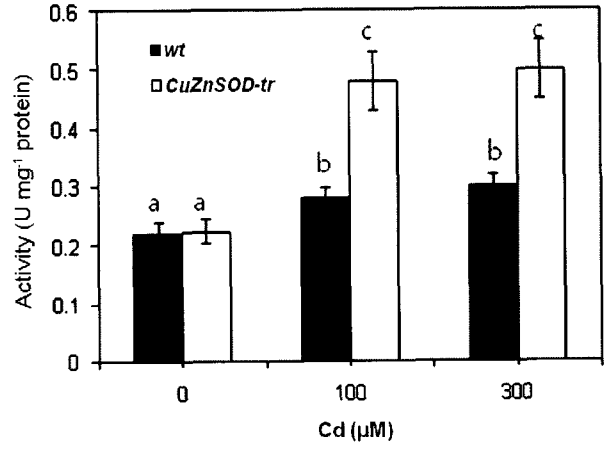

Fig. 9. Activity of catalase in *Arabidopsis* seedlings grown on media containing Cd for 21 d. Bars showing the same letter are not significantly different (P = 0.05, ANOVA; post hoc test LSD). Error bars indicate SE. n = 4.

As a member of the ascorbic acid-glutathione cycle, APX is one of the most important enzymes, performing a crucial function in the elimination of toxic H_2O_2 from plant cells (Asada, 1992). The increased activity of APX may be associated with its localization in chloroplasts or plastids, where reduced activity of FeSOD and CuZnSOD, an indicator of decreased H_2O_2 production, were observed. Meanwhile, CAT is more relevant to Cd-induced H_2O_2 outside the chloroplasts. The changes in enzyme activities observed in this study indicate that the overproduction of H_2O_2 may occur inside or outside of the chloroplasts and that the activities of antioxidant enzymes might prove insufficient to remove ROS, including H_2O_2. Excessive levels of ROS subsequently damage cell organelles, including those associated with photosynthesis or respiration, ultimately resulting in severe cellular damage and chlorosis of leaves. H_2O_2 itself is a powerful inhibitor of metabolism, including carbon fixation (Kaiser 1976). Further, the oxidation-reduction of metal ions by H_2O_2 and O_2^- via the Haber-Weiss reaction generates the most toxic hydroxyl radical (· OH) (Imlay and Linn 1988). The maintenance of higher activities of SOD, CAT, and APX in transgenic *CuZnSOD-tr* showed that higher SOD activities must accompany the appropriate activities of H_2O_2 removal enzymes to protect plants against oxidative damage.

Taken together, upon exposure to Cd, the transgenic *CuZnSOD-tr* maintained better growth, lower oxidative stress, and higher activities of endogenous SOD and H_2O_2 scavenging enzymes as compared to non-transgenic *wt*. The stable growth of *CuZnSOD-tr* upon exposure to Cd may be due to lower oxidative stress, and the higher levels of activity of SOD and H_2O_2 scavenging enzymes and lower H_2O_2 accumulation might contribute to lower oxidative stress. The high SOD activity of the Cd hyperaccumulator *T. caerulescens* (Boominathan et al. 2003) was also consistent with this explanation. H_2O_2 levels can be controlled at low, nontoxic levels in association with a strong CAT or APX induction response. As other heavy metals also evidenced increases in lipid peroxidation and SOD, APX, and CAT activities (Reddy et al. 2005) and metal-resistant lines evidenced reduced oxidative stress with low levels of metal accumulation (Ezaki et al. 2000), metal stress may frequently induce significant changes in antioxidant systems. Metal-mediated ROS generation appears to be a cause of ATP depletion, as well as the simultaneous loss of growth capability (Yamamoto et al. 2002). Therefore, the acquisition of antioxidant functions may constitute a mechanism underlying metal tolerance. Antioxidant activities may perform a crucial function in this regard, and the cell membranes in tolerant plants evidence stability superior to those of susceptible plants (Zhang et al. 2007).

The monitoring of SOD transcripts and SOD activity after the application of Cd stress indicates that the principal level of control is post-translational (Jacob et al. 2001). Our data also showed that the transgenic line evidenced high SOD activity and stable growth under Cd stress conditions. The maintenance of cell integrity via the over-expression of Cu/ZnSOD may maintain the stability of the activities of other enzymes and metabolism. The results of the present experiment show that stable SOD expression is crucial for the protection of plants from Cd-induced oxidative stress and the Cd tolerance of *CuZnSOD-tr* overexpressing Cu/ZnSOD could be the consequence of the maintenance of higher SOD activities, as well as H_2O_2 scavenging enzymes.

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