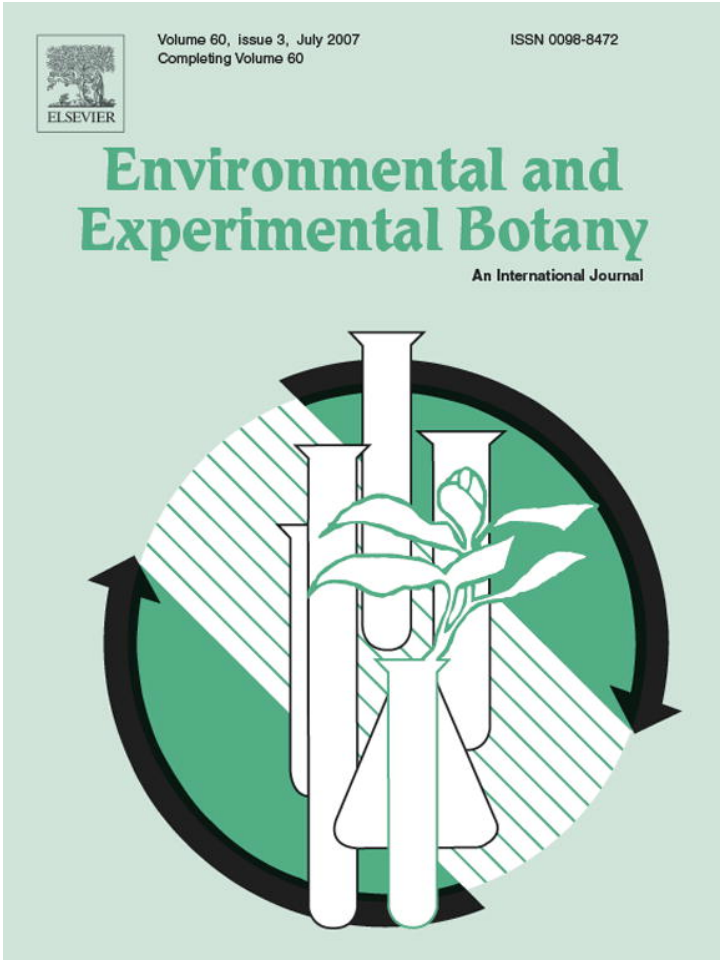

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Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L.

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Abstract

Changes in the activity of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and the contents of malondialdehyde (MDA), chlorophyll, free proline and phytochelatins (PCs) in *Solanum nigrum*, the newly discovered Cd-hyperaccumulator were examined and compared with a non-hyperaccumulator *Solanum melongena*. It was indicated that leaf SOD and POD activity of *S. nigrum* was significantly higher than that of *S. melongena*. The Cd treatments significantly increased root SOD activity, leaf POD activity, and CAT activity and free proline content in the leaves and roots of *S. nigrum*. On the contrary, the Cd treatments decreased SOD activity, and did not change CAT activity in the leaves and roots of *S. melongena*. Moreover, there were no significant differences in free proline levels in the roots of *S. melongena*. These results validated that *S. nigrum* had a greater capacity than *S. melongena* to adapt to oxidative stress caused by Cd and free proline accumulation might be responsible for the tolerance of *S. nigrum* to Cd. Treated with 10 μg Cd g^{-1}, growth of *S. nigrum* and its contents of chlorophyll and MDA were basically unaffected. In contrast, there were a decrease in the growth and chlorophyll content, and an increase in MDA in the roots of *S. melongena*. Although lipid peroxidation was promoted in both the hyperaccumulator and non-hyperaccumulator by high Cd stress, the greater increase took place in the tissues of *S. melongena*. The PCs level in roots of *S. nigrum* was significantly lower than that of *S. melongena*. On the contrary, the content of leaf PCs was much higher in *S. nigrum* than that in *S. melongena*. These results further suggested that antioxidative defense in the Cd-hyperaccumulator might play an important role in Cd tolerance, and PCs synthesis is not the primary reason for Cd tolerance although PCs in *S. nigrum* increased significantly by Cd.

Keywords: Antioxidant enzyme; Cadmium; Free proline; Hyperaccumulator; Phytochelatin; *Solanum nigrum*; Tolerance

1. Introduction

Throughout the world, over 400 species of terrestrial plants have been identified as hyperaccumulators of various heavy metals (Baker et al., 2000; Zhou et al., 2006), which are capable of taking up and storing high levels of heavy metals without suffering metal toxicity or cell damage. Consequently the exploitation of hyperaccumulators that can remove toxic heavy metals from metal-contaminated soils is currently of considerable commercial interest. At present some progresses have been made in our understanding of the ability of hyperaccumulators to tolerate and accumulate metals within their tissues (Zhou and Hua, 2004; Zhou and Sun, 2004; Wei et al., 2006). However, it is still difficult to fully understand the physiological, biochemical and molecular mechanisms involved in metal hyperaccumulation, which obstructs the optimization of the phytoextraction technique and its further commercial application.

The oxidative stress induced by heavy metals in plants can take place possibly by generating active oxygen species (AOS) such as superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$) (Salin, 1987; Foyer et al., 1994; Dat et al., 2000; Wang and Zhou, 2006). These oxygen species cause lipid peroxidation, enzyme inactivation and DNA damage, resulting in dramatic reduction of growth and productivity, finally causing plant death. Unlike copper (Cu) and iron (Fe), which induce oxidative stress via Fenton-type reaction, redox-inactive metals, such as cadmium (Cd) and lead (Pb) do not directly generate AOS. Cd as one of the aggressive heavy metals was found to produce oxidative

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stress. Cd-induced H_2O_2 accumulation has been observed in several plant species (Somashekaraiah et al., 1992; Sanità and Gabbrielli, 1999). H_2O_2 is a necessary substrate for the cell wall stiffening process, which is considered to be one of the mechanisms resulting in growth inhibition. As H_2O_2 diffuses freely across membranes, it possibly induces widespread damage in cell compartments (Foyer et al., 1997). The association between H_2O_2 accumulation and Cd toxicity in plants suggests that antioxidative defense may play a key role in Cd tolerance.

The importance of antioxidative systems is generally emphasized in preventing oxidative stress by scavenging AOS. The antioxidative system comprises antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase, ascorbate peroxidase and glutathione reductase, as well as nonenzyme antioxidants such as ascorbic acid and glutathione. Superoxide anion radicals produced in different cell compartments are rapidly converted into H_2O_2 in a reaction catalyzed by SOD (Noctor and Foyer, 1998). The accumulation of H_2O_2 is prevented in the cells by various antioxidant enzymes, which are mainly located in peroxisomes. As a very important enzyme for plant respiration, POD can participate in lignin biosynthesis and convert H_2O_2 to H_2O. Regulation of antioxidative enzymes can provide plants with an additional protective ability against oxidative stress. Antioxidative responses and tolerance to oxidative stress are dependent on the plant species and environmental conditions. There are many indications that antioxidative defense plays an important role in hyperaccumulators (Boominathan and Doran, 2003).

The accumulation of "stress metabolites" in plants may also be induced by heavy metals. Among these metabolites, proline is probably the most widespread in plants. In addition, proline accumulation is not only regarded as an indicator of environmental stress but also considered as an important protective role against heavy metal stress (Alia-Saradhi, 1991; Sharma et al., 1998). The free proline has been found to chelate Cd ion in plants and form a nontoxic Cd-proline complex (Sharma et al., 1998). The cumulative capacity of free proline is a manifestation of the self-protection ability of plants exposed to different metal stresses.

Complexation with phytochelatins (PCs) has been identified as an important mechanism for detoxifying toxic metals such as Cd, Pb and Hg in various plant species (Cobbett, 2000; Gupta et al., 1998; Scarano and Morelli, 2002). However, PCs do not contribute to the Cd tolerance observed in metal-tolerant populations of *Silene vulgaris* L. (De Knecht et al., 1995) and other species (Schat et al., 2002). It was also showed by Ebbs et al. (2002) that increased PCs production is not the primary mechanism by which Cd tolerance is achieved in hyperaccumulator *Thlaspi caerulescens*. Since the above-mentioned plant species are dissimilar taxonomically and morphologically, their tolerance mechanisms may differ greatly. In particular, there may be a different role of PCs in metal tolerance between species.

Solanum nigrum L. is a newly discovered Cd-hyperaccumulator (Wei et al., 2004). In a pot-culture experiment, *S. nigrum* could accumulate up to 124.6 μg Cd g^{-1} DW in leaves, indicating no phytotoxic symptoms and reduction in growth. There have been no detailed studies on Cd tolerance and hyperaccumulation of *S. nigrum*. Therefore, the aims of our study were to investigate the changes in lipid peroxidation, antioxidative enzyme activities, free proline and PCs accumulation in *S. nigrum* and to assess the role of antioxidative metabolism and proline and PCs accumulation in Cd tolerance by *S. nigrum*, in comparison with a closely botany-related species *Solanum melongena*.

2. Materials and methods

2.1. Soil preparation and plant culture

Soil samples were collected from an agricultural field in the Shenyang Station (123°41′N and 41°31′E) of Experimental Ecology, Chinese Academy of Sciences. The fresh soil samples were air dried, passed through a sieve of 4.0 mm, and thoroughly mixed with basal fertilizers. The tested soil contained 1.5% organic matter, had a pH of 6.5. Basal fertilizers applied were 150 mg N kg^{-1} dry soil as urea, 60 mg P kg^{-1} and 80 mg K kg^{-1} as KH_2PO_4. There were five Cd-level treatments corresponding to each plant species: control (no Cd addition) and treatments T_1–T_4, at the following concentrations: 10, 25, 50 and 100 μg g^{-1} air-dried soil, and each treatment was triplicated. Cd was applied as $CdCl_2 \cdot 2.5H_2O$ and mixed thoroughly with the soil samples, and equilibrated for 14 days before the pot culture. Table 1 lists the mean Cd contents in the control and contaminated soils (T_1–T_4) before planting as measured using soil samples collected from each pot.

Seeds of *S. nigrum* were collected from a non-contaminated field in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences. Seeds of *S. nigrum* and *S. melongena* were sown into the pots with a diameter of 9.0 cm and a depth of 12.0 cm, each filled with 1.0 kg of soil samples. The pots

Table 1
Total Cd and 1.0 M HCl extractable Cd content in the control (CK) and Cd treatments (T_1–T_4) before planting

Treatment	*S. nigrum* (μg g^{-1} DW)		*S. melongena* (μg g^{-1} DW)	
	Total Cd	Extractable Cd	Total Cd	Extractable Cd
CK	0.3 ± 0.04	0.2 ± 0.05	0.2 ± 0.04	0.1 ± 0.08
T_1	10.7 ± 0.47	4.5 ± 0.25	10.4 ± 0.15	4.6 ± 0.21
T_2	27.1 ± 1.22	10.1 ± 0.90	25.5 ± 2.7	10.4 ± 0.15
T_3	50.7 ± 0.87	25.2 ± 1.62	50.2 ± 4.89	25.5 ± 2.70
T_4	104.0 ± 4.23	38.5 ± 0.47	102.3 ± 13.26	37.7 ± 1.87

Mean values ± standard deviations are given.

were kept in a controlled growth chamber (temperature: 30 °C in day and 20 °C at night; light: 12 h in day and 12 h at night, and 60–70% of the relative humidity).

Five weeks after germination, samples of both plants were collected and analyzed, respectively.

2.2. Plant growth measurements

Plants were harvested by cutting the shoots at the soil surface and the roots were carefully separated from the soils. The shoots and roots were rinsed with distilled water, wiped with tissue paper, and weighed. Divided leaves, stems and roots were dried at 105 °C for 30 min, then at 70 °C for determination of dry weight.

2.3. Determination of Cd content

Before the Cd analysis, dried plant and soil samples were ground using a ball mill. The plant and soil powders were digested with concentrated $HNO_3/HClO_4$ (87:13, v/v) solution for determining the total Cd concentration. Extractable Cd in soils was determined by weighing 1.0 g of soil into 50 mL polyethylene vials, to which 25 mL of 1.0 M HCl was added. The soil suspension was shaken for 8.0 h at 25 °C, and then centrifuged at 3000 rpm for 5 min. The supernatant was filtered at 0.45 μm Millipore filter. The Cd concentration was determined using a Hitachi atomic absorption spectrophotometer (AAS) (Wei et al., 2004).

2.4. Assays of enzyme activity and lipid peroxidation

The 0.5 g fresh weight of leaves and roots was homogenized in 50 mM cold Na-phosphate buffer (pH 7.8), 0.1 mM EDTA and 1% (w/v) PVP using a prechilled mortar and pestle in an ice bath. The supernatant from the centrifuging at 13,000 rpm for 30 min at 4 °C was used for further analyses. The activity of SOD was measured as described by Somashekaraiah et al. (1992). The activity of POD and CAT was determined using guaiacol and H_2O_2 substrates, respectively, as described previously (Wu and von Tiedemann, 2002; Chance and Maehly, 1955). The level of lipid peroxidation was determined in terms of 2-thiobarbituric acid (TBA) reactive metabolite, chiefly malondialdehyde (MDA). MDA was measured as described by Liu et al. (2004) and expressed as nmol g^{-1} fresh weight.

2.5. Determination of chlorophyll, free proline and PCs

The content of chlorophyll was determined in 80% acetone extract of 0.1 g leaf (Hegedüs et al., 2001) and expressed as mg g^{-1} fresh weight. For the analysis of free proline, 0.5 g fresh weight of leaves and roots was homogenized with 5 mL of 3% sulfosalisylic acid, and the homogenate was cooled after heating for 10 min at 100 °C. After centrifugation at 3000 rpm for 10 min, the content of free proline in the supernatant was measured using ninhydrin reagent at 520 nm (Zhang et al., 1990) and expressed as μg g^{-1} fresh weight. Phytochelatin analysis was carried out as described by Keltjens and Van Beusichem (1998) and expressed as nmol PC-SH g^{-1} fresh weight.

2.6. Statistical analysis

All measurements were carried out in triplicate. Two-way analysis of variance (ANOVA) was performed on all data sets.

3. Results

3.1. Plant growth and Cd bioaccumulation

There was a slight decrease in dry leaf, stem and root biomass of *S. nigrum* with increasing Cd concentration in the soils (Table 2), however, growth of *S. nigrum* was basically unaffected under the condition of treatments T_1 and T_2 compared with the control. On the contrary, increased Cd in the soils reduced dry leaf, stem and root biomass of *S. nigrum* by 33–55%, 32–49% and 30–52% in the treatments T_3 and T_4, respectively. Compared with *S. nigrum*, growth of Cd-treated *S. melongena* was severely inhibited (Table 2). Dry leaf and stem biomass of *S. melongena* was affected with significant ($P < 0.05$) decreases observed in all the treatments. Under the highest soil Cd treatment (T_4), dry leaf and stem biomass of *S. melongena* was inhibited by 83% and 73%, respectively. Compared with the control, dry root biomass decreased 32% in the treatment T_1, and 37–67% in the other treatments (T_2, T_3 and T_4).

The Cd accumulation in *S. nigrum* and *S. melongena* is shown in Fig. 1. According to Fig. 1, the accumulation of Cd in leaves and roots of the two species was proportional with the increasing concentration of Cd in the soils. In all the treatments, the concentration of Cd in leaves of *S. nigrum* was always higher than that in roots, with the ratio of leaf Cd/root Cd concentrations varying between 1.2 and 2.3, and furthermore, the Cd concen-

Table 2
Dry biomass of *S. nigrum* and *S. melongena* grown in different Cd treatments

Treatment	Dry biomass of *S. nigrum* (g plant^{-1})			Dry biomass of *S. melongena* (g plant^{-1})		
	Root	Leaf	Stem	Root	Leaf	Stem
CK	0.095 ± 0.012	0.23 ± 0.011	0.17 ± 0.006	0.057 ± 0.006	0.18 ± 0.016	0.134 ± 0.012
T_1	0.092 ± 0.018	0.21 ± 0.011	0.15 ± 0.009	0.039 ± 0.006	0.10 ± 0.011	0.078 ± 0.007
T_2	0.077 ± 0.011	0.19 ± 0.017	0.14 ± 0.015	0.036 ± 0.004	0.07 ± 0.004	0.063 ± 0.012
T_3	0.063 ± 0.006	0.16 ± 0.018	0.12 ± 0.005	0.023 ± 0.003	0.04 ± 0.003	0.044 ± 0.002
T_4	0.043 ± 0.006	0.11 ± 0.014	0.09 ± 0.005	0.019 ± 0.003	0.03 ± 0.002	0.036 ± 0.003

Mean values ± standard deviations are given.

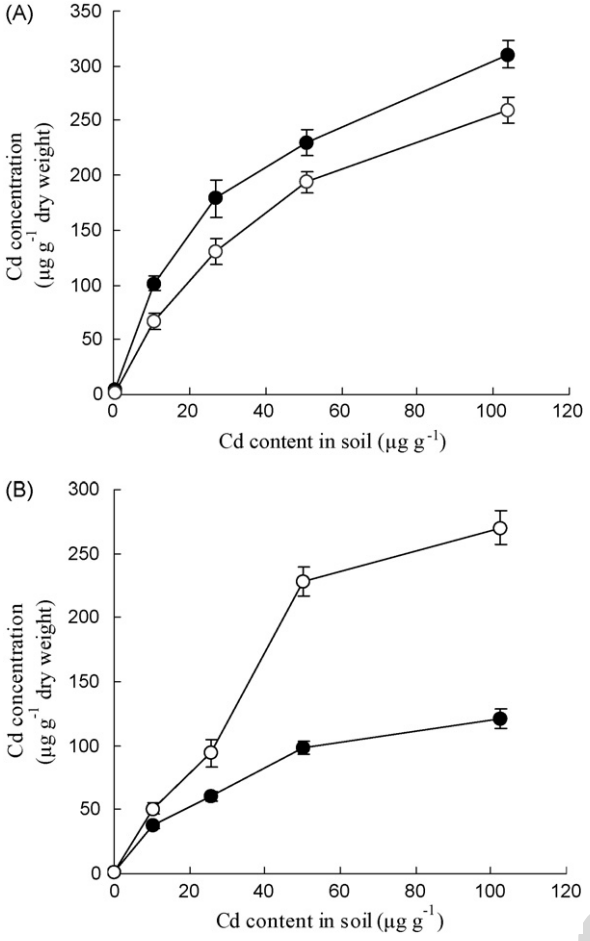

Fig. 1. Cd accumulation in leaves (●) and roots (○) of *S. nigrum* (A) and *S. melongena* (B) grown in different Cd treatments.

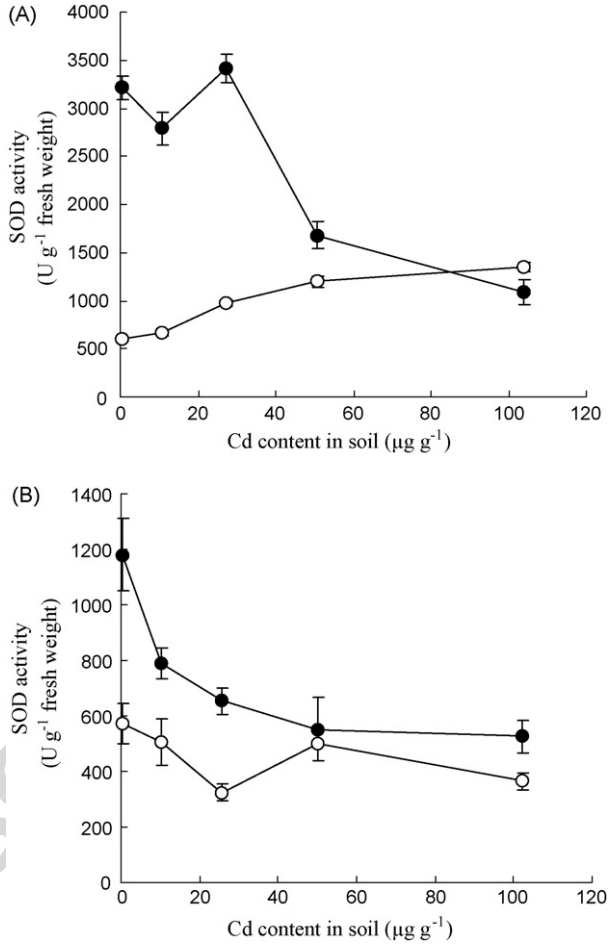

Fig. 2. SOD activity in leaves (●) and roots (○) of *S. nigrum* (A) and *S. melongena* (B) subjected to different Cd concentrations.

trations in leaves of Cd-treated *S. nigrum* greatly exceeded the threshold value of 100 μg Cd g^{-1} DW in leaves of a plant, which is used to define a Cd-hyperaccumulator (Baker et al., 1994). On the contrary, *S. melongena* had higher Cd concentrations in the roots than those in the leaves, which is unexpected for a hyperaccumulating species. The concentration of Cd in leaves of *S. nigrum* was two to three folds as much as that of *S. melongena*. The maximum concentration of Cd extracted from the soil by the *S. nigrum* leaves was 310 μg Cd g^{-1}, but only 121 μg Cd g^{-1} was extracted by the *S. melongena* leaves.

3.2. Antioxidative responses and oxidative stress

Treated with the different Cd concentrations, the endogenous SOD activity in *S. nigrum* leaves was about 2.0–5.2 folds higher than in *S. melongena* leaves. The difference in the SOD activity between the two species was statistically significant under all the Cd treatments ($P < 0.05$). In general, the activity of SOD in *S. nigrum* leaves was decreased with an increase of Cd concentration except for treatment T_2, however, there were no significant differences between the control and treatment T_2 ($P > 0.05$). Similar to *S. nigrum*, Cd had significant effects on the inhibition of the SOD activity in *S. melongena* leaves. As shown in Fig. 2, the SOD activity was much lower in the roots than in the leaves of both *S. nigrum* and *S. melongena*. Moreover, *S. nigrum* had higher SOD activity in the roots than *S. melongena*. Unlike in leaves, the activity of SOD in *S. nigrum* roots was greatly increased with the increasing concentration of Cd in the soils. As far as *S. melongena* was concerned, the SOD activity in Cd-treated roots was 13–43% of the SOD activity in the control.

The endogenous POD activity in leaves of *S. nigrum* (Fig. 3A) was roughly 1.2–2.8 folds as much as that in *S. melongena* (Fig. 3B). The difference in the POD activity between the two species was statistically significant under all the Cd treatments ($P < 0.05$). Concretely speaking, the increased POD activity was observed in the leaves of both *S. nigrum* and *S. melongena*. There was a great difference between the activity of POD in leaves of *S. nigrum* treated with the increasing Cd concentration and that with the control. The POD activity in leaves of *S. melongena* treated with T_1–T_3 was increased and reached the highest level. On the contrary, the activity of POD in leaves of *S. melongena* treated with T_4 was decreased, but about 1.7 folds higher than the control. It is well shown that the POD activity was much higher in the roots than in the leaves of both *S. nigrum* and *S. melongena*. The Cd treatments T_2 and T_3 had a negligible effect on the POD activity in *S. nigrum* roots compared with the control. In contrast, the activity of POD in roots of *S. nigrum* increased by about 126% and 129% under the condition of treatments T_1

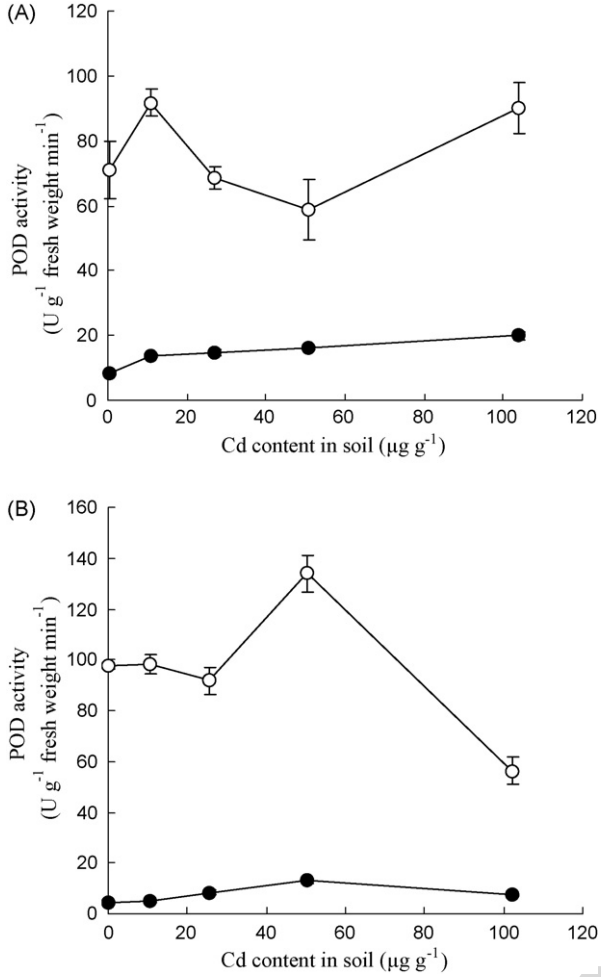

Fig. 3. Effect of Cd on the POD activity in leaves (●) and roots (◯) of *S. nigrum* (A) and *S. melongena* (B).

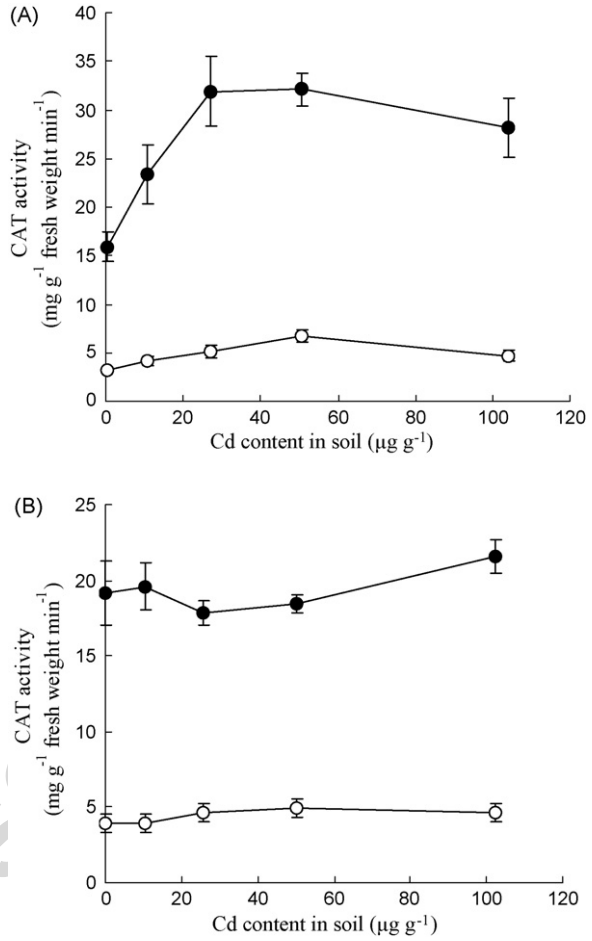

Fig. 4. CAT activity in leaves (●) and roots (◯) of *S. nigrum* (A) and *S. melongena* (B) subjected to different Cd concentrations.

and T_4, respectively. In *S. melongena*, there was no essentially alteration in the POD activity in roots under the condition of treatments T_1 and T_2 relative to the control ($P > 0.05$), but the POD activity in roots of *S. melongena* was enhanced when it was grown at 50 μg Cd g^{-1} air-dried soil (T_3) and it has to be emphasized that the elevated POD activity was strongly inhibited with the highest Cd concentration (T_4).

Changes in the activity of CAT in *S. nigrum* and *S. melongena* were shown in Fig. 4. Although the CAT activity in *S. melongena* leaves was slightly higher than that in *S. nigrum* under the control, there was no significant alteration at any Cd concentration applied ($P > 0.05$). In contrast, the CAT activity in leaves of *S. nigrum* was significantly enhanced by Cd treatments at an average of 147–202% compared with the control. The CAT activity in roots of *S. nigrum* had a change, which was similar to that in leaves, but was lower than in leaves. In the same way, the CAT activity in roots of *S. melongena* was also lower than that in leaves. On the whole, the application of Cd increased the CAT activity in roots of *S. melongena*. However, the effects were not significant under all Cd treatments ($P > 0.05$).

Lipid peroxidation in *S. nigrum* and *S. melongena* was assessed by measuring the concentration of MDA (Fig. 5). The enhanced MDA level in leaves of the two species was detected with the increasing concentration of Cd in the soils, whereas there were no significant ($P > 0.05$) differences in the MDA level in leaves of *S. nigrum* treated with low Cd (T_1 and T_2) and the control. Furthermore, the lowest Cd treatment (T_1) did not significantly increase the MDA level in leaves of *S. melongena*. In general, the MDA level in leaves of *S. melongena* was about 2.3–2.6 folds as much as that in leaves of *S. nigrum* under all the Cd treatments. Under the control, the MDA level in roots of *S. nigrum* was approximately 1.8 folds as much as that in roots of *S. melongena*. The application of Cd increased the MDA level in roots of both species, although the difference of MDA in roots of *S. nigrum* was not significant between treatment T_1 and the control. The greater change was observed in *S. melongena* roots.

3.3. Changes in chlorophyll and accumulation of free proline and PCs

As a characteristic symptom, chlorophyll content in the leaves could be decreased (Fig. 6) with the increased Cd accumulation in leaves. In *S. nigrum*, the content of chlorophyll was decreased by Cd treatments. However, the effect was not significant under the condition of treatments T_1 and T_2 ($P > 0.05$). On the contrary, the content of chlorophyll in *S. melongena* was proportionally reduced with the increasing Cd concentration in the

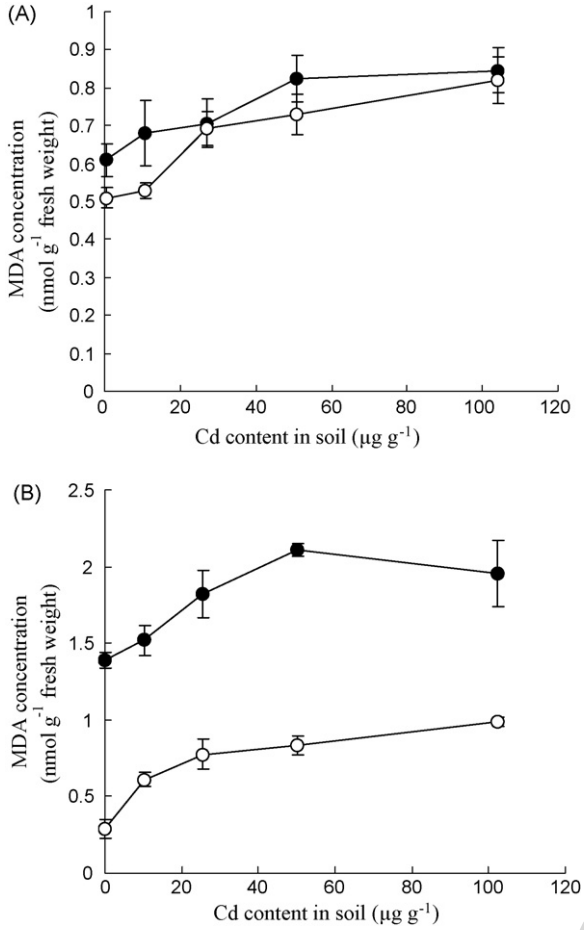

Fig. 5. MDA concentrations in leaves (●) and roots (○) of *S. nigrum* (A) and *S. melongena* (B) grown in different Cd treatments.

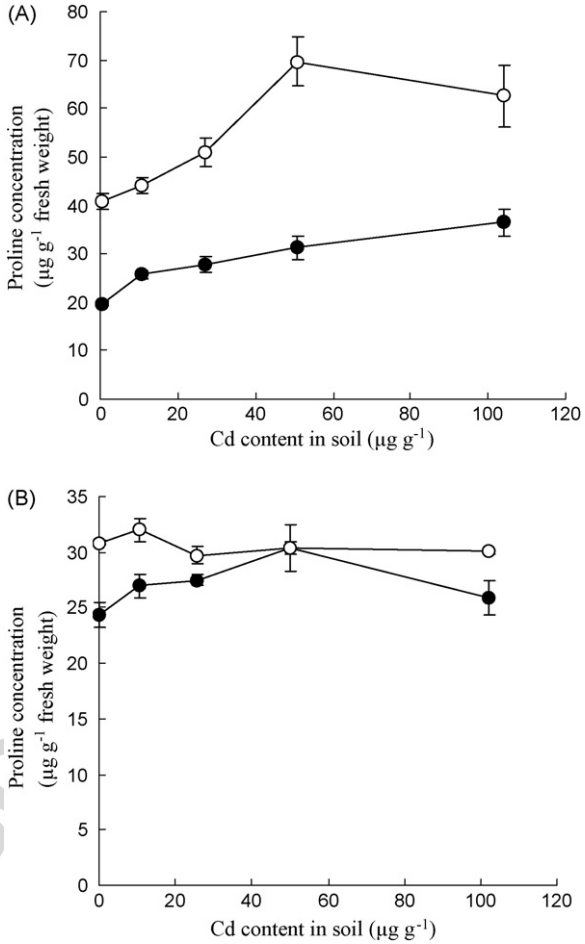

Fig. 7. The content of proline in leaves (●) and roots (○) of *S. nigrum* (A) and *S. melongena* (B) grown in different Cd treatments.

soils. The difference in chlorophyll content between species was statistically significant under all the Cd treatments ($P < 0.05$).

The alteration in the content of free proline in *S. nigrum* and *S. melongena* was depicted in Fig. 7. The concentration of free proline in *S. nigrum* leaves was slightly lower than that in *S. melongena* under the control, whereas Cd treatments significantly increased free proline level in the leaves of *S. nigrum* at the rate of 131–184% under the control. In *S. melongena* leaves, free proline level was also enhanced at the rate of only 125% under the control and reached the highest level when the plants were treated with 50 μg Cd g^{-1} dry soil (T_3), and declined to the level under the control when the plants were treated with the highest Cd (T_4). The concentration of free proline in the roots of *S. nigrum* was roughly 1.3–2.3 folds as much as that of *S. melongena*. The difference between the two species was statistically significant under all the Cd treatments ($P < 0.05$). The application of Cd increased free proline level in the roots of *S. nigrum*. In contrast with *S. nigrum*, there was no significant alteration in free proline in the roots of *S. melongena* ($P > 0.05$).

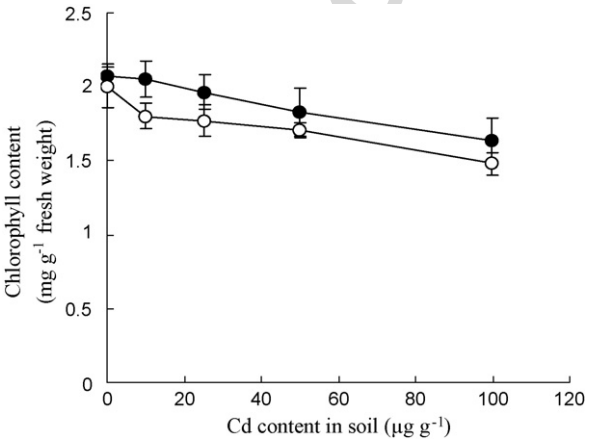

Fig. 6. Changes in the chlorophyll content of *S. nigrum* (●) and *S. melongena* (○) leaves grown in different Cd treatments.

The enhanced concentration of PCs in roots of the two species was detected with the increasing concentration of Cd added to the soils, whereas there were no significant ($P > 0.05$) differences in the roots of *S. melongena* treated with low Cd (T_1 and T_2) and the control (Fig. 8). In the presence of Cd, more PCs accumulated in *S. melongena* roots (about 1.2–2.0 folds), despite the higher Cd accumulation in roots of *S. nigrum*. The level of PCs in leaves of *S. nigrum* linearly enhanced with the increasing Cd concentration in the soils and reached the maximum value of 1208.0 nmol SH g^{-1} FW, about 2.0 folds as much as that in the control. In *S. melongena* leaves, the content of PCs increased up to 718 nmol SH g^{-1} FW at the rate of only 26%

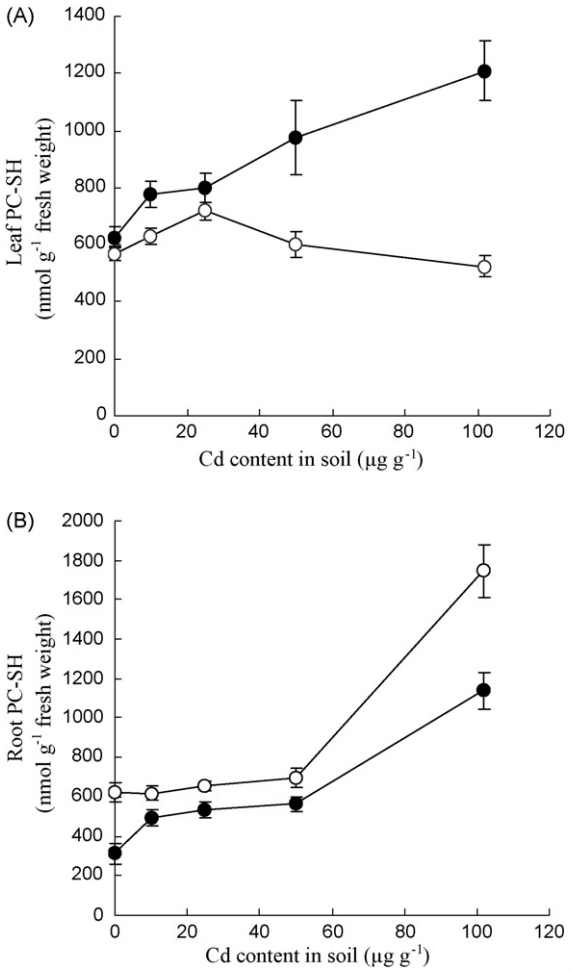

Fig. 8. Effects of Cd on the concentration of PCs in leaves (A) and roots (B) of *S. nigrum* (●) and *S. melongena* (◯).

under the control when the plants were treated with 25 μg Cd g^{-1} (T_2), and declined obviously at higher soil Cd concentrations (T_3 and T_4). In other words, leaf PCs synthesis by *S. nigrum* was greater than that by *S. melongena*, which is consistent with higher Cd accumulation in the leaves of *S. nigrum* than that of *S. melongena*.

4. Discussion

SOD is one of the stress-resistant enzymes and can catalyze the disproportionation of two $O_2^{\bullet -}$ radicals to H_2O_2 and O_2. H_2O_2 is also toxic to plant cells, which can be removed by CAT. Therefore, the combination of SOD and CAT plays an important role in the resistance of a plant to environmental stress. In our present study, the SOD activity in roots of *S. nigrum* was enhanced with the increasing Cd in the soils. Simultaneously, the increased CAT activity could be observed in the roots of *S. nigrum* with the increasing Cd concentration. This result is consistent with the recent reports by Srivastava et al. (2005) and Kertulis et al. (in press), who concluded that antioxidant enzymes SOD and CAT were significantly induced upon exposure of the plants to arsenic in the nutrient medium in arsenic hyperaccumulator *Pteris vittata*. Conversely, the Cd treatments inhibited the SOD activity in roots of *S. melongena* and had a negligible effect on the activity of CAT. These results indicated that the roots of *S. nigrum* had the greater capacity than the roots of *S. melongena* to adapt oxidative stress caused by Cd, as confirmed by the greater increase of MDA level in the roots of *S. melongena* compared with that in the roots of *S. nigrum* with the increasing Cd concentration in the soils. Because the roots are the principal entry of Cd into a plant, and AOS in the form of H_2O_2 is a common response to Cd in a non-hyperaccumulator species (Stroiński and Zielezińska, 1997; Shützendübel et al., 2001), the cooperation of SOD and CAT activities in the roots of Cd-hyperaccumulators represents an effective defense strategy.

In addition, it has to be emphasized that the Cd treatments inhibited SOD activity in the leaves of *S. nigrum*, except for Cd treatment T_2, in contrast to POD and CAT. It was suggested that Cd-induced reduction of SOD could be responsible for an inactivation of the enzyme by H_2O_2 produced in different compartments, where SOD catalyses the disproportionation of superoxide radicals (Vitoria et al., 2001). These results also suggested that the enhanced POD and CAT activities may promote the removal of H_2O_2 when SOD in the leaves of *S. nigrum* is inactivated, which fits well to the earlier work by Lee and Shin (2003).

Amongst various enzymes involved in the removal of AOS, POD can be considered as one of the key ones, since both of its extra- and intracellular forms are participating in the breakdown of H_2O_2 (Foyer et al., 1994). The POD activity was much higher in the roots than in the leaves of both the hyperaccumulator and the non-hyperaccumulator, which coincides with the earlier study on barley roots by Cd treatments (Hegedüs et al., 2001). It was suggested that the elevated POD activity could be the consequence of either the ionic microenvironment or the tissue specific gene expression in the roots (Blinda et al., 1996). In our present work, the POD activity in *S. nigrum* leaves was gradually increased with the increasing soil Cd concentration, whereas the POD activity in *S. nigrum* roots was increased with Cd treatments T_1 and T_4. In contrast with the results of POD, the Cd treatments decreased the SOD activity in *S. nigrum* leaves and enhanced the SOD activity in its roots. These results indicate that the leaves and roots of *S. nigrum* operate with different mechanisms in respect to the AOS elimination.

Proline can play an important protective role against heavy metal stress. It has been demonstrated that free proline could chelate with Cd ion in plants and form a nontoxic Cd-proline complex (Sharma et al., 1998). In our work, with the increasing Cd concentration in the soils, the enhanced concentrations of free proline were observed in both the leaves and the roots of *S. nigrum*. In contrast, the application of Cd did not significantly change the level of free proline in the roots of *S. melongena*. The concentration of free proline was increased in the leaves of *S. melongena* with Cd treatments T_1–T_3, and there was the greater increase in free proline in the leaves of *S. nigrum* than that of *S. melongena*. Thus, it could be suggested that free proline might play an important protective role against Cd stress and *S. nigrum* had the stronger self-protection ability than *S. melongena*.

The oxidative stress induced by non-redox heavy metals can be demonstrated by MDA formation. There were no significant

differences in MDA levels in the leaves and roots of *S. nigrum* between Cd treatment T_1 and the control, indicating that the AOS metabolism in *S. nigrum* was enhanced by protection reactions of oxidative stress at low Cd. Correspondingly, the low Cd treatment (T_1) did not cause significant changes in chlorophyll content and the growth of *S. nigrum*. These results suggested that the antioxidative defense in *S. nigrum* might play an important role in Cd tolerance. In addition, Cd treatment T_2 did not significantly decrease the dry biomass of *S. nigrum* roots, but the enhanced MDA level was observed in *S. nigrum* roots. The result showed that Cd-induced oxidative stress occurs in the hyperaccumulator tissues even though the growth is unaffected by Cd stress, which fits well to the earlier study on the Cd-hyperaccumulator, *T. caerulescens* (Boominathan and Doran, 2003).

There are many indications that the production of the high level of metal–phytochelatin complexes is involved in metal tolerance in plants (Zenk, 1996; Citterio et al., 2003; Gupta et al., 1998). In the present study, leaf PCs synthesis in the two *Solanum* species was both significantly increased when soil Cd was 25 $\mu g\, g^{-1}$ or less, which had no effects on the leaf growth of *S. nigrum*, but severely inhibited that of *S. melongena*. In *S. nigrum*, leaf PCs production was promoted with the increasing level of Cd, demonstrating increased PCs accumulation in *S. nigrum* leaves was not due to higher phytotoxicity but increasing uptake of Cd ions into the cytoplasm probably. On the contrary, PCs synthesis in *S. melongena* leaves was decreased at 50 and 100 $\mu g\, Cd\, g^{-1}$, which caused more severe phytotoxicity in *S. melongena*, indicating the activity of leaf PCs synthase had reached saturation. It is thus clear that PCs accumulation is not the primary mechanism by which Cd tolerance is achieved in *S. nigrum*. Even as described by Yen et al. (1999), it is the formation of stable complexes that is important for metal tolerance rather than the ability to synthesize PCs.

5. Conclusion

In contrast with *S. melongena*, *S. nigrum* is equipped with superior antioxidative defense to adapt to the oxidative stress during exposure to Cd, which was associated with significantly higher SOD activity and POD activity in the leaves, and further increases in POD activity of the leaves and SOD activity of the roots with Cd treatments. Moreover the Cd application significantly increased CAT activity in both the leaves and the roots of *S. nigrum*. Treated with low Cd (T_1), the growth of *S. nigrum* and its chlorophyll content and the level of MDA were basically unaffected. These results showed that the antioxidative defense in the Cd-hyperaccumulator might play an important role in Cd tolerance. In addition, the application of Cd significantly increased the level of free proline in both the leaves and the roots of *S. nigrum*. On the contrary, the Cd treatments had a little effect on free proline accumulation in *S. melongena*. It can be concluded that free proline accumulation plays an important role in Cd tolerance in *S. nigrum*. Similarly to proline, Cd-induced PCs accumulation in *S. nigrum*, but Cd tolerance does not seem to be due to PCs production.

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