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REGULAR ARTICLE

Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress

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Abstract The present study aims to elucidate the role of antioxidative enzyme in the adaptive responses of metal-accumulators (*Thlaspi caerulescens* and *Brassica juncea*) and non-accumulator plant (*Nicotiana tabacum*) to Cadmium stress. When seedlings of plants were grown in hydroponic condition for a period of 4 days in the presence of 200 or 400 μM $CdCl_2$, photosynthetic rate, transpiration rate and stomatal conductance in metal-accumulators decreased more slowly than that in tobacco. MDA content and electrolyte leakage increased with elevated Cd concentration and exposure time in all plant species, while the oxidative damage in tobacco was more serious than that in metal-accumulators. The activities of SOD and CAT in metal-accumulators were significantly higher than that in tobacco under normal condition, whereas there was no significant difference in the activity of POD between Indian mustard and tobacco. The activities of antioxidative enzymes increased rapidly in metal-accumulators in response to the Cd treatments, especially SOD and CAT. In tobacco, CAT activity declined rapidly by exposure to the Cd treatment, though the activity of SOD and POD was enhanced, indicating that the antioxidative enzymes in tobacco could not fully scavenge ROS generated by Cd toxicity. These results collectively indicate that the enzymatic antioxidation capacity is one of the important mechanisms responsible for metal tolerance in metal-accumulator plant species.

Keywords Antioxidative enzyme · *Brassica juncea* · Cadmium · Heavy metal tolerance · *Nicotiana tabacum* · *Thlaspi caerulescens*

Abbreviations

Cd	cadmium
ROS	reactive oxygen species
MDA	malondialdehyde
SOD	superoxide dismutase
CAT	catalase
POD	peroxidase
EC	electrical conductivity

Introduction

Cadmium (Cd) is one of the most toxic heavy metals in plants, due to its high solubility in water and phytotoxicity (Clemens 2006). The presence of excessive amount of Cd in soil and water causes a range of plant responses including leaf chlorosis, stunted growth and even death (Baryla et al. 2001;

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Mallick and Mohn 2003). Cd toxicity may decrease stomatal density and conductance to CO_2, thus reducing leaf photosynthesis (Poschenrieder and Barcelo 1999; Baryla et al. 2001). Before the occurrence of leaf chlorosis and death, heavy metal toxicity causes the generation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen and associates changes in antioxidative enzyme activities (Gratão et al. 2005). The excessive ROS reacts with lipids, pigments and proteins, resulting in membrane damage, inhibition of photosynthesis and enzyme inactivation (Scandalios 2005).

In contrast to the essential metals like copper and iron, Cd does not seem to induce production of ROS through Fenton-type reactions or Haber–Weiss reactions (Prasad 1995). However, Cd does cause oxidative stress in plants. For instance, malondialdehyde (MDA) content was increased by Cd in bean (*Phaseolus vulgaris*; Chaoui et al. 1997), sunflower (*Helianthus annuus*; Gallego et al. 1996), and pea (*Pisum sativum*; Sandalio et al. 2001), suggesting oxidative stress caused by Cd might be induced through indirect mechanisms, such as inactivation of enzymes by interacting with functional SH-group of them, disruption of the electron transport chain and interaction with nucleic acids (Van Assche and Clijsters 1990; Smeets et al. 2005). It is known that species with higher level of SH-compounds (such as *Brassica* species) are more likely to tolerate heavy metal toxicity than those non-SH species (Clemens 2006). However, it is unknown whether metal-accumulator species are genetically better equipped to tolerate heavy metal induced oxidative damages, as an important mechanism contributing to the overall heavy metal tolerance in plants.

Major ROS-scavenging enzymes in plant include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and peroxidase (POD; EC 1.11.1.7). SOD is a key enzyme in protecting cells against oxidative stress, which constitutes primary line of defense as to dismutate superoxide radicals to H_2O_2 (Alscher et al. 2002; Fatima and Ahmad 2005). Degradation of H_2O_2 to water and oxygen is carried out by CAT in peroxisomes or by POD in vacuoles, cell wall and cytosol (Mittler 2002). For example, in mung bean (*Phaseolus aureus*) seedlings, Cd induced elevated POD but decreased CAT activities (Shaw 1995). In Cd-treated brahmi (*Bacopa monnieri*), the activities of SOD and POD in leaves were enhanced, whereas the CAT activity decreased significantly (Singh et al. 2006). In pea plant, both the SOD and CAT activities were decreased, while POD did not change significantly, either in activity or accumulation of transcript (Romero-Puertas et al. 2007). However, in metal-accumulator species, such as black nightshade (*Solanum nigrum*) and Indian mustard (*Brassica juncea*), the activities of all three enzymes were significantly enhanced upon Cd exposure (Sun et al. 2007; Mobin and Khan 2007), suggesting the specific differences in antioxidative enzyme responses to Cd toxicity. As a result, it is hypothesized that in addition to metal tolerance mechanisms, such as complexing of metal ions by phytochelatins, compartmentalization in vacuoles, immobilization at the cell, and synthesis of stress proteins, metal-accumulator species may be genetically more capable of metabolizing ROS through up-regulating the important antioxidative enzyme activities than the non-accumulators.

Metal-accumulators are capable of taking up and storing elevated concentrations of Cd, Ni, Zn, Cu, and other heavy metals without suffering metal toxicity or cell damage (Boominathan and Doran 2003). *T. caerulescens* and Indian mustard, which grow on soils with large variation of Cd concentrations, are recognized as Zn/Cd accumulators and used as the model plants to study mechanism of Cd accumulation (Baker et al. 2000; Zhou et al. 2006). In the present work, the antioxidative enzymes of metal-accumulators (*T. caerulescens* and Indian mustard) and the non-accumulator tobacco plant were compared for their responses to Cd treatments over a time course, in order to clarify the difference in antioxidative response between metal-accumulator and tobacco plants.

Materials and methods

Plant cultivation and treatment

Plants of *T. caerulescens*, Indian mustard and wild tobacco (NC89) were used in this study. Seeds of *T. caerulescens* and Indian mustard were kindly provided by Dr. Mark G.M. Aarts (Netherlands) and National Germplasm Resources Lab, USA (IP: 173874) respectively. Seeds were sown under sterile condition in Petri dishes containing MS medium, solidified with 0.8% (*w*/*v*) agar. The cultures were maintained at 60–70% relative humidity and day/night temperatures of 22±3°C under a 16 h photope-

riod with a photosynthetic photon flux density of 165 μmol m^{-2} s^{-1}. After germination, the seedlings were transferred into half strength Hoagland solution for plant propagation under the same culture condition. Solution pH was maintained close to 6.5 by adding diluted HCl or NaOH. The nutrient solution (1 l) in the plastic growth containers was continuously aerated with pumps and renewed every 4 days. Seedlings of 6-week-old Indian mustard and 8-week-old *T. caerulescens* and tobacco were treated with different concentrations of $CdCl_2$ (200 or 400 μM) maintained in half strength Hoagland solution for different time periods of 0, 1, 2, 3 and 4 day, both in triplicate. After harvesting, plants were washed with double distilled water, blotted and the youngest fully developed leaves were taken for assays of various parameters.

Photosynthesis, transpiration and gas exchange

Rate of photosynthesis (P_n), transpiration rate (E) and stomatal conductance (g_s) were recorded on fully expanded leaves of second youngest nodes at 0, 1, 2, 3, 4 days separately after $CdCl_2$ treatment using an intelligent portable photosynthesis system (LCpro+, ADC, UK) between 11:00 and 13:00 at light saturation intensity. These observations were recorded on three plants in a treatment.

Evaluation of oxidative stress markers

Fresh material (about 0.5 g) was homogenized in 0.1% (*w*/*v*) cold trichloroacetic acid. The homogenate was centrifuged at 15,000×*g* for 25 min. The supernatant obtained was used for the determination of hydrogen peroxide and lipid peroxidation levels. The hydrogen peroxide was measured spectrophotometrically after reaction with KI (Alexieva et al. 2001). The reaction mixture consisted 0.5 ml 0.1% trichloroacetic acid leaf extract supernatant, 0.5 ml of 100 mM K-phosphate buffer (pH 6.8) and 2 ml reagent (1 M KI *w*/*v* in fresh double-distilled water H_2O). The blank probe consisted of 0.1% TCA in the absence of leaf extract. The reaction was developed for 1 h in dark and the absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated according to a standard curve with known concentrations of H_2O_2. Lipid peroxidation was measured by the amount of MDA, a product of unsaturated fatty acid peroxidation. MDA concentration was determined by the thiobarbituric acid reaction (Heath and Packer 1968).

Electrolyte leakage

The degree of membrane integrity was assessed by the percent of electrolyte leakage from the upper fully expanded leaves. One leaf per plant from each of the treatments was immersed in 10 ml of Milli 'Q' water and incubated in a water bath at 25°C for 2 h. The suspension medium was measured for the initial electrical conductivity (EC1). The samples were then boiled at 100°C for 15 min to release all the electrolytes, cooled and the final electrical conductivity (EC2) was measured. The percent leakage of electrolytes was calculated using the formula (EC1/EC2)×100%.

Assays of antioxidative enzyme activity

Fresh leaf samples (0.5 g) were homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (*w*/*v*) at 4°C. The homogenate was filtered through four layers of cheese cloth and centrifuged at 15,000×*g* for 20 min at 4°C. Supernatant was used to measure the activities of enzymes.

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971). The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitroblue tetrazolium, 2 μM riboflavin, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The test tubes were shaken and placed 30 cm below light source consisting of 15 W fluorescent lamp for reading the absorbance at 560 nm. The activity of SOD was expressed as unit per milligram protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of nitroblue tetrazolium under light.

Catalase was estimated as the decline in absorbance at 240 nm due to the decline of H_2O_2 extinction. The reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was started by adding H_2O_2 (Cakmak and Horst 1991). One unit of activity is determined by the variety of 0.01 min^{-1} at 240 nm. Enzyme activity was expressed as unit per milligram protein.

The determination of POD activity was based on the method as described by Nickel and Cunningham (1969). Activity was measured by the increase in absorbance at 470 nm due to guaiacol oxidation. The reaction mixture contained 25 mM guaiacol, 10 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was started by adding H_2O_2. One unit of activity is determined by the variety of 0.01 min^{-1} at 240 nm. Enzyme activity was expressed as unit per milligram protein.

Protein determination

In all the enzymatic preparations protein was determined by the method of Bradford (1976) using bovine serum albumin (BSA, Sigma) as standard.

Statistical analysis

The results were based on three replicates from two independent experiments at least. To confirm the variability of data and validity of results, all the data were subjected to the analysis of variance (ANOVA). To determine the significant difference between treatments, least significant difference (LSD) was estimated.

Results

Metal-accumulator species are more tolerant to metal toxicity

When seedlings of plant were grown in hydroponic conditions for a period of 4 days in the presence of 200 or 400 μM $CdCl_2$, *Thlaspi caerulescens* grew well up to 400 μM $CdCl_2$ for one week, while the leaves of Indian mustard showed significant wilting and chlorosis symptoms at the concentration of 400 μM $CdCl_2$ for 3 days (Fig. 1). In contrast to accumulators, the necrosis and wilting in the leaves of tobacco were observed at the concentration of 200 μM $CdCl_2$, the leaves did not recover after transfer back to normal condition (Fig. 1). Furthermore, although the photosynthesis rate of both metal-accumulator and tobacco plants continued to decrease during the exposure to 200 or 400 μM $CdCl_2$ stress, the rate of reduction was lower in metal-accumulators (Fig. 2A and B). Similarly, the transpiration rate and stomatal conductance declined less in metal-accumulators than that in tobacco under Cd stress (Fig. 2C–F).

Cadmium induces oxidative stress

The increased accumulation of lipid peroxides is indicative of enhanced production of toxic oxygen species. As shown in Fig. 3, MDA content was increased continually with enhanced Cd concentrations

Fig. 1 Effect of cadmium on plant growth. Seedlings were grown in half strength Hoagland solution in the absence or presence of 400 μM $CdCl_2$ (*T. caerulescens* or Indian mustard) or 200 μM $CdCl_2$ (tobacco). *Left*, control; *right*, 4 day

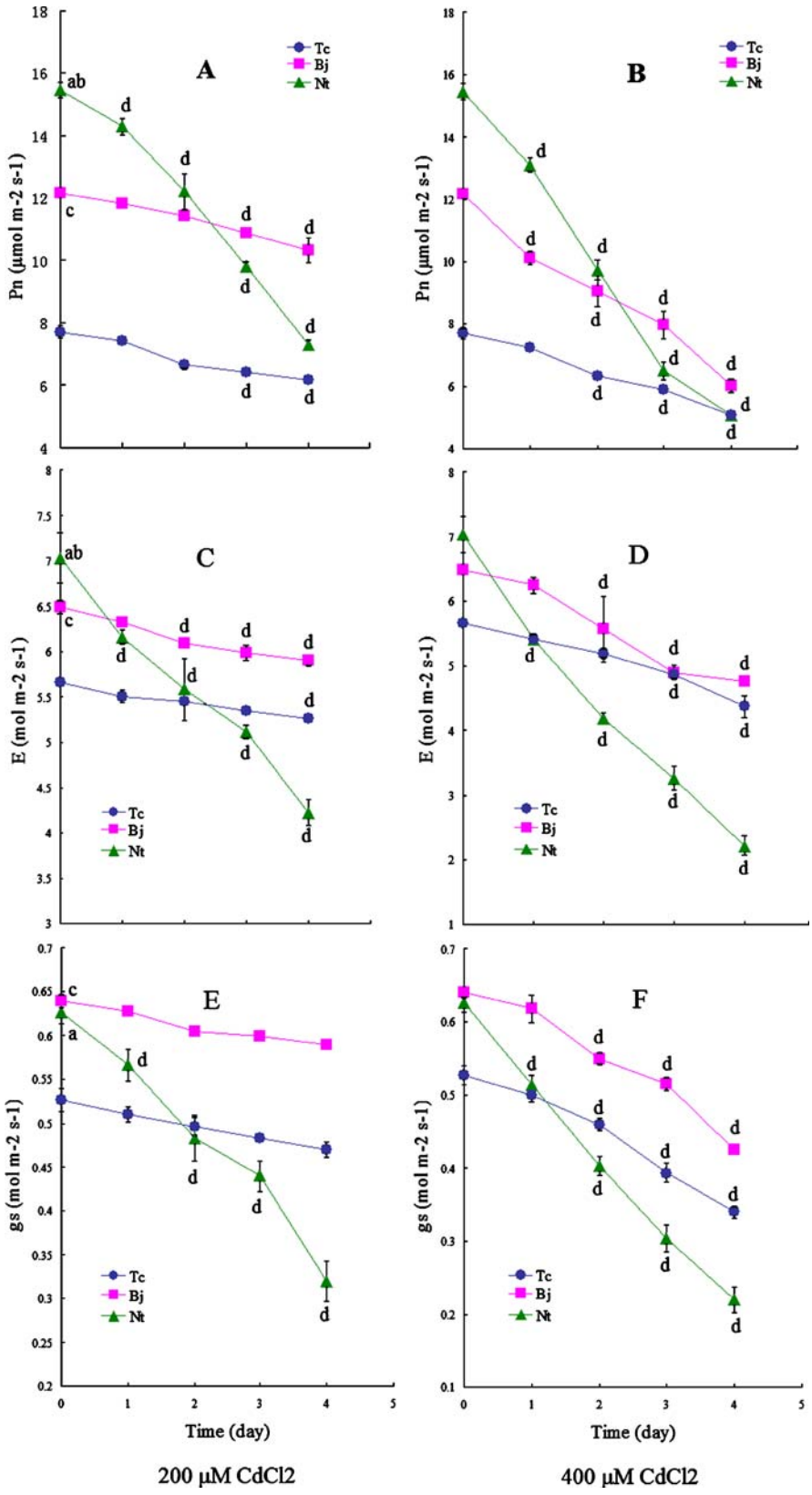
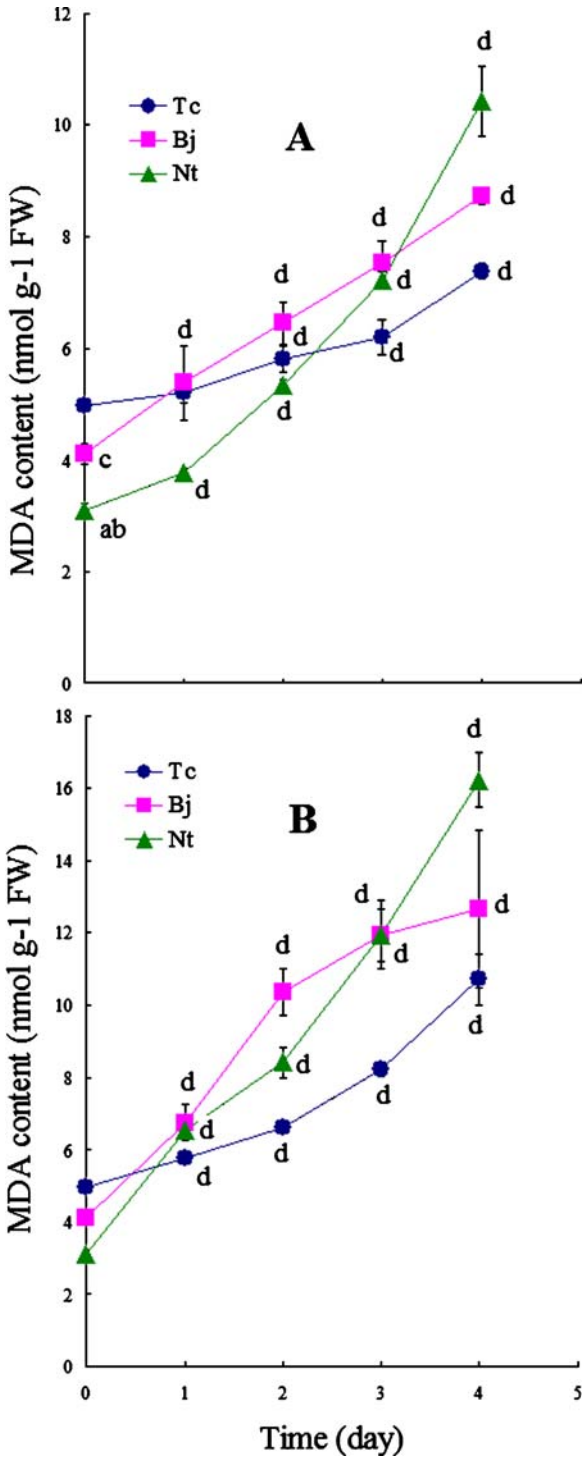


Fig. 2 Effect of cadmium on the photosynthetic rate (P_n) (**A**, **B**), transpiration rate (E) (**C**, **D**) and stomatal conductance (g_s) (**E**, **F**) in metal-accumulators (*T. caerulescens* and Indian mustard) and tobacco plant. Values are means of triplicates±SD and vertical bars represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ (*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; *d* denotes values that differ significantly from the control)

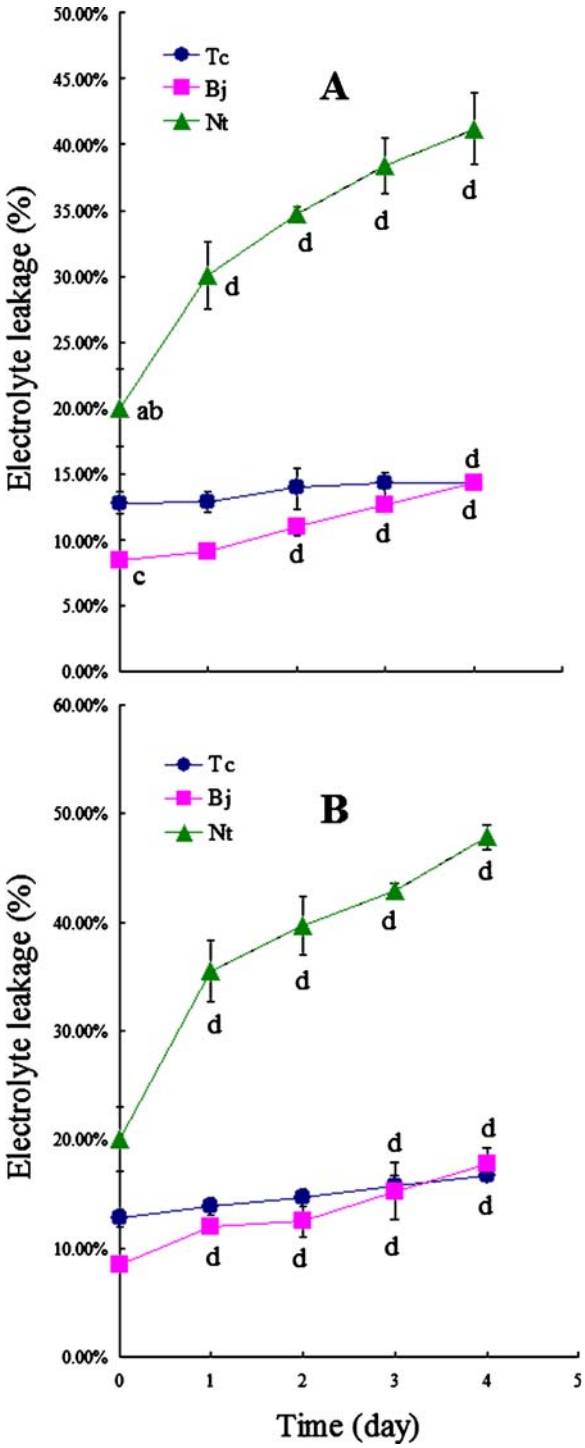


◀ **Fig. 3** MDA content in leaves of three plants treated with different Cd concentrations. **A**, 200 μM $CdCl_2$; **B** 400 μM $CdCl_2$. Values are means of triplicates±SD and *vertical bars* represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; d denotes values that differ significantly from the control]

and exposure periods in leaves of both the accumulator and tobacco plants compared to appreciate control. The treatment of 200 μM $CdCl_2$ led to 48.5% and 112.4% increase in MDA level in *T. caerulescens* and Indian mustard at day 4, respectively. In contrast, with the same $CdCl_2$ concentration, the MDA content in tobacco increased by 237.9% in comparison to the control (Fig. 3A). Similar results were observed in 400 μM $CdCl_2$ treatment (Fig. 3B). In addition, the increased MDA level in Indian mustard was higher than that in *T. caerulescens* at all Cd concentrations.

The electrolyte leakage increased in concentration-time dependent manner in leaves of both metal-accumulators and tobacco plants under Cd stress (Fig. 4). However, the electrolyte leakage in tobacco increased significantly at day 1 after exposure to 200 or 400 μM $CdCl_2$, and then continued to increase at a lower rate, while the electrolyte leakage in the metal-accumulator increased slowly (Fig. 4A and B). For example, the increase was 0.86% in *T. caerulescens*, 8.06% in Indian mustard, and 50.07% in tobacco during the first day at 200 μM $CdCl_2$, compared to the appreciate control, respectively (Fig. 4A). Moreover, the electrolyte leakage in Indian mustard increased faster than that in *T. caerulescens* upon $CdCl_2$ treatments for 4 days (Fig. 4), indicating the membrane damage imposed by Cd in *T. caerulescens* was the lowest, followed by Indian mustard and tobacco.

The accumulation of H_2O_2 in leaves was measured to assess the development of oxidative stress induced by Cd toxicity. The endogenous H_2O_2 level in metal-accumulator species was higher than that in tobacco plants under normal condition, which might be due to the genetically differences among plant species (Fig. 5). However, the level of H_2O_2 in tobacco increased by 68.0% at day 4 after 200 μM $CdCl_2$ addition, while the increases of H_2O_2 in *T. caerulescens* and Indian mustard were 23.4% and 24.9%, respectively, in comparison to the control (Fig. 5). The H_2O_2 content in tobacco was higher than that in metal-accumulators after 4-day $CdCl_2$ exposure. This coincided with the results of lipid peroxidation and membrane injury.



◀ **Fig. 4** Electrolyte leakage in leaves of three plants treated with different Cd concentrations. **A** 200 μM $CdCl_2$; **B** 400 μ*M* $CdCl_2$. Values are means of triplicates±SD and *vertical bars* represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; *d* denotes values that differ significantly from the control]

Activity of antioxidative enzymes

The activity of SOD was about 2-fold higher in accumulators than in tobacco plant under normal condition (Fig. 6). A concentration-time dependent increase in the activity of SOD was observed in both metal-accumulators and tobacco plants upon Cd exposure, and the enzyme activity in metal-accumulators was significantly higher than that in tobacco under 200 or 400 μM $CdCl_2$ treatments (Fig. 6A and B), suggesting metal-accumulators were equipped with a higher level of SOD activity and capacity for scavenging superoxide radical.

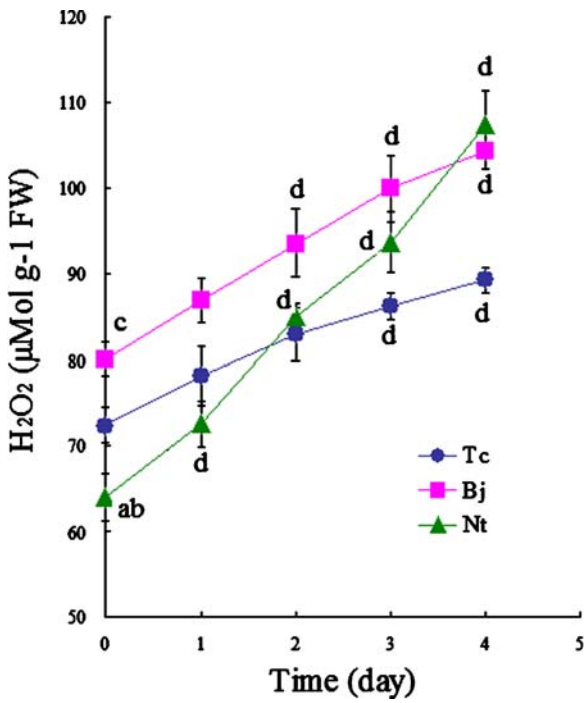
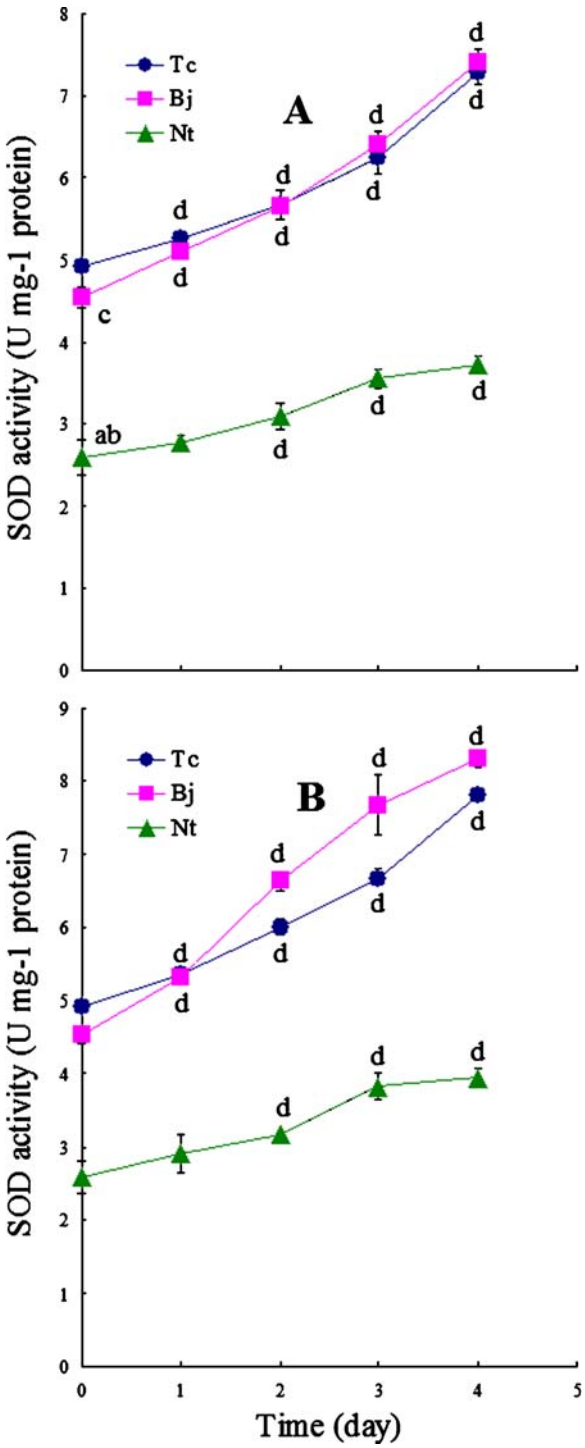


Fig. 5 H_2O_2 content in leaves of three plants treated with 200 μM $CdCl_2$. Values are means of triplicates±SD and vertical bars represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (Nt) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; *d* denotes values that differ significantly from the control]



◀ **Fig. 6** SOD activity in leaves of three plants treated with different Cd concentrations. **A** 200 μM $CdCl_2$; **B** 400 μM $CdCl_2$. Values are means of triplicates±SD and *vertical bars* represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; d denotes values that differ significantly from the control]

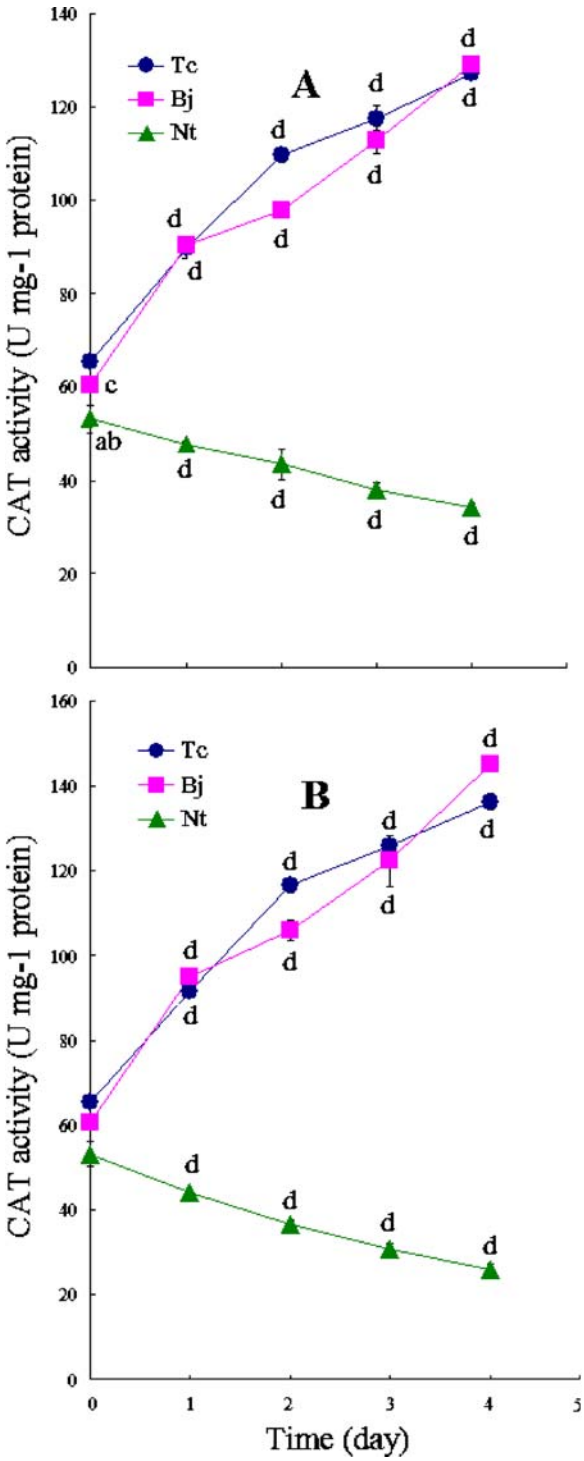
The activities of CAT in metal-accumulator and tobacco plants were shown in Fig. 7. The activity of CAT in metal-accumulators was higher than that in tobacco plant. In all $CdCl_2$ treatments, the CAT activity of both *T. caerulescens* and Indian mustard was enhanced dramatically, by contrast, a significant reduction in CAT activity was observed in tobacco (Fig. 7).

There was no significant difference in the activity of POD between tobacco and Indian mustard under normal condition, and this enzyme activity was increased in leaves of three examined plants at all Cd concentrations and exposure periods (Fig. 8). However, the POD activity in tobacco increased more rapidly, and was higher than that in metal-accumulators on day 4 after the treatment, indicating the increase of POD activity in tobacco induced by Cd was the highest, followed by Indian mustard and *T. caerulescens*.

Discussion

The reduction in photosynthesis and the occurrence of oxidative stress were induced by cadmium

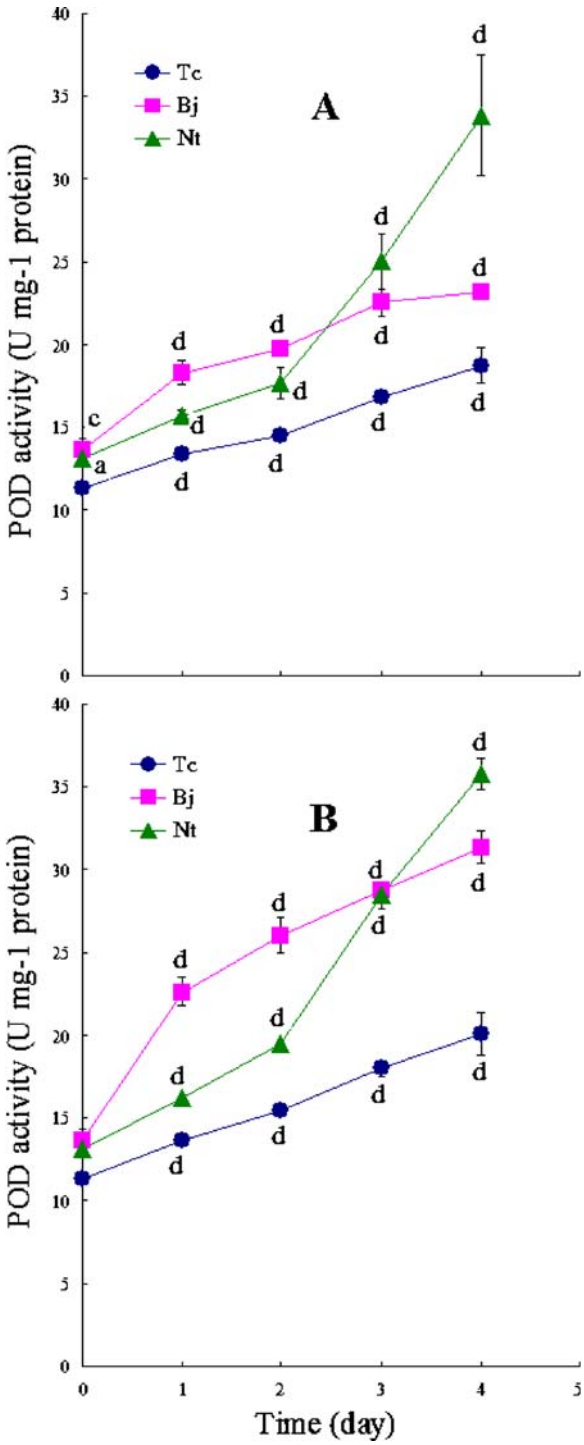
Cd toxicity in plants depends on the concentration of active Cd^{2+} species and the exposure time, and genetically determined tolerance of the plant species concerned. *T. caerulescens* is Cd hyperaccumulator, Indian mustard a Cd accumulator, while tobacco is non-accumulator. The metal-accumulator species were more tolerant to Cd toxicity than the non-accumulator species. Our results showed that the concentration of 200 μM $CdCl_2$ adversely affected the growth of tobacco, more than that of Indian mustard. In contrast, the growth of *T. caerulescens* was only affected at the concentration of 400 μM $CdCl_2$, indicating the metal-accumulator species were more tolerant of Cd stress than the non-accumulator. The decrease in the net photosynthetic rate was negatively related to the increase in Cd concentration and exposure time, which may have contributed to the growth reduction by Cd toxicity. Comparatively, the decreases of P_n in metal-accumulators were less than



◀**Fig. 7** CAT activity in leaves of three plants treated with different Cd concentrations. **A** 200 μM $CdCl_2$; **B** 400 μM $CdCl_2$. Values are means of triplicates±SD and *vertical bars* represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; *d* denotes values that differ significantly from the control]

that of tobacco at the same Cd concentration. This is because metal-accumulators may have a higher level of light-saturated electron transport capacity through PSII and thus, less oxidative photoinhibition damages may have occurred in leaf cells, compared to non-accumulator plant, in response to heavy metal toxicity (Papazoglou et al. 2005).

The plant cell membranes are considered as primary sites of heavy metal injury, and the membrane destabilization is frequently attributed to lipid peroxidation (Singh et al. 2006). Increased production of MDA by Cd exposure has been observed in pea (Chaoui et al. 1997; Metwally et al. 2005), rice (Shah et al. 2001) and sunflower seedlings (Gallego et al. 1996). Similarly, the increase in electrolyte leakage upon Cd exposure has been reported in brahmi (Mishra et al. 2006) and Indian mustard (Mobin and Khan 2007). In this study, there was a significant increase in the level of both MDA content and electrolyte leakage upon Cd stress. Meanwhile, the electrolyte leakage was positively correlated with MDA content and the increasing Cd concentration throughout the treatment period. These results indicated that the Cd treatments produced oxidative damage in both metal-accumulator and tobacco plants, whereas the magnitude of Cd-induced oxidative damages was much higher in tobacco than that in the leaves of Indian mustard and *T. caerulescens* as reflected by MDA levels. These observations agree with the results from H_2O_2 evaluation, that the accumulation of H_2O_2 in tobacco was higher than metal-accumulators. H_2O_2 itself is a powerful inhibitor of metabolism including carbon fixation (Kaiser 1976), and the oxidation-reduction of metal ions by H_2O_2 and O_2^- through the Haber–Weiss reaction produces the most toxic hydroxyl radical (Imlay and Linn 1988). The increased lipid peroxidation observed here was probably due to the harmful effect of excessive levels of H_2O_2 or its ROS derivatives in the cellular compartments (Bowler et al. 1992). Excessive levels of ROS may have resulted in damage to cell organelles including the photosynthetic apparatus, ultimately leading to severe cellular damage and



◀**Fig. 8** POD activity in leaves of three plants treated with different Cd concentrations. **A** 200 μM $CdCl_2$; **B** 400 μM $CdCl_2$. Values are means of triplicates±SD and *vertical bars* represent standard deviations. *Values carrying different letters* are significantly different at $P \leq 0.05$ [*a* denotes significant difference between tobacco (*Nt*) and *T. caerulescens* (*Tc*) under normal condition; *b* denotes significant difference between tobacco and Indian mustard (*Bj*) under normal condition; *c* denotes significant difference between Indian mustard and *T. caerulescens* under normal condition; d denotes values that differ significantly from the control]

chlorosis of the leaves. These results support that the metal accumulator species had a higher antioxidation capacity through up-regulating the antioxidative enzymes in leaf cells.

Antioxidation was one of the metal-tolerance mechanisms in metal-accumulator species

The enzyme SOD is an essential component of plant antioxidation system as it dismutates superoxide radicals to H_2O_2 and O_2 in the cytosol, mitochondria and chloroplast (Salin 1987). Cadmium treatment decreased the activity of SOD in non-accumulator plants, such as bean (Somashekaraiah et al. 1992), sunflower (Gallego et al. 1996) and pea plants (Sandalio et al. 2001), while it increased the enzyme activity in Cd-accumulators, such as candargy (*Alyssum lesbiacum*) (Schickler and Caspi 1999), Indian mustard (Mobin and Khan 2007) and black nightshade (Sun et al. 2007). The role of SOD enzyme in co-tolerance was further supported by using transgenic plants overexpressing *SOD* in response to external oxidative damages caused by heavy-metal toxicity (Lee et al. 2007). Our results showed the increases in SOD activities in all plants under 200 or 400 μM $CdCl_2$ treatments compared to the appreciate control, moreover, the SOD activities in metal-accumulators were significantly higher than that in tobacco under both normal condition and Cd stress, indicating a high level of SOD activity might protect metal-accumulator plants from oxidative damage induced by Cd toxicity. In addition, there was more pronounced increase in the SOD activity in Indian mustard with the increase in Cd concentrations and exposure time in comparison to *T. caerulescens*. This is possibly due to that the higher level of superoxide radical generation resulting in higher cellular damage in Indian mustard as confirmed by the greater increase of MDA level in the leaves of Indian mustard compared with that in *T. caerulescens*. The enhanced cellular damage seems to reflect deterioration on the equilibrium between generation of ROS and defense mechanisms towards removal of ROS in

Indian mustard. Thus, the maintenance of the overall defense system of *T. caerulescens* seems to be better than that of Indian mustard.

CAT is one of the key enzymes in the scavenging of H_2O_2 to water and molecular oxygen via two electron transfer. An increase in the activity of CAT upon Cd exposure was reported in metal-accumulators, such as candargy (Schickler and Caspi 1999), Indian mustard (Mobin and Khan 2007) and black nightshade (Sun et al. 2007). However, an overall decline in CAT activity was also associated with Cd toxicity in bean (Chaoui et al. 1997), mung bean (Shaw 1995), sunflower (Gallego et al. 1996), Scot pine (*Pinus silvestris*; Schutzendubel et al. 2001), and pepper (*Capsicum annuun*; León et al. 2002). The decline in CAT activity is regarded as a general response to many stresses and it is supposedly due to inhibition of enzyme synthesis or a change in assembly of enzyme subunits (MacRae and Ferguson 1985). Our results revealed a high level of and a large increase of the CAT activity in metal-accumulators with the increasing Cd concentrations and exposure time, whereas its activity was significantly inhibited in tobacco.

The maintenance of a high CAT activity in metal-accumulators under Cd stress represents an important feature of metal-accumulator tolerant of Cd toxicity relative to tobacco. A decrease of enzymic free radical scavengers caused by heavy metals may contribute to a shift in the balance of free-radical metabolism towards H_2O_2 accumulation (De Vos and Schat 1991). In parallel to the reduced CAT activity, the H_2O_2 content in tobacco was increased rapidly in response to the Cd treatments, compared to metal-accumulators. In addition, the increase in CAT activity is considered as an indirect evidence of an enhanced oxidative damage (Smirnoff 1995). The CAT activity in Indian mustard was enhanced nearly 1.2 times more than that in *T. caerulescens* on day 4 at 400 μM $CdCl_2$, indicating Indian mustard experienced a greater oxidative damage at highly toxic Cd level than *T. caerulescens*.

The activity of POD in both metal-accumulator and tobacco plants was enhanced with the increasing Cd concentrations and exposure time. The induction of POD activity under environmental stress conditions has also been reported in various plant species (Wada et al. 1998; Lagriffoul et al. 1998; Radotic et al. 2000; Piquery et al. 2000; Singh et al. 2006), suggesting POD played an important protective role against various stress in plants. In addition, it is worthy to notice that the CAT activity decreased in Cd-treated tobacco plants as we mentioned above, but due to the sharp rise of POD activity in connection with the accumulation of H_2O_2, the decreased CAT activity might be compensated by the increased POD activity for detoxification of H_2O_2 toxicity in tobacco. Similar patterns of POD and CAT activity have been observed under NaCl salinity (Mittal and Dubey 1991), toxicity caused by O_2 (Foster and Hess 1980) and Fe ion (Hendry and Brocklebank 1985). Therefore, a higher level of POD activity in tobacco upon Cd exposure could partially resist to the Cd-induced oxidative damage.

In conclusion, compared to tobacco plant, metal-accumulators are equipped with superior antioxidative defense to adapt to the oxidative stress induced by Cd toxicity. Both MDA content and electrolyte leakage in Indian mustard increased more rapidly than that in *T. caerulescens* at all $CdCl_2$ treatments, accordingly, the activities of all antioxidative enzymes in Indian mustard increased faster than that in *T. caerulescens*, suggesting the oxidative stress induced by Cd in Indian mustard was severer than that in *T. caerulescens*, and the coordinated increase of the activities of antioxidative enzymes was effective in protecting the plant from the accumulation of ROS under Cd stress.

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