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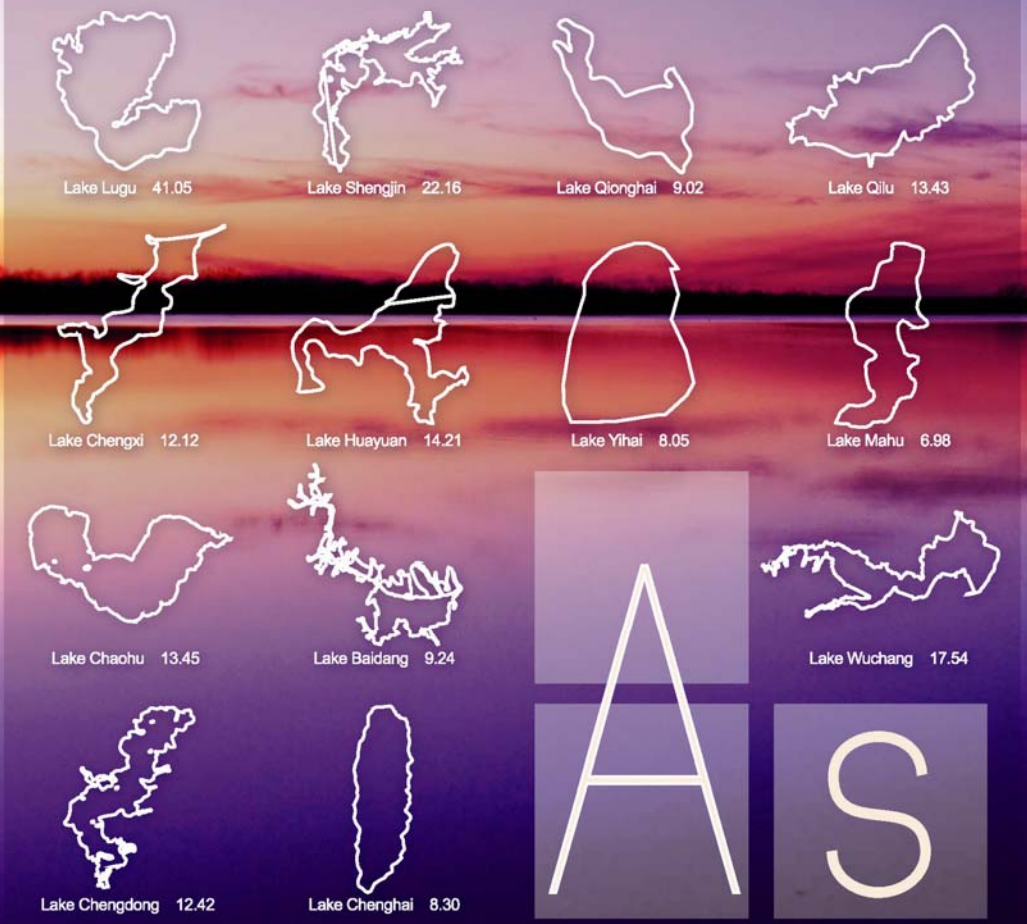

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Cadmium accumulation and tolerance of two castor cultivars in relation to antioxidant systems

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ABSTRACT

To investigate the effects of Cd on tolerance and antioxidant activities of castor, two different castor (*Ricinus communis* L.) cultivars (Zibo No. 5 and Zibo No. 8) were used for a hydroponic experiment (0, 1 and 2 mg/L Cd) and a pot experiment using Cd-contaminated soil (34 mg/kg) with the addition of ethylenedinitrilotetraacetic acid (EDTA). The results indicated that there were significant differences between the two cultivars with respect to Cd uptake in shoots (113–248 mg/kg for Zibo No. 5 and 130–288 mg/kg Zibo No. 8), biomass tolerance indexes (64.9%–74.6% for Zibo No. 5 and 80.1%–90.9% for Zibo No. 8) in the hydroponic experiment and survival rates (0% for Zibo No. 5 and 100% for Zibo No. 8) determined by the addition of EDTA in the pot experiment, suggesting that Zibo No. 8 has higher tolerance than Zibo No. 5. Moreover, the castor cultivars have low bioconcentration factors (4.80% for Zibo No. 5 and 5.43% for Zibo No. 8) and low translocation factors (<1%). Consequently, Zibo No. 8 can participate in Cd phytostabilization in highly Cd-polluted areas. The results indicated that glutathione (GSH) as a non-enzymatic antioxidant, and antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX), were cultivar- and dose-dependent. The higher tolerance of Zibo No. 8 compared with Zibo No. 5 can be attributed to the higher GSH levels in the root and higher GPX activity in the leaf.

Introduction

Cadmium (Cd) pollution has caused worldwide concern because of its high toxicity to plants, animals and humans. Furthermore, it is one of the most ubiquitous pollutants in soil (Huang et al., 2011). When Cd-contaminated land is used for crop planting, Cd is easily transferred from the soil to human body *via* the food chain, endangering human health (Jarup, 2003). In China, at least 13,330 ha farmland is contaminated by Cd, according to a recent soil survey from 11 provinces (Biao and Nan, 2000). Therefore, many Chinese people are confronted with potentially serious

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health risks due to Cd pollution. Hence, it is of utmost importance to remediate Cd-contaminated soil.

Phytoremediation can be applied for the reduction of heavy metals from polluted soil using green plants (Shi and Cai, 2009). It is generally accepted that phytoremediation technology is cheap, convenient, and not harmful to the environment (McGrath and Zhao, 2003). However, the key point of phytoremediation is to search for the most efficient plant to treat, for example, Cd-polluted soil. Castor (*Ricinus communis* L.) is a C_3 plant of the Euphorbiaceae family from tropical Africa. It develops large biomass and a strong root system, and can be planted in a wide range of geographical environments in China. Some evidence has been provided that castor could phytoremediate soil polluted by Cd (Lu and He, 2008; Shi and Cai, 2009; Huang et al., 2011). This plant is characterized by a high tolerance to Cd concentrations exceeding 200 mg/kg (Shi and Cai, 2009), revealing a higher remediation efficiency compared to Indian mustard (*Brassica juncea* L.), which is considered to be a potential phytoremediator (Bauddh and Singh, 2012a,b). In addition, castor is an important oil crop for industry, but not edible for humans or animals (Olivares et al., 2013), and grown on marginal lands that are usually unsuitable for food crops (Berman et al., 2011). In addition, castor is a perennial plant, which can constantly remove Cd from contaminated soil (Bauddh and Singh, 2012a). It is also an excellent rotation and companion crop (Olivares et al., 2013), which is able to phytoremediate Cd-polluted soil in cooperation with other plants such as Indian mustard (Bauddh and Singh, 2012a). Consequently, castor can be cultivated for phytoremediation and for bioenergy production, which simultaneously addresses two critical global problems — increasing energy demands and remediation of Cd-polluted soil. Thus, it is a highly valuable renewable resource.

Cadmium, a non-essential toxic heavy metal, leads to alterations of the morphology and physiology of plants, caused by oxygen free-radical-mediated oxidative stress and peroxidation of membrane lipids (Tappel, 1973; Foyer et al., 1994; Chaoui et al., 1997; Szollosi et al., 2009). For plants' self-protection, plant cells induce the activity of oxygen radical detoxifying enzymes, such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX), and non-enzymatic antioxidants such as glutathione (GSH), in order to resist to oxidative stress caused by toxic metal concentrations (Vanassche and Clijsters, 1990; Chaoui et al., 1997). Antioxidants play an important role in the defensive mechanism of plants against Cd, as found in the citrus rootstock of citrumelo (Podazza et al., 2012), safflower (Shi et al., 2010) or Indian mustard (Liu et al., 2011). Glutathione is also active in many plants, for instance in garden cress (Gill et al., 2012) and wheat (Sun et al., 2005). However, reports about the Cd tolerance mechanisms of differently tolerant castor cultivars are few.

Therefore, the goals of the present study were (1) to compare the Cd tolerance, uptake and accumulation in two different castor cultivars; (2) to investigate the tolerance mechanisms of the castor cultivars exposed to Cd stress with the focus on antioxidant enzymes (GPX, CAT, and SOD) and a non-enzymatic antioxidant (GSH); and (3) to verify whether the castor cultivars can grow in a highly Cd-polluted soil (34 mg/kg) for phytoremediation.

1. Materials and methods

1.1. Selection and preparation of plant cultivars

Seeds of castor Zibo No. 5 and Zibo No. 8 cultivars, with high cadmium-phytoextraction ability and adaptability to the conditions in many parts of China, were obtained from Zibo Academy of Agricultural Sciences, Zibo City, Shandong Province, China. Castor seeds were initially grown on artificial non-polluted soil for 2 to 3 weeks until the seedlings developed two healthy tender leaves. These uniform seedlings were used for hydroponic experiments and pot experiments in the greenhouse located at the Center for Environmental Remediation, Institute of Geographical Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, China.

1.2. Hydroponic experiments

1.2.1. Plant culture

The uniform seedlings of the castor plants were transplanted to 1 L pots containing 400 mL of half-strength Hoagland's solution with the following composition: 2.5 mmol/L $Ca(NO_3)_2$, 2.5 μmol/L KNO_3, 0.5 mmol/L KH_2PO_4, 0.5 mmol/L $MgSO_4$, 25 μmol/L H_3BO_3, 2.25 μmol/L $MnCl_2$, 1.9 μmol/L $ZnSO_4$, 0.15 μmol/L $CuSO_4$, 0.05 mmol/L $(NH_4)_6Mo_7O_{24}$ and 5 μmol/L Fe-EDTA. $CdCl_2{\cdot}2.5H_2O$ (guaranteed reagent) was used for providing Cd pollution. The Cd salt was added to the hydroponic culture. The pH of the nutrient solution was maintained at (6.0 ± 0.1) by the addition of 0.1 mol/L NaOH. Eight replicates were each treated with 0, 1 or 2 mg/L Cd: three replicates for the determination of biomass and Cd concentration, and the others for analysis of physiological indexes. Plants were kept in a greenhouse at temperatures of 25/15 °C during the day/night and a 16 hr photoperiod of about 300 mE/(m^2·sec) intensity, as well as 60% average relative humidity. The nutrient culture was replaced every 3 days, and the seedlings of castor grew for 3 weeks in the nutrient culture.

1.2.2. Growth parameters

At the end of the experiments, the plants for determination of biomass and Cd concentration were harvested and washed by distilled water, and then divided into two parts: root and shoot. All plant parts were dried in an oven at 70 °C for 48 hr to constant weight. The dry weights were measured by electronic balance.

Roots and shoots were ground in a mill, digested in flasks on an electric heating plate at 60 °C and treated with concentrated HNO_3 (guaranteed reagent). The temperature was then increased to 110 °C and kept stable until the sample solution became clear (Alexander et al., 2006). The sample volume was adjusted to 25 mL with ultrapure water. The Cd concentration of the sample was measured by flame atomic absorption spectroscopy (ContraAA 700, Analytikjena, Germany). A reference material GBW07603 (GSV-2) was used to monitor the Cd recovery of the plant samples (recovery: 90% ± 10%).

1.2.3. Measurement of antioxidant enzymes

After washing with distilled water, the plants were divided into three parts for analysis of physiological indexes: root, stem and leaf. These parts were stored in liquid nitrogen to maintain the activity of their enzymes. The leaves and roots were used for the analysis of antioxidants. Fresh samples were homogenized with the extracting solutions and ground with a chilled mortar and pestle, and then centrifuged at 10,000 ×*g* for 25 min at 4 °C. The supernatants were stored at 4 °C and used for the analysis of antioxidants.

Glutathione was extracted using a 5 mmol/L EDTA-TCA solution and analyzed according to the method developed by Eyer and Podhradsky (1986).

Enzyme extraction was carried out according to the method described by Knörzer et al. (1996). Superoxide dismutase activity was based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Giannopolitis and Ries, 1977). SOD activity was defined as the amount of protein inhibiting 50% of the initial reduction of NBT under light. One SOD activity unit was expressed as U/mg fresh weight (fw). Catalase activity was analyzed by the consumption of H_2O_2 at 240 nm (Aebi, 1984). Reduction of 0.01 units at A_{240} per min was considered as one unit of enzyme activity (U), and CAT activity was expressed as U/mg fw. Guaiacol peroxidase was determined by H_2O_2-induced guaiacol oxidation at 470 nm (Chance and Maehly, 1955). An increase of 1 unit at A_{470} per min was considered as one unit of enzyme activity (U), and GPX activity was expressed as U/mg fw.

GSH and antioxidant enzymes were analyzed using an ultraviolet–visible spectrophotometer (UV-5100B, Metash, China) at the Center for Environmental Remediation, Institute of Geographical Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, China.

1.3. Pot experiment

1.3.1. Collection and preparation of Cd contaminated soil

The heavy-metal-polluted soil was sampled from topsoil (0–20 cm) near a metal smelting factory in Gejiu County, Yunnan Province, China. The soil was air-dried and sifted through a 1 cm sieve. The basic concentrations of the soil were as follows: total N: 1.40 g/kg, total P: 0.760 g/kg, available P: 12.1 mg/kg, organic matter: 6.65 g/kg, cation exchange capacity: 116 mmol/kg, Cd: 34 mg/kg and pH: 7.36.

1.3.2. Ethylenedinitrilotetraacetic acid (EDTA) treatments

During the pot experiments each pot was filled with 0.5 kg soil. Each of the uniform plants was transplanted to one single pot. Eight pots were used for each cultivar. To four pots, EDTA solution (6 mmol/kg) was added after 40 days, and the other four pots were not treated by EDTA and represented the control group (CK). After adding the EDTA solution, the plants in the four pots with the EDTA-treated Zibo No. 5 withered and died (at day 69), while Zibo No. 8 grew normally. After 69 days, the rest of the plants were harvested, washed with tap water to clean the root surfaces from soil particles, and finally rinsed three times with deionized water. The determination method for Cd concentrations in roots and shoots was based on the description above.

The extracting solution for determining available Cd consisted of 0.1 mol/L $Ca(NO_3)_2$ and was applied according to the following procedure: 8 g soil was treated with 20 mL of 0.1 mol/L $Ca(NO_3)_2$, mixed in a shaker for 2 hr at 25 °C, centrifuged at 5000 ×*g* for 15 min, and then filtered through quantitative paper (Conder et al., 2001). The concentration of available Cd was determined by inductively coupled plasma optical emission spectrometry (Optima 5300 DV, PerkinElmer, America) at the Physical and Chemical Analysis Center, Institute of Geographical Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, China.

1.4. Tolerance index, bioconcentration factor and translocation factor

Tolerance index (TI) was defined as the ratio of the castor biomass after Cd treatments to that of the control group. Cadmium bioconcentration factor (BCF) was defined as the ratio of Cd in shoot or root of the plant to that in the nutrient solution or soil. Cadmium translocation factor (TF) was described as the ratio of Cd in the shoot to that in the root. The indexes are defined as follows:

$$\mathrm{TI} = \frac{W_{\mathrm{Cd}}}{W_{\mathrm{control}}} \quad (1)$$

where, W_{Cd} (g) and $W_{control}$ (g) represent the biomass after Cd treatment and the biomass of the control group, respectively.

$$\mathrm{BCF} = \frac{C_{\mathrm{tissue}}}{C_{\mathrm{medium}}} \quad (2)$$

where, C_{tissue} (mg/kg) and C_{medium} (mg/L or mg/kg) represent the Cd concentration in the shoot or root and the Cd concentration in the nutrient solution or soil, respectively.

$$\mathrm{TF} = \frac{C_{\mathrm{shoot}}}{C_{\mathrm{root}}} \quad (3)$$

where, C_{shoot} (mg/kg) and C_{root} (mg/kg) represent the Cd concentration in the shoot and the Cd concentration in the root, respectively.

1.5. Statistical analysis

Data were analyzed using the two-way analysis of variance test or independent samples *T*-test in SPSS 18.0 software. All measurements were conducted with triplicates and data presented are mean values, which were compared by Duncan's test with $p < 0.05$ considered as a significant difference.

2. Results

2.1. Growth of the two castor cultivars

The Cd hydroponic experiment showed that increasing Cd concentrations led to decreasing biomass of the two castor cultivars (Table 1). Compared with the control group, the decrease of root biomass of Zibo No. 5 was 24.2% and 40.3% after the addition of 1 and 2 mg/L Cd solution, respectively; the decreased values of root biomass of Zibo No. 8 were 20.3% and 25.4%. Similarly, the shoot biomass decreased by 26.3% and 34.2% for Zibo No. 5, and by 8.57% and 20.0% for Zibo No. 8. The tolerance index of Zibo No. 8 was higher compared to Zibo No. 5 (Table 1). According to the two-way analysis of variance test analysis, the root biomass and shoot biomass of the two cultivars were significantly influenced by Cd doses ($p < 0.01$). In the pot experiment, with the EDTA addition, the root biomass and shoot biomass of Zibo No. 5 decreased by 54.3% and 28.7%, while those of Zibo No. 8 increased by 14.4% and 22.4% (Table 2).

2.2. Cd uptake of the two castor cultivars

Under hydroponic conditions, Cd uptake by roots and shoots of the two castor cultivars increased with increasing Cd doses (Table 1). For the treatments with 1 and 2 mg/L Cd solution, the Cd concentrations in the shoots increased from 113 to 248 mg/kg for Zibo No. 5 and from 130 to 288 mg/kg for Zibo

Table 1 – Effects of Cd stress on biomass, Cd concentration, BCF, TF, and TI of two castor cultivars in hydroponic experiment.

Cd treatment (mg/L)	Biomass (g/plant)		Cd concentration (mg/kg dry weight)		BCF		TF (%)	TI (%)
	Root	Shoot	Root	Shoot	Root	Shoot		
Zibo No. 5								
0	0.062a	0.38a						
1	0.047ab	0.28b	2307b	113b	2307a	113b	4.9b	74.6
2	0.037b	0.25b	2538a	248a	1269b	124a	9.8a	64.9
Zibo No. 8								
0	0.059a	0.35a						
1	0.047b	0.32a	1899a	130b	1899a	131a	6.9b	90.9
2	0.044b	0.28a	2090a	288a	1045b	144a	13.8a	80.1
Analysis of variance								
Cd	14.8^{**}	9.3^{**}	17.8^{**}	534.9^{***}	1102.6^{***}	8.3^{*}	828.9^{***}	
Cultivar	0.1	0.7	73.6^{***}	20.7^{**}	122.9^{**}	19.4^{**}	214.0^{***}	
Cd × cultivar	0.8	1.5	0.2	3.2	10.4^{*}	0.1	25.1^{**}	

BCF: bioconcentration factor; TF: translocation factor; TI: tolerance index.
Mean values ($n = 3$) with different letters in the same column for each cultivar are significantly different according to the Duncan's test or independent samples *T*-test ($p < 0.05$).
The effect of Cd dose and cultivar was analyzed by the two-way analysis of variance test, and significant effects were detected in biomass, Cd concentration, BCF and TF; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

No. 8. Accordingly, the BCF increased from 113 to 124 for Zibo No. 5 and from 131 to 144 for Zibo No. 8. Two-way analysis of variance results indicate that Cd uptake in the shoots was significantly impacted by the cultivar ($p < 0.01$) and Cd concentrations ($p < 0.001$). As seen in Table 1, the TF ranged from 4.9% to 13.8%. A significant effect of Cd ($p < 0.001$) and cultivar ($p < 0.001$), as well as Cd × cultivar interaction ($p < 0.01$), was detected in the TF.

In the pot experiment the Cd concentrations in the roots and shoots of Zibo No. 8 were 1.2 and 1.1 times higher, respectively, than those of Zibo No. 5, when compared with the control group (CK) (Table 2). Under EDTA treatment, there was no significant difference in the Cd concentration in the roots of Zibo No. 8, while the Cd concentration in the shoots showed a significant increase ($p < 0.05$) compared to the control group. However, the Cd concentrations of Zibo No. 5 under EDTA treatment are not indicated (Table 2), because of the death of Zibo No. 5 during the experiment.

2.3. Effect of Cd on antioxidant systems of the two castor cultivars

Under hydroponic conditions, Cd treatment changed the GSH level and antioxidant enzyme activities for both castor cultivars (Fig. 1). Under Cd stress, the GSH, SOD and POD activities in the root were higher than those in the leaf. The glutathione level in the root of Zibo No. 5 was lower than that in the root of Zibo No. 8, and showed a significant increase in the root of Zibo No. 8, when treated by the high Cd solution (2 mg/L) (Fig. 1a). As seen in Fig. 1a, the variation of GSH activity for the leaves of both cultivars increased at the 1 mg/L Cd solution and decreased at the 2 mg/L Cd solution. Moreover, the GSH concentration for Zibo No. 8 changed significantly compared with the control group. Two-way analysis of variance results indicate that the GSH activity in the roots and leaves was significantly affected by Cd dose and cultivar, and a Cd × cultivar interaction ($p < 0.001$) was observed in the roots.

Table 2 – Effects of EDTA on biomass, Cd concentration, BCF, and TF of two castor cultivars and available Cd in pot experiment.

	Biomass (g/plant)		Cd concentration (mg/kg dry weight)		BCF		TF (%)	Available Cd
	Root	Shoot	Root	Shoot	Root	Shoot		(mg/kg)
Zibo No. 5								
CK	0.0823a	0.407a	176	1.63	5.20	0.0480	0.924	0.0256b
EDTA	0.0376b	0.290a	NA	NA	NA	NA	NA	3.43a
Zibo No. 8								
CK	0.0871a	0.379a	205a	1.84b	6.04a	0.0543b	0.898b	0.0311b
EDTA	0.0996a	0.464a	216a	8.84a	6.36a	0.261a	4.10a	3.54a

Mean values ($n = 3$) with different letters in the same column for each cultivar are significantly different according to the Duncan's test or independent samples *T*-test ($p < 0.05$).
NA means not available because of the death of Zibo No. 5.

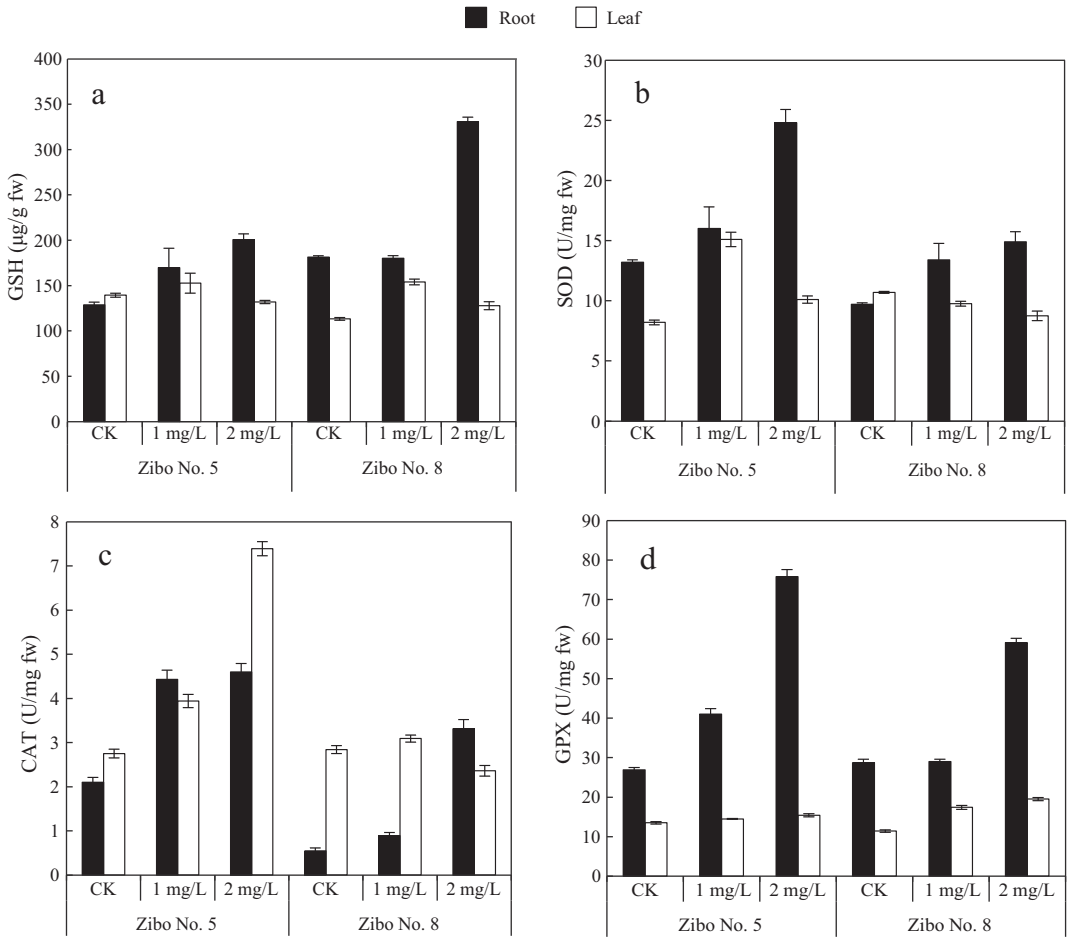

Fig. 1 – Glutathione (GSH, a), superoxide dismutase (SOD, b), catalase (CAT, c) and guaiacol peroxidase (GPX, d) responses of castor cultivars after exposing to 1 and 2 mg/L Cd for 3 weeks. Data are means ± SE of three individual triplicates.

As seen in Fig. 1b and c, the SOD and CAT activities for roots and leaves of Zibo No. 5 were higher after the Cd treatments compared with Zibo No. 8. SOD and CAT activities were inhibited for Zibo No. 8 leaves, while other enzyme activities increased, compared to the control group. GPX activities increased in both cultivars with increasing Cd concentration. Under Cd treatment, Zibo No. 5 showed high GPX activity in the roots compared with Zibo No. 8, whereas Zibo No. 8 displayed high GPX activity in the leaves (Fig. 1d). Two-way analysis of variance results indicate that SOD, CAT and GPX activities in the roots and leaves were significantly affected by Cd dose and cultivar, and a Cd × cultivar interaction ($p < 0.001$) was observed in the roots.

3. Discussion

A tolerance index based on biomass exposed to heavy metals was used to evaluate the heavy metal toxicity in the plants (Shi and Cai, 2009). Zibo No. 8 revealed higher TI than Zibo No. 5 under hydroponic conditions (Table 1), showing the higher Cd tolerance of Zibo No. 8 compared with Zibo No. 5. Baker (1981) suggested two basic strategies of metal tolerance in plants: (1) the "exclusion" strategy, in which the concentrations of heavy metals in the shoots are maintained at a constant low level when grown in heavy-metal-contaminated soils; (2) the "accumulator" strategy, in which metals are actively concentrated within plant tissues, implying that internal metal detoxification mechanisms exist. The results indicate higher BCF of shoots and higher TF of Zibo No. 8 compared with Zibo No. 5 after treatment with the Cd solutions, suggesting the existence of an internal Cd detoxification mechanism in Zibo No. 8. The BCFs of castor cultivars under soil condition were lower than those under hydroponic conditions, considering that Cd in soil occurs in complicated forms because of its association with many physicochemical environments, that in turn impact Cd availability (Li et al., 1995).

Previous studies reported that the BCF values of the aboveground organs of castor were less than 1 (Shi and Cai, 2009; Huang et al., 2011; Varun et al., 2012). Previous reports indicated that on a per-plant basis, castor removed more Cd from soil than Indian mustard, which has been suggested as a potential phytoremediator (Nouairi et al., 2009; Bauddh and Singh, 2012b). Even though the Cd concentration was higher in shoots of Indian mustard than in those of castor, the greater biomass of castor resulted in a higher total amount of Cd accumulated by castor compared with Indian mustard (Bauddh and Singh, 2012a). In a previous study, the maximum BCF value among the tested 30 castor cultivars was 0.747 for

stems and 0.822 for leaves at 5.396 and 2.396 mg/kg Cd pollution, respectively (unpublished). Castors showed low bioconcentration factors in the shoot (0.0480 for Zibo No. 5 and 0.0543 for Zibo No. 8) and low translocation factors (<1%) at 34 mg/kg Cd polluted soil (Table 2). However, the BCFs in stems of Zibo No. 5 and Zibo No. 8 were 0.490 and 0.612 at 2.396 mg/kg Cd, respectively, and 0.554 and 0.586 at 5.396 mg/kg Cd, respectively (unpublished). Considering the Cd concentration in the aboveground tissues and the biomass of the aboveground tissues, Zibo No. 5 and Zibo No. 8 could effectively remove Cd from polluted soil. However, Zibo No. 8 had low extraction efficiency and could participate in Cd phytostabilization in highly Cd-polluted soil. Thus, the Cd levels in the soil impacted the extraction efficiency of the castor cultivars.

Ethylenedinitrilotetraacetic acid (EDTA) is the most commonly used and effective metal chelator (Hong et al., 1999; Cutright et al., 2010), enhancing metal concentrations in soil solutions (Do Nascimento et al., 2006). In the pot experiment, the EDTA treatment increased the concentration of available Cd in the Cd-polluted soil significantly: 134 times for Zibo No. 5 and 114 times for Zibo No. 8 compared to the control group (Table 2). The result was a significant increase of Cd concentrations in the shoots of Zibo No. 8 (4.8 times compared with the control group) and the death of Zibo No. 5. Results also showed that the Cd concentrations in the roots and shoots of the Zibo No. 5 that died during the experiment were significantly higher compared with the control group (CK) (data not shown). However, Cd concentrations in the roots of Zibo No. 8 showed no significant increase, which confirms that Zibo No. 8 is characterized by higher Cd tolerance compared to Zibo No. 5 and an internal Cd detoxification mechanism.

Cd toxicity in plants is attributed to the production of excess reactive oxygen species (ROS), such as hydroxyl radicals (OH^-), superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2), which cause oxidative damage. The activity of antioxidants, such as GSH, SOD, CAT and GPX, represents an important protective mechanism to diminish oxidative damage due to heavy metal contamination. GSH belongs to the non-enzymatic antioxidant system, eliminates cytotoxic H_2O_2, and reacts with singlet oxygen, superoxide radical and hydroxyl radical (Larson, 1988; Blokhina et al., 2003). Moreover, the increase of GSH is crucial for Cd detoxification, as it is the monomeric substrate of phytochelatins, which form complexes with cadmium and sequester it into the vacuoles (Groppa et al., 2007). Nevertheless, the decrease of GSH levels in the leaves of Zibo No. 8 at high Cd concentrations (2 mg/L Cd) in contrast to lower Cd concentrations (1 mg/L Cd) (Fig. 1a) could be attributed to the increase of phytochelatin concentrations, which results in a higher resistance of plants to oxidative stress (Galli et al., 1996). The accumulation of phytochelatins is an important mechanism of Cd detoxification in plants. Similar to results obtained in the present study (Fig. 1a), the variation of GSH level seems to be associated with the Cd concentration and plant species (Seregin and Ivanov, 2001).

Superoxide dismutase is a metalloenzyme that catalyzes the dismutation of superoxide radicals in H_2O_2 and O_2, and plays an important role in protecting cells against the toxic effects of superoxide radicals produced in different cellular compartments (Del Rio et al., 2002). Catalase and guaiacol peroxidase are the most important enzymes for the regulation of the intracellular H_2O_2 level affected by SOD-catalyzed reactions (Blokhina et al., 2003). The increase of SOD activities may be attributed to the increased production of active oxygen species, which results in the increase of expression of gene-encoding SOD (Bowler et al., 1992; Fatima and Ahmad, 2005). In contrast, the decrease of SOD activity (Fig. 1b) can be attributed to enzyme inactivation by H_2O_2 produced in different cell compartments during enzymatic and non-enzymatic processes (Luna et al., 1994). The increase of CAT could be attributed to the increased H_2O_2 production, and subsequent degradation to water and O_2. However, CAT activity in the leaves of Zibo No. 8 at a Cd concentration of 2 mg/L decreased (Fig. 1c), which might be attributed to enzyme inhibition, because Cd bound to the thiol group of this enzyme (Ouzounidou et al., 1997) or caused disruption of protein synthesis as well as the damage of proteins (Srivastava and Dubey, 2011). The CAT activity in the leaves of Zibo No. 5 increased, but decreased in those of Zibo No. 8 (Fig. 1c). Previous experimental results have also shown that variations of CAT activities under oxidative stress can be contradictory: increase, decrease or no change (Boscolo et al., 2003), which can be attributed to different plant species, plant tissues, or durations and concentrations of metal exposure (Radic et al., 2010). GPX is not only part of the antioxidant defense system, but also the main peroxidase isoenzyme involved in lignin synthesis, which is important for the binding of Cd to cell walls (Podazza et al., 2012), an important tolerance mechanism protecting the protoplast from the toxic effect of Cd (Wojcik et al., 2005). GPX activity increased significantly under Cd exposure (Fig. 1d), confirming the results of previous studies (Schutzendubel et al., 2001; Uraguchi et al., 2006; Kovacik and Klejdus, 2008).

The present study indicates that castor is characterized by high Cd tolerance and that the degree of Cd tolerance depends on the cultivar. The higher tolerance of Zibo No. 8 compared with Zibo No. 5 can be attributed to the higher GSH levels in the root (Fig. 1a) and the higher GPX activity in the leaf (Fig. 1d). The higher GSH, SOD and GPX activities of the root compared with those of the leaf (Fig. 1a, b, d) may be one reason for the protection system against excessive Cd stress in the root.

4. Conclusions

This study clearly demonstrated that Zibo No. 8 showed a great tolerance against Cd, which makes it more suitable for the phytostabilization of highly Cd-polluted soils. Glutathione, as a non-enzymatic antioxidant, and antioxidant enzymes including superoxide dismutase, catalase and guaiacol peroxidase, were cultivar- and dose-dependent. The higher tolerance of Zibo No. 8 compared with Zibo No. 5 can be attributed to the higher GSH level in the root and higher GPX activity in the leaf. GSH, SOD and GPX activities were organ-dependent, and the higher GSH, SOD and GPX activities of the root compared with the leaf induced the effective detoxification in the root. Furthermore, subcellular compartmentation is the underlying mechanism of Cd detoxification and additional work will be carried out in the future in order to draw a clear picture of this mechanism.

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