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BRIEF COMMUNICATION

Comparison of antioxidant responses to cadmium and lead in *Bruguiera gymnorrhiza* seedlings

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Abstract

Seedlings of mangrove plant *Bruguiera gymnorrhiza* cultured in sand with Hoagland's nutrient solution were treated with 1 to 30 mM $Cd(NO_3)_2$ or $Pb(NO_3)_2$ for 2 months. In all Cd/Pb treatments, the malondialdehyde content increased while the chlorophyll content declined. Peroxidase (POD) and superoxide dismutase (SOD) activities in roots increased at moderate Cd/Pb concentrations (1 - 10 mM), whereas decreased at higher concentrations (20 - 30 mM). Catalase (CAT) activity in roots was inhibited by 1 - 10 mM Cd but enhanced by 1 - 10 mM Pb. The activities of POD, SOD and CAT in leaves were less affected by Cd and Pb than in roots. A new SOD and three CAT isoenzymes were induced by Pb. In contrast, no additional SOD and CAT isoenzymes were induced by Cd.

Additional key words: antioxidant enzymes, catalase, mangrove, peroxidase, superoxide dismutase.

Cadmium (Cd) and lead (Pb) are important pollutants in mangrove ecosystems. It is well known that Cd and Pb have no biological function and are extremely toxic, but they can be easily absorbed by plants and enter the food chain. Cd and Pb toxicities have been shown to be linked to oxidative stress. Both elements can induce the production of reactive oxygen species (ROS). We concentrated on three antioxidative enzymes, peroxidases (POD, EC 1.11.1.7), superoxide dismutases (SOD, EC 1.15.1.1) and catalases (CAT, EC 1.11.1.6), which play very important roles in ROS detoxification under adverse conditions (Monnet *et al.* 2006, Radić *et al.* 2006).

Bruguiera gymnorrhiza is one of the major mangrove species. There are some reports on its biochemical response to environmental stresses, such as wastewater, ammonium and salinity (Takemura *et al.* 2000, Ye and Tam 2002, Miyama and Tada 2004). However, information on the response of mangrove plants to heavy metals is quite scarce. In this work, we compared the variation of antioxidant enzyme activities and their isoenzyme patterns in *B. gymnorrhiza* seedlings under Cd and Pb stress.

Mature *Bruguiera gymnorrhiza* propagules, collected from Guangxi Yingluo Bay in the south of China, were grown in moistened sand under greenhouse conditions (12-h photoperiod, irradiance of 800 μmol m^{-2} s^{-1}, day/night temperature of 25/20 °C and relative humidity of 70 - 80 %). When the 3rd pair of leaves appeared, Hoagland's nutrient solution containing different Cd or Pb concentrations (1 - 30 mM) was added every 4 d to each pot. Cadmium was added as $Cd(NO_3)_2$ and lead as $Pb(NO_3)_2$. Seedlings were treated for 2 months. To avoid the precipitation of PO_4^{3-} and Pb^{2+}, no PO_4^{3-} was added to the Hoagland solution but 0.25 % KH_2PO_4 containing 0.01 % *Tween 20* was sprayed on the leaves every morning and night.

Samples of fresh tissue (0.6 g) from the 3rd leaf pairs and roots were washed and ground in a mortar with 4 cm^3 of 100 mM phosphate buffer (pH 6.8) containing 10 % polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 11 000 *g* for 20 min. All the processing was carried out at 4 °C. The supernatant was used for the

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Abbreviations: CAT - catalase; MDA - malondialdehyde; PAGE - polyacrylamide gel electrophoresis; POD - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
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enzyme activity determination and the electrophoresis of POD, SOD and CAT isoenzymes.

Peroxidase was determined by monitoring the formation of tetraguaiacol from guaiacol in the presence of H_2O_2 (Zhang *et al.* 1995). SOD activity was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium according to Beauchamp and Fridovich (1971). Catalase (CAT) activity was measured at 405 nm by an assay of hydrogen peroxide based on the formation of its stable complex with ammonium molybdate (Góth 1991). Non-denaturing discontinuous polyacrylamide gel electrophoresis (PAGE) was conducted in 4 % stacking gels and different concentrations of separating gels (10 % for POD and CAT, 12.5 % for SOD, and 0.4 % starch in the gels for CAT). 60 μg protein were applied onto all the lanes. POD isoenzyme was visualized according to Cuypers *et al.* (2002). SOD isoenzyme was detected by the method of Mascher (2002). Catalase isoenzyme was revealed according to Ding and Zhang (1999).

Chlorophyll content was determined in leaf acetone extract by *Beckman DU-650* spectrophotometer at 663 nm (chlorophyll *a*) and at 646 nm (chlorophyll *b*) (Wang 2001). The level of lipid peroxidation was estimated by measuring MDA content according to Heath and Packer (1968).

Significant decrease in growth and chlorophyll content was observed when Cd and Pb were applied at concentrations 20 or 30 mM (Table 1). There was no leaf spot caused by Cd and Pb toxicity at any of the tested concentrations, but 30 mM Cd caused lamina withering. The Cd/Pb concentration and the total chlorophyll content were negatively correlated [r^2 (Cd) = -0.926, r^2 (Pb) = -0.942, $P < 0.01$]. Under 30 mM Pd treatment, the chlorophyll content was only 33 % of the control.

MDA content was markedly raised over that of the control (Table 1). The Cd/Pb concentration and MDA content were positively correlated [r^2 (Cd, root) = 0.964, r^2 (Cd, leaf) = 0.957; r^2 (Pb, root) = 0.970, r^2 (Cd, leaf) = 0.961, $P < 0.01$]. An 8-fold increase was detected in the roots when 20 mM Cd was supplied. Similarly, Cd and Pb induced lipid peroxidation in other plants (Mi and Hyun 2003, Reddy *et al*. 2005).

The POD and SOD activities in roots firstly increased at the moderate concentrations (1 - 10 mM) of Cd and Pb and then decreased at higher concentrations (20 - 30 mM) (Table 1, Fig. 1). The activities in the leaves were less affected than in the roots. The roots might play a role as a barrier to prevent heavy metals from entering the stems and leaves. Increasing POD activity helps in H_2O_2 scavenging and so limits production of further free radicals (Demirevska-Kepova *et al*. 2004). Result from PAGE (Fig. 1) revealed that no new POD isoform was observed in all treatment. All SOD isoforms were inhibited by KCN and H_2O_2, and so were identified as to be Cu,Zn-SOD. In our study, the activity of leaf SOD LS-1 was inhibited by 30 mM Cd, while the activities of LS-1 and LS-2 were gradually enhanced by 30 mM Pb and an additional isoenzyme LS-3 was detected at the highest Pb concentration.

Each Cd treatment, from 1 to 30 mM, resulted in a significant decrease in the CAT activity in the roots. Only about one third of the activity of the control was detected under 1 mM Cd exposure. In contrast, significant decline ($P < 0.001$) in roots did not occur until 20 and 30 mM Pb were applied. The activities of two CAT isoenzymes in roots RC-1 and RC-2 were strongly inhibited by 1 mM Cd and were hardly detectable by PAGE, which was in accordance with the results from the determination of enzyme activitiy. In comparison to Cd, the activities of RC-1 and RC-2 increased at 1 and 10 mM Pb. Moreover,

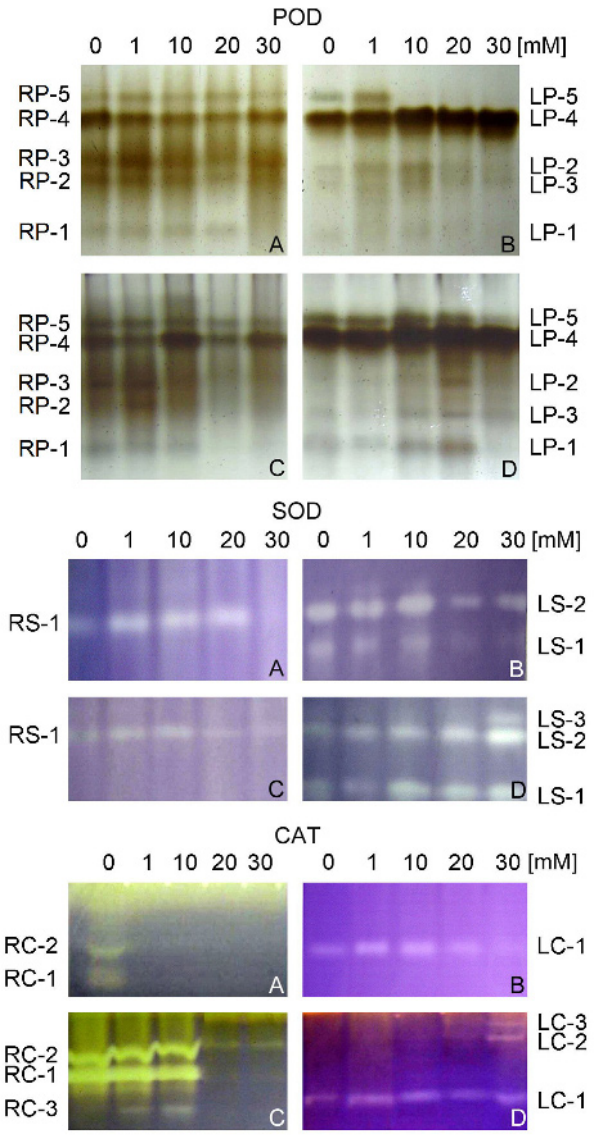

Fig. 1. Effects of Cd/Pb on POD, SOD and CAT isoforms in *B. gymnorrhiza* seedlings. *A* - isoform from roots of seedlings grown in different Cd concentrations; *B* - isoform from leaves of seedlings grown in different Cd concentrations; *C* - isoform from roots of seedlings grown in different Pb concentrations; *D* - isoform from leaves of seedlings grown in different Pb concentrations. RP - root POD, LP - leaf POD, RS - root SOD, LS - leaf SOD, RC - root CAT, LC - leaf CAT.

Table 1. Effects of Cd and Pb on plant height increment, chlorophyll content (Chl), MDA content, POD, SOD and CAT activities in *B. gymnorrhiza* seedlings. Values followed by same letters are not significantly different at ($P < 0.05$). Means ± SE, $n = 6$.

Parameter	Control	Metal	1 mM	10 mM	20 mM	30 mM
Plant height	11.6 ±1.2a	Cd	9.2±1.6a	9.5±1.1a	7.1±1.8c	6.9±2.3c
increment [cm]		Pb	10.5±1.9a	12.7±2.5b	6.4±2.0c	7.7±2.2c
Chl	0.82±0.07a	Cd	0.63±0.05b	0.64±0.12b	0.56±0.07d	0.51±0.06d
[mg g^{-1}(f.m.)]		Pb	0.63±0.02b	0.49±0.07c	0.32±0.04e	0.27±0.04e
MDA in roots	0.63±0.16a	Cd	1.70±0.44b	4.21±0.74c	7.22±0.76d	6.15±0.97e
[nmol g^{-1}(f.m.)]		Pb	1.95±0.39b	4.35±0.54c	5.70±0.37e	6.08±0.88e
MDA in leaves	0.13±0.08a	Cd	0.60±0.08b	1.80±0.11c	2.11±0.20c	2.33±0.29e
[nmol g^{-1}(f.m.)]		Pb	0.64±0.10b	1.12±0.21d	1.33±0.16d	2.34±0.34e
POD in roots	1002±62.2a	Cd	1187±77.8a	1142±67.2a	1122±73.2a	832±66.3d
[U mg^{-1}(protein)]		Pb	1377±62.1b	1392±54.3b	984±48.8c	1131±78.4a
POD in leaves	273±13.2a	Cd	442±20.1b	446±25.3b	452±22.3b	312±19.6d
[U mg^{-1}(protein)]		Pb	438±21.2b	508±32.6c	495±19.4c	522±17.7c
SOD in roots	133±8.2a	Cd	172±13.4a	272±15.4c	304±17.8c	130±16.5a
[U mg^{-1}(protein)]		Pb	211±12.1b	241±10.3b	128±13.2a	167±11.1a
SOD in leaves	33±1.2a	Cd	35±1.7a	62±3.3c	43±3.4b	32±1.1a
[U mg^{-1}(protein)]		Pb	42±1.8b	55±2.7b	55±1.6b	103±4.6d
CAT in roots	877±78.2a	Cd	331±40.4b	340±38.4b	389±44.4b	310±32.2b
[U mg^{-1}(protein)]		Pb	1087±61.1c	1335±92.5d	508±39.7e	638±33.3f
CAT in leaves	122±5.5a	Cd	168±10.4b	195±10.3c	88±9.9d	79±9.7d
[U mg^{-1}(protein)]		Pb	200±11.1c	211±11.5c	159±10.2b	244±12.2e

three additional CAT isoenzymes, RC-3, LC-2 and LC-3were induced by Pb. The fact that new SOD and CAT isoenzymes were induced by Pb while no additional SOD and CAT isoenzyme indicated the different regulation mechanisms in the expressions of SOD and CAT isoforms between Cd and Pb stress. Phytochelatins (Jiang *et al*. 2001), glutathione (Wójcik *et al*. 2011) and antioxidant enzymes are believed to function as detoxifying agents for heavy metals.

In conclusion, the resistance of *B. gymnorrhiza* seedlings to Cd and Pb is closely related to the three antioxidant enzymes. Increased activities of POD, SOD and CAT are actively involved in counteracting the oxidative stress. We found that the chlorophyll content, the activities and the isoforms of the antioxidant enzymes experienced significant changes even though there were no visible Cd or Pb toxicity symptoms in the seedlings.

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