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Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids

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Abstract

This paper analyzes the detoxification mechanisms adopted by amino- and organic acids to alleviate Cd toxicity. In addition, with our published data on Zn–Cd interactions, the influence of Zn (200 μM) supplements on the detoxification mechanisms of amino- and organic acids have also been studied. The experimental studies on metal uptake, lipid peroxidation levels, estimation of reduced and oxidized glutathione levels as well as γ-glutamylcysteine synthetase activity in amino acid supplemented Cd treatments indicated glutathione-mediated detoxification system, which was also enhanced by Zn (200 μM) supplements. However Zn did not aid in glutathione synthesis, but maintained the ratio of reduced and oxidized forms. The supplementation of organic acids to Cd treatments indicated detoxification through the mechanism of chelation. Zn seemed to be less influential on organic acids-mediated detoxification mechanism as compared to amino acid mediated detoxification system.

Keywords: Amino acids; Chelation; Detoxification; Glutathione; Organic acids; Phytochelatins; *Ceratophllum demersum*

1. Introduction

Heavy metal pollution is of considerable importance and relevant to the present scenario due to the increasing levels of pollution and its obvious impact on human health through the food chain (Hadjiliadis, 1997; Prasad, 2001). Aquatic ecosystems act as one of the major receptacle other than terrestrial ecosystem for various contaminants generated through the unregulated release of effluents from mines, smelters, industries, excessive usage of agrochemicals, and from aerial deposition (Adriano, 2001; Kabata-Pendias, 2001). Locations adjacent to agricultural areas pose a high risk to aquatic habitats because of the potential for significant pesticide runoff after rainfall events. The survival of a plant in a heavy metal contaminated environment is determined by its sensitivity to metal toxicity (Prasad, 1997). Therefore plants have to adapt themselves to the prevailing conditions for their survival, resulting in acquisition of a wide range of metal-tolerance mechanisms (Ernst et al., 1992). Cadmium, a group IIB transition element

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Abbreviations: EDTA: Ethylenediaminetetraacetic acid; PMSF: Phenylmethylsulfonylfluoride; PVPP: Phenylmethylsulfonylfluoride; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid.

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is not known to possess any metabolic significance to the living system as against Zn, another group IIB element known to be one of the most essential micronutrient for the plant system (Vallee and Falchuk, 1993). Ore extraction of Zn from mines and non-ferrous metal production processes in smelters with subsequent release of Zn effluents to the environment is normally accompanied by Cd environmental pollution (Sterkeman et al., 2000) because zinc ores (ZnS) generally containing 0.1–5% and sometimes even higher cadmium (Adriano, 2001). Thus, this association of Cd and Zn in the environment, and hence the interactive functions are of considerable importance of study.

A number of organic acids, amino acids and some members of mugineic acids occur in plant tissues and are possible ligands for metal complexation, thereby conferring metal tolerance (Łobiński and Potin-Gautier, 1998; Hall, 2002). Amino acids stabilizing various macromolecules structurally and aiding in vital cellular functions, are present in living systems up to concentrations of several millimoles per liter and are therefore potential ligands for metal ion binding (Pohlmeier, 2004). Low molecular weight organic acids especially citric, oxalic and malic acids are capable of forming complexes with metals which can affect their fixation, mobility and availability to plants. Metal–organic acid interactions in the soil–plant system are found to be important for solubilizing metals from highly insoluble phases (Cieslinski et al., 1998; Jones, 1998; Wu et al., 2003). There have been several reports concerning the detoxification of trace metals like Cd, Al, Ni and Zn by amino acids (Krämer et al., 1996; Peraza et al., 1998; Kerkeb and Krämer, 2003) as well as organic acids (Sagner et al., 1998; Jonnarth et al., 2000; Ma, 2000; Elkhatib et al., 2001), but no reports concerning their behavior in a multiple metal contaminated environment, a point not often considered in many experiments. Bioconcentration of Cd (Kumar and Prasad, 2004a,b) and Zn–Cd interactions have been well studied in *Ceratophyllum demersum* L. where potentially antagonistic interaction is known to occur between the two (Aravind and Prasad, 2003, 2004a,b,c, 2005). In this study we highlight the specific detoxification mechanisms adopted by amino acids (cysteine, glutamic acid and glycine) and organic acids (citric, oxalic and malic acids) when supplemented to *C. demersum* L., already existing in a Cd and Zn coexisting system.

2. Materials and methods

2.1. Plant material and growth conditions

Ceratophyllum demersum L. plants were collected from local ponds and maintained in multiple sets of aquaria in the following conditions. The plants were supplied with 0.1% Hoaglands nutrient solution containing 1 mM $NH_4H_2PO_4$, 10 mM KNO_3, 2 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, as macronutrients, 46 μM H_3BO_3, 9 μM $MnCl_2 \cdot 4\,H_2O$, 0.76 μM $ZnSO_4 \cdot 7\,H_2O$, 0.32 μM $CuSO_4$, 0.55 μM H_2MoO_4, as micronutrients and 78 μM Fe-EDTA as the iron source at a temperature of 26–30 °C, solution pH 6.0, under the natural day and night cycle in shade conditions (without the influence of direct sunlight) as found exactly in the natural environment. The plants were not provided with any supplementary light source, as it would be inhibitory for their growth. After the plants were acclimatized to laboratory conditions for more than one month they were used for further experiments.

2.2. Supplementation of amino- and organic acids to cadmium treatments

Plant material (2 g) was transferred to a 250 ml glass beaker with 200 ml of 0.1% Hoaglands nutrient solution (containing 0.764 nmol Zn) and the chosen concentration of the metals. Cd treatment of 10 μM concentration was given to the plants using $CdCl_2$. Amino acids: cysteine (Cys), glutamic acid (Glu) and glycine (Gly) and organic acids: citric acid, oxalic acid and malic acid were supplemented in the concentration range of 0.1–1.0 mM. Zn supplementation (200 μM), (most effective concentration, refer Aravind and Prasad, 2003, 2004a,b,c, 2005) was done using $ZnCl_2$ to the amino- and organic acids supplemented Cd (10 μM) treated plants. After a period of one week as standardized earlier (Aravind and Prasad, 2003) the plants were used for further experiments.

2.3. Quantification of cadmium and zinc content

Plants (2 g) were washed thoroughly with 10 mM EDTA to remove metals adsorbed to the surface. They were oven-dried at 80 °C for two days and acid-digested with 3:1 $HNO_3/HClO_4$. This solution was analyzed for Cd and Zn content using atomic absorption spectrometer (GBC 932 plus, Australia) (Greger and Lindberg, 1987).

2.4. Estimation of lipid peroxidation

Plant material (300 mg) was homogenized with 3 ml of 0.5% (w/v) TBA in 20% (w/v) TCA. The homogenate was incubated at 95 °C for 30 min and the reaction was stopped in ice. The samples were centrifuged at $10\,000 \times g$ for 10 min and the absorbance of the resulting supernatant was recorded at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of malondialdehyde (MDA)-155 $mM^{-1}\ cm^{-1}$ was used in the calculation according to Heath and Packer (1968).