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ScienceDirect
Ecotoxicology and Environmental Safety 71 (2008) 76–85
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L.☆

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Received 28 April 2006; received in revised form 21 September 2007; accepted 13 October 2007
Available online 21 December 2007

Abstract

A hydroponics experiment using Indian mustard (*Brassica juncea* L.) was conducted to investigate the effect of different concentrations (10–160 μM) of cadmium (Cd) and a fixed concentration (500 μM) of ethylene diamine tetra acetic acid (EDTA) on Cd accumulation and its toxicity for 14 and 28 days (d). The results showed that Cd alone and Cd + EDTA increased total dry biomass production, photosynthetic pigments and total protein content of *B. juncea* up to 160 μM with respect to control for 14 d (hormesis effect). Further, on treatment with Cd at 160 μM for 28 d, dry biomass of root and shoot, total protein content and total chlorophyll decreased up to 73%, 58%, 67% and 53% respectively, while in the case of Cd + EDTA, the decrease in the above parameters was 38%, 50%, 57% and 46% with respect to their control. It was observed that the maximum Cd accumulation after 28 d in the root and shoot was 1925 and 977 mg kg^{-1} dry weight (dw), respectively, while in the case of Cd + EDTA it was 1013 and 2316 mg kg^{-1} dw, respectively. Levels of phytochelatins (PCs), glutathione reductase (GR; EC 1.6.4.2), non-protein thiols (NP-SH) and glutathione (GSH) were monitored as plants primary and secondary metal detoxifying responses. Glutathione reductase showed three-fold increased activity for Cd and 2.2-fold for Cd + EDTA at 160 μM after 14 d followed by decreased activity after 28 d with respect to control. Maximum synthesis of PCs was found at 10 μM of Cd exposure followed by a gradual decline after 28 d. This may be correlated with reduced level of GSH, probably due to reduced GR activity, resulting in enhanced oxidative stress as also proved by phenotypic changes in plants such as browning of roots and yellowing of leaves. Thus, the capacity of *B. juncea* to accumulate and tolerate high concentrations of Cd, through enhanced level of PCs, GSH, NP-SH and GR suggests its applicability for phytoremediation.

Keywords: *Brassica juncea*; Cadmium; EDTA; Glutathione; Glutathione reductase; Hormesis effect; Phytochelatins; Phytoremediation

1. Introduction

Agricultural soils in many parts of the world are slightly to moderately contaminated by Cd due to long-term use of phosphatic fertilizers, sewage sludge application, as well as dust from smelters. Cadmium, being a non-essential element, poses several toxic effects to humans, animals and plants. Heavy metals, including Cd, cannot be degraded like other pollutants and the clean up usually requires their removal. It is reported that Cd-phytoextraction from agricultural soils could be easily implemented due to ease of Cd mobility in the soil–plant system as compared to other heavy metals (Davis, 1984; Robinson et al., 2000). Plant-based clean up technology (Phytoremediation) offers a number of advantages over traditional clean up methods as well as over other bioremediation technologies. Heavy metal phytoextraction has emerged as a promising, cost effective alternative to the conventional engineering-based remediation methods that usually involve excavation and removal of contaminated soil layer, physical stabilization or washing of contaminated soils with strong acids that change the soil property. These conventional methods are neither cost effective nor ecofriendly (Robinson et al., 2000; Pollard et al., 2002).

Brassica juncea (Brassicaceae), a fast growing and high biomass-producing plant, seems a suitable species for phytoextraction, as it can compensate lower Cd

☆In the present manuscript, the study performed is on plants and not on animals.

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doi:10.1016/j.ecoenv.2007.10.030

accumulation with much higher biomass yield. The use of several chelaters, including ethylene diamine tetra acetic acid (EDTA), increased the solubility of metals in the soil facilitating their easy availability, uptake and translocation from root to shoot in the vascular plant (Blaylock et al., 1997; Bricker et al., 2001; Wu et al., 2004). In this experiment, we have used EDTA to observe its effect on accumulation as well as toxicity. The use of EDTA showed two major advantages: (i) it increased metal uptake as well as translocation of Cd from root to shoot and (ii) lowered the toxicity of Cd. This EDTA-mediated advantage is crucial for a successful phytoremediation technology.

The knowledge of Cd toxicity/detoxification mechanism is of critical importance from the practical standpoint of optimizing Cd phytoremediation by using *B. juncea*. The proposed mechanisms for metal detoxification in plants include chelation of the metal by ligands and sequestration of metals away from sites of metabolism in the cytoplasm, notably into the vacuole or cell wall (Salt et al., 1995; Bricker et al., 2001). Organic ligands containing sulfur donor centers form stable complexes with many elements, including Cd. Plants can produce low molecular weight thiols that have high affinity for toxic metals (Bricker et al., 2001). The most important/critical low molecular weight biological thiols are glutathione (GSH) and cysteine. GSH, a sulfur-containing tripeptide thiol with the formula γ-glutamate-cysteine-glycine, is a direct structural unit for phytochelatins (PCs) (Cobbett, 2000) and is a very important antioxidant.

PCs are small metal-binding peptides with the structure $(\gamma\text{-Glu-Cys})_n$–Gly, where the value of n varies from 2 to 11, and their synthesis is induced by several metals, particularly by Cd (Grill et al., 1985). These are considered important components of toxic metal chelation/detoxification in higher plants. PCs biosynthesis involves the enzyme γ-glutamyl cysteine dipeptidyl transpeptidase, also known as PC synthase (PCS; EC 2.3.2.15), with GSH as the substrate. The functional significance of PCs can be attributed to the presence of thiol (–SH) group, responsible for the coordination of metals (Cobbett, 2000; Hall, 2002). Apart from this, Cd alone and in combination with EDTA apparently induced the formation of both GSH and non-protein thiols (NP-SH) at higher concentrations. Reducing substances such as L-ascorbic acid and GSH form an ascorbate–glutathione cycle together with ascorbate peroxidase (APX), dehydro ascorbate reductase (DHAR) and glutathione reductase (GR). This cycle performs effectively to scavenge hydrogen peroxide in the cell (del Rio et al., 1998). Further, the antioxidative responses of the plant against Cd toxicity are not clear, because Cd does not belong to the transition metals group, such as copper, iron and zinc, which induced oxidative stress via Fenton-type reactions. In view of this, the present study is focused on finding the possibility of *B. juncea* as a Cd phytoremediator. Therefore, the plants have been exposed to Cd at low, moderate and high concentrations and evaluated for metal accumulation in root and shoot. Effects on total dry biomass production, photosynthetic pigments and protein content of the plant were also monitored as gross effect, while the level of GSH, NP-SH, GR activity and PCs were estimated to know the capacity of *B. juncea* to withstand oxidative stress induced by Cd as well as its detoxification by PCs.

2. Materials and methods

2.1. Preparation of Hoagland and $CdCl_2 \cdot H_2O$ solution

The salts and chemicals used in this study were purchased from BDH, AnalR grade and Loba chemicals. Bovine serum albumin and GSH were from Sigma Chemical Company, USA. Hoagland solution was prepared by the method of Hoagland and Arnon (1950). In brief, Hoagland solution was prepared by making macronutrient ($Ca(NO_3)_2 \cdot 4H_2O$, 118.89 g l^{-1}; KNO_3, 50.25 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 24.08 g l^{-1}; KH_2PO_4, 13.61 g l^{-1}), micronutrient (H_3BO_3, 2.86 g; $MnSO_4 \cdot H_2O$, 1.54 g; $ZnSO_4 \cdot 7H_2O$, 0.22 g; $CuSO_4 \cdot 5H_2O$, 0.089 g; $H_2MoO_4 \cdot H_2O$, 0.09 g were dissolved in 1 l of distilled water) solutions; and EDTA-K salt (2.50 g) and $FeSO_4 \cdot 7H_2O$ (2.50 g) dissolved in 1 l of distilled water. For preparation of 100% Hoagland solution, 10.0 ml of each macronutrient, 1.0 ml of micronutrient and 1.0 ml of EDTA-K salt and $FeSO_4 \cdot 7H_2O$ was made up to 1.0 ml by the addition of distilled water. A stock solution of Cd (1000 μM) was prepared by dissolving 21 mg $CdCl_2 \cdot H_2O$ in 100 ml of double-distilled water. Required dilutions (10–160 μM) of this solution were used.

2.2. Seed germination

Seeds of *B. juncea* procured from Chandra Shekhar Azad Agriculture University, Kanpur, U.P., India, were soaked for 3–4 h followed by several washings with double-distilled water. They were germinated on moistened filter paper. Healthy and similar size seedlings were selected and transferred for growth to hydroponic culture medium for 10 days (d).

2.3. Treatment of plants with Cd and Cd+EDTA

Two sets of beakers each containing 400 ml of Hoagland medium were taken. In one set, Cd at the concentration of 10, 20, 40, 80 and 160 μM was maintained, whereas the other set had 500 μM of EDTA along with the aforesaid Cd concentrations. The concentrations of Cd present in the nominal exposure solutions of each treatment were measured prior to experimentation and immediately following each daily renewal by atomic absorption spectrophotometer and found to be 9.9 ± 0.01, 19.9 ± 0.03, 39.6 ± 0.05, 79.8 ± 0.06, 159.4 ± 0.08 μM for 10, 20, 40, 80 and 160 μM, respectively. Subsequently, about 10 healthy plants of similar size were taken for each concentration. Both sets of beakers were kept in plant growth chamber under controlled conditions at 25 ± 2 °C, illuminated for 12 h daylight period (light intensity approximately 500 μmol m^{-2} s^{-1} for growth). In our preliminary experiment, we did not observe significant toxicity to plant growth after 14 d exposure, while after increasing duration of exposure to 28 d, significant hampering of growth was observed. Hence, we studied various parameters for these two exposure durations.

2.4. Dry biomass study

At the end of experimental exposure, harvesting was done. The harvested plants were blotted on the tissue and filter paper and roots and shoots separated manually. They were kept in an oven at 65 ± 5 °C for 1 week and weighed on an electronic balance to determine any effect on dry biomass production.