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Research article

Effect of lead on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus*

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ABSTRACT

A screenhouse experiment was conducted to assay the effect of Lead (Pb) on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus* after 6 weeks. Results revealed a good Pb tolerance and accumulation potential of *C. didymus* towards the increasing Pb concentrations (500, 900, 1800, 2900 mg kg^{-1}) in soil. The content of Pb in roots and shoots elevated with higher Pb levels and reached a maximum of 3684.3 mg kg^{-1} and 862.8 mg kg^{-1} Pb dry weight, respectively, at 2900 mg kg^{-1} treatment. Pb exposure stimulated electrolyte leakage, H_2O_2 level, MDA content and the activities of antioxidant machinery (SOD, CAT, APX, GPX and GR). However, at the highest Pb concentration, the activities of SOD and CAT declined. The H_2O_2 level and MDA content in roots increased significantly up to ~500% and 213%, respectively, over the control, at 2900 mg kg^{-1} Pb treatment. Likewise, concurrent findings were noticed in shoots of *C. didymus*, with the increasing Pb concentration. The present work suggests that *C. didymus* exhibited a good accumulation potential for Pb and can tolerate Pb-induced oxidative stress by an effective antioxidant defense mechanism.

1. Introduction

Rapid industrialization, injudicious use of agricultural fertilizers, faulty mining activities, and improper management of solid wastes pose a serious threat to the terrestrial ecosystems due to the persistent release of toxic heavy metals (Dinakar et al., 2008). Lead (Pb), a heavy metal, has no biological role in plants and its toxicity can cause physiological, biochemical and morphological alterations like seed dormancy, stunted growth and reduced chlorophyll content in plants (Shahid et al., 2012). Agency for Toxic Substances and Disease Registry (ATSDR, 2005) has listed Pb, as the number one heavy metal pollutant. In soil, concentration of Pb more than 30 ppm can cause toxicity in large number of plant species (Ruley et al., 2004; Xiong, 1997). Exposure to Pb at higher level induces oxidative burst in plants by generating reactive oxygen species (ROS) and causes death of the plant cell. Pb-induced toxicity promotes the accumulation of superoxide ion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), that inhibits the activity of enzymes, leading to peroxidation of lipids in the cells (Israr et al., 2011; Kaur et al., 2015).

The cell organelles of the plants have developed certain antioxidative response to combat the effect of ROS, generated due to oxidative stress. The response of antioxidant enzymes helps to measure the tolerance potential of a plant species towards oxidative stress. Various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR) have the potential to counteract the effect of ROS (Shakoor et al., 2014). Superoxide dismutase (SOD) sequesters the noxious superoxide ion ($O_2^{\bullet -}$) by breaking it into H_2O_2 and oxygen. H_2O_2 is further subsequently detoxified with the help of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) that convert H_2O_2 into water and molecular oxygen. Another crucial antioxidant enzyme glutathione reductase (GR) balances the GSH/GSSG ratio in the cells and helps to combat oxidative stress (Qiu et al., 2008).

Abbreviations: H_2O_2, hydrogen peroxide; $O_2^{\bullet -}$, superoxide ion; $^1O_2$, singlet oxygen; ROS, reactive oxygen species; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPX, guaiacol peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase; GSSG, oxidized glutathione; GSH, reduced glutathione.

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In order to minimize the hazardous impact of heavy metals on the environment some low cost and viable methods like phytoremediation must be employed. Thus, it is pertinent to investigate certain wild plant species that can be used for remediation purposes. Therefore, *Coronopus didymus*, a wild annual herb of Brassicaceae, was selected for the present study. It grows along the roadsides and gardens during winters (October–February) in northern parts of India. The purpose of the work is to evaluate and assess: (i) the accumulation of Pb in plant tissue, its effect on biomass and ROS generation, (ii) the effect of different concentrations of Pb (500–2900 mg kg^{-1}) on antioxidative response in roots and shoots of *C. didymus* and (iii) the tolerance mechanism of plant correlated with the increased Pb concentrations. The present work will be helpful to evaluate the detoxification mechanism adopted by *C. didymus* in Pb-contaminated soils.

2. Materials and methods

2.1. Plant material and Pb treatments

The seeds of *C. didymus* were collected locally from a non-contaminated site at Panjab University campus, Chandigarh, India. Seeds were surface cleaned, disinfected, sterilized (sodium hypochlorite) followed by washing with distilled water. Seeds were grown in plastic tray having 10 kg soil in a screenhouse. After 15 day, the seedlings were rooted out carefully from the tray and transplanted in the plastic pots having 1 kg of soil with different treatments of Pb. Treatment of Pb in soil was given in the form of lead nitrate [Pb $(NO_3)_2$; MW = 331.21; purity: 99%; Merck Ltd., Mumbai, India]. The experimental set up consisted of 5 treatments, amended with 500, 900, 1800 and 2900 mg kg^{-1} Pb and a parallel treatment without Pb amendment served as control. Four replicates (pots) were maintained for each Pb treatment having five plants per pot. After 6 weeks, plants were harvested for determination of biomass, H_2O_2, lipid peroxidation (in terms of MDA accumulation), Pb concentration and antioxidative response.

2.2. Plant biomass and metal accumulation

The plant material (roots and shoots) was dried in an oven at 75 °C for 72 h to determine the biomass. For determination of heavy metal content, the plant material (100 mg) was crushed and digested in 10 ml mixture of $HNO_3/HClO_4$ (4:1, *v/v*) and heated at a temperature of 150 °C until the formation of white fumes. Distilled water was added to raise the total volume of the digested material to 20 ml. The content of Pb in plant (roots and shoots) was determined by atomic absorption spectrophotometer (AAS).

2.3. Membrane integrity

The degree of membrane integrity loss was estimated by measuring the amount of electrolyte leakage (EL) as per the method given by Singh et al. (2007). The EL was calculated as: $EL = (E_1 - E_2) \times 100$, and expressed in terms of percent (%) value. E_1 was the initial conductivity of the bathing medium, whereas E_2 was the conductivity of the medium after boiling the contents for 30 min.

2.4. Detection of H_2O_2 and MDA content

To determine the H_2O_2 and MDA content, 100 mg of treated and untreated roots and shoots were crushed in 10 ml of 0.1% TCA (trichloroacetic acid) in pre-chilled mortar and pestle and centrifuged at 12,000×*g* for 15 min at 4 °C using cold centrifuge (Sigma Inc., USA). The supernatant was stored at 4 °C for further estimation.

The content of H_2O_2 was measured in the plant tissue as per Velikova et al. (2000). To 0.5 ml of supernatant (TCA extract) or distilled water (as blank), 0.5 ml of phosphate buffer (10 mM; pH = 7.0) and 1 ml of potassium iodide (1 M) solution was added. The absorbance of the reaction mixture was read at 390 nm and the content of H_2O_2 was calculated using an extinction coefficient ($\varepsilon = 0.28\ \mu M^{-1}\ cm^{-1}$) and expressed in terms of nmol g^{-1} f wt.

Lipid peroxidation of the plant tissue was measured in terms of malondialdehyde content as per Heath and Packer (1968). To 1 ml of supernatant, 4 ml of 0.5% TBA in 20% TCA was added. The mixture was heated at 95 °C for 30 min, cooled over ice, followed by centrifugation at 10,000×*g* for 10 min. The absorbance of the homogenized mixture was read at 532 nm, and corrected at 600 nm for non-specific absorbance. The MDA content was calculated using an extinction coefficient ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$) and expressed as nmol g^{-1} f wt.

2.5. Assays for antioxidative enzymes

2.5.1. Extraction of enzymes

100 mg plant tissue (roots and shoots) was crushed in 10 ml of 100 mM phosphate buffer (pH = 7.0) using pre-chilled mortar and pestle and centrifuged at 15,000×*g* for 25 min at 4 °C. The supernatant was collected and stored at 4 °C until use.

2.5.2. Antioxidant enzymes activities

The SOD activity was measured to quantify the ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) as per the method of Beauchamp and Fridovich (1971). To 1.8 ml reaction mixture [containing 50 mM phosphate buffer (pH = 7.4), 13 mM methionine, 0.1 mM EDTA, 13 μM Riboflavin, 0.05 M Na_2CO_3 and 63 μM NBT], 0.2 ml of enzyme extract or distilled water (as control) was added. Reaction tubes were kept under two 15 W fluorescent lamps for 15 min for the development of blue color and later these test tubes were transferred in the dark in order to stop the reaction. The other set of reaction tubes were kept in the dark. The non-irradiated samples did not develop any color and served as control. The OD (optical density) of both irradiated and non-irradiated samples was measured at 560 nm and the specific enzymatic activity was determined in terms of EU (enzyme unit) mg^{-1} protein. One unit of SOD represented the quantity of enzyme required for 50% inhibition in NBT photoreduction.

The CAT activity was determined in terms of the rate of H_2O_2 disappearance and was determined as per the method given by Cakmak and Marschner (1992). To 1.8 ml reaction mixture [containing 25 mM phosphate buffer (pH = 7.0) and 10 mM H_2O_2], 0.2 ml of enzyme extract was added. The increase in OD was measured at 240 nm for 1 min. The CAT activity was calculated by using an extinction coefficient ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) and expressed as EU mg^{-1} protein.

The activity of APX was measured by the method given by Nakano and Asada (1981). To 1.8 ml reaction mixture [containing 25 mM phosphate buffer (pH = 7.0), 0.1 mM EDTA, 0.25 mM ascorbic acid and 1.0 mM H_2O_2], 0.2 ml of enzyme extract was added. The decrease in the absorbance was measured at 290 nm for 1 min. The activity of enzyme was calculated using an extinction coefficient ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) and expressed as EU mg^{-1} protein.

The activity of GPX was determined in terms of oxidation of guaiacol as per the method given by Egley et al. (1983). To 1.8 ml of reaction mixture [containing 25 mM phosphate buffer (pH = 7.0), 0.05% guaiacol and 1 mM H_2O_2], 0.2 ml of enzyme extract was added. The increase in the OD was measured at 470 nm and the enzyme activity was calculated using an extinction coefficient