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LEAD (Pb)-INHIBITED EARLY ROOT GROWTH IN WHEAT INVOLVES ALTERATIONS IN ASSOCIATED BIOCHEMICAL PROCESSES

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ABSTRACT
The present study investigated the effect of Lead (Pb; 50 and 500 μM) on biochemical alterations during the early radicle growth. It was observed that Pb-exposure significantly enhanced the contents of water-soluble proteins and carbohydrates in wheat radicle after 24 h in a dose-response manner. Proteins and carbohydrates content increased by 2.3- and 3.5-folds, respectively at 500 μM Pb-exposure. On the other hand, the activities of hydrolyzing enzymes proteases and amylases were drastically reduced. At 500 μM, activities of proteases, α-amylases, and β–amylases decreased by 92, 55, and 82% respectively. In addition, Pb-exposures significantly enhanced the activities of enzymes - peroxidases, polyphenol oxidases by 2.6- and 2.2- times, respectively, over of control at 500μM Pb. Enhancement in the activities of these enzymes indicate their upregulation / induction in response to Pb-induced toxicity in wheat radicle and provide protection. The study concludes that Pb-induced toxicity in emerging wheat seedlings involves the biochemical alterations in terms of macromolecules and the activities of related enzymes to cope with the Pb-stress.

INTRODUCTION

Lead (Pb), is one of the heaviest non-essential metal released into the natural environment from a range of anthropogenic activities (Ekmekçi *et al*., 2009). Upon release, it gets accumulated in the soil and causes toxicity to plants and animals. Since Pb accumulated in the soil cannot be degraded or transformed into other non-harmful forms, world over efforts are being made to cleanse Pb-contaminated soil (Sharma and Dubey, 2005). In the soil, Pb is present in the forms of Pb-phytochelatins, Pb-nitrate, Pb-acetate, Pb-sulfide and Pb-citrate that are readily available and absorbed into the plants (Lopez *et al*., 2009). However, it depends upon the Pb-concentration and its bioavailability in the soil (Ekmekçi *et al*., 2009). Further, plants show a great deal of variation in their response to Pb-toxicity (Sharma and Dubey, 2005). Pb has been reported to inhibit seed germination, reduce growth and dry mass accumulation, negatively affect photosynthesis, transpiration and other physiological parameters in plants (Sharma and Dubey, 2005). Earlier studies have shown that Pb is a potent root inhibitor (Ramos *et al*., 2002). In general, it has been postulated that growth inhibition and visible injury in the plants are the secondary effects of heavy metals, whereas primary target sites are the biochemicals and enzymes involved in physiological processes (Sharma and Dubey, 2005). However, not many details are available regarding the effect of Pb on the changes in the macromolecular content and the activities of enzymes associated with the early germination and seedling growth of plants. We therefore, conducted a series of experiments to explore the effect of Pb on the content of macromolecules (proteins and carbohydrates) and the activities of hydrolyzing enzymes (α-amylases, β-amylases, proteases) and oxidoreductase enzymes (peroxidases and polyphenol oxidase) in wheat. For the present study, wheat has been chosen as target species at the germinating stage since; it is a widely grown cereal crop in India.

MATERIALS AND METHODS

Lead supplied as lead nitrate (Mol. wt.: 331.21; purity 99%) was purchased from Merck Ltd., Mumbai, India). Certified seeds of wheat (*Triticum aestivum* L. var. PBW 502) used in the present study were purchased locally from the seed store. All other chemicals and reagents used in the study for biochemical estimations and enzymatic assay were of reagent grade and procured from the best available sources.

Dose-response assay

Effect of lead on early growth of wheat was studied under laboratory conditions in a dose response manner. Before use, wheat seeds were surface sterilized with 0.1% (w/v) sodium hypochlorite solution, washed thoroughly under running tap water and then rinsed with distilled water. Pre-imbibed wheat seeds (for 4 h at 25 ºC) were allowed to germinate in 15 cm diameter Petri dishes (20 seeds per Petri dish, 5 replicates) lined with a single layer of Whatman # 1 filter circle moistened

with 7 mL of respective Pb solution (50 and 500 μM) or distilled water (for control). All the Petri dishes were placed in an environmentally controlled growth chamber set at day /night temperature of 20/10 ($\pm$2) ºC, relative humidity of 75 ($\pm$2)%, and a photoperiod of 12 h (7.30-19.30) at a photosynthetic photon flux density (PPFD) of approximately 240 μ mol photons $m^{-2}s^{-1}$. After 24 h, roots were collected and washed in 10 mM $CaCl_2$ to remove lead accumulated on their surface. These were stored at -80ºC and used for various biochemical estimations.

Estimation of protein and carbohydrate content

Nearly 200 mg of root tissue was homogenized in 10 mL of distilled water. After passing through a double layer of muslin cloth, the sample was centrifuged at 15,000 g for 15 min and the supernatant was collected for the estimations. Water-soluble protein content was determined using Folin-Ciocalteu reagent against bovine serum albumin as a standard as per Lowry *et al.*, (1951). Estimation of carbohydrate content was done using anthrone reagent against a standard of glucose (Loewus, 1952).

Preparation of enzyme extract

Crude enzyme extracts were prepared by homogenizing nearly 100 mg of root tissue in a pre-chilled pestle and mortar with 5 mL of 0.1 M phosphate buffer (pH = 7). Homogenates were centrifuged at 15,000 g for 25min at 4ºC rotor temperature in a Sigma Centrifuge. The fraction of supernatant thus obtained was used for determining the activities of proteases, amylases (α–, β-), peroxidases and polyphenol oxidases (PPO). The supernatant was stored at – 4ºC before enzyme assays. An aliquot of the supernatant was used to determine protein content using bovine serum albumin standard as per Lowry *et al.,* (1951).

Enzyme assays

Protease activity was estimated using Casein (1% in 0.1 M phosphate buffer, pH = 6) as a substrate (Basha and Beevers, 1975). Activity of α-Amylases was assessed as per Muentz (1977) using starch as a substrate; whereas β- Amylase was estimated following the method of Bernfeld (1951) with modifications suggested by Dure (1960). Peroxidases were assayed using 0.2 M hydrogen peroxide as a substrate following Malik and Singh (1980). Activity of polyphenol oxidases was determined using catechol (0.01 M in 0.1 M phosphate buffer, pH = 6) as a substrate as per Van Lelyveld and Pretorious (1973).

Data analysis

The experiments were performed in a randomized design with five replicates, each consisting of a single Petri dish with 20 seeds each. All the experiments were repeated and the data presented is of a single experiment since the differences between two experiments were less than 5%. The data were analyzed by one-way ANOVA and means were separated using post hoc Tukey's test at $p < 0.01$ and $p < 0.05$.

RESULTS AND DISCUSSION

The present study revealed that upon Pb treatment water soluble protein and carbohydrate content increased significantly ($p < 0.05$) in the roots of *T. aestivum* (Table 1). The effect was concentration dependent. The protein content increased by 91% and 229% at 50 and 500 μM Pb treatment, respectively, as compared to control (Table 1). These observations are supported by earlier observations that heavy metals induced the accumulation of total soluble proteins in emerging seedlings (Suzuki *et al.*, 2002; Cuypers *et al.*, 2005; Mishra and Dubey, 2006) suggested that these metal-induced proteins play a significant role either in detoxification and/or in the maintenance of heavy metal homeostasis.

On the other hand, the activity of protein-hydrolyzing enzyme, proteases, decreased with the increasing Pb concentration. At 500 μM Pb treatment, the protease activity decreased nearly 92% over control (Table 2). A decline in activity of proteases indicated the failure of the plant to hydrolyze proteins under Pb-stress. Earlier, Balestrasse *et al.*, (2003) also reported an inhibition of protease activity in soybean root nodules treated with Cd. Likewise, a decline in protease activity was also recorded in roots and shoots of rice plants (Shah and Dubey, 1997) and in *Lemna minor* when exposed to excess Pb^{2+} and Cd^{2+} (Mohan and Hosetti, 1997).

Table 1: Effect of Pb on the content of water-soluble proteins and carbohydrates in wheat roots

Lead (μM)	Proteins (mg/mL^{-1})	Carbohydrates (μg mg^{-1} FW)
0	0.318 ± 0.001^{a}(0)	20.63 ± 0.02^{a}(0)
50	0.609 ± 0.001^{b} (+91.51)	45.53 ± 0.05^{b} (+120.70)
500	1.045 ± 0.002^{c} (+228.62)	92.77 ± 0.00^{c} (+349.68)

Data represented as mean $\pm$ S.E of five independent replicates; Figures in parenthesis represent percent increase over control. Different alphabets within a column at a particular treatment represent significant difference from control at $p < 0.05$ applying post hoc Tukey's test

Parallel to protein content, a progressive increase was observed in carbohydrate content in response to lead in a dose-response manner. A ~1.2-fold increase was observed in carbohydrate content at 50 μM Pb concentration and it enhanced further to ~3.5 times over control at 500 μM concentration (Table 1). These observations are supported by an earlier observation of El-Said Deef (2007) who reported an accumulation of reducing sugars in the leaves of *Rosmarinus officinalis* upon exposure to Cu. The observed increase in carbohydrate

Table 2: Effect of Pb on the specific activities of enzymes - proteases, α and β- amylases, peroxidases and polyphenol oxidases, in the root tissue of wheat

Lead(μM)	Proteases (μg h^{-1} mg protein^{-1})	α-Amylases (μg min^{-1} mg protein^{-1})	β-Amylases (μg min^{-1}mg protein^{-1})	Peroxidases (Kat s^{-1} mg protein^{-1})	Polyphenol oxidases (Kat s^{-1} mg protein^{-1})
0	332.93 ± 1.16^{a} (0)	24.65 ± 0.00^{a}(0)	17.48 ± 0.01^{a}(0)	0.071 ± 0.002^{a}(0)	0.210 ± 0.005^{a}(0)
50	126.90 ± 0.76^{b}(-61.88)	16.78 ± 0.00^{b}(-31.93)	13.46 ± 0.00^{b}(-22.98)	0.124 ± 0.001^{b}(+74.65)	0.340 ± 0.001^{b}(+61.90)
500	27.58 ± 1.14^{c}(-91.72)	11.03 ± 0.00^{c}(-55.26)	3.36 ± 0.00^{c}(-80.77)	0.185 ± 0.001^{c}(+160.56)	0.461 ± 0.001^{c}(+119.52)

Data represented as mean $\pm$ S.E of five independent replicates. Figures in parenthesis represent percent increase (+)/decrease (-) over control. Different alphabets within a column at a particular treatment represent significant difference from control at $p < 0.05$ applying post hoc Tukey's test.

content in the present study could be attributed to the decreasing activity of amylolytic enzymes upon Pb-stress.

It was observed that Pb-exposure significantly decreased activity of α-amylases ranging from 32 to 55% over that of control (Table 2). Likewise, a decline was observed in the activity of β-amylases and a reduction of ~80% over control was noticed at 500 μM (Table 2). The decreased activity of amylases suggests the inability of the plant to meet the increased energy demands of the tissue in response to Pb-induced stress. Earlier, Chugh and Sawhney (1996) reported a similar decrease in the activity of amylases in pea seeds upon exposure to Cd. In general, the starch degrading enzymes have been found to be sensitive to heavy metals like Cr (Dua and Sawhney, 1991), Cd (Chugh and Sawhney, 1996).

In the present study, a significant increase was noticed in the activity of peroxidases (PODs) (Table 2). It indicated the induction of Pb-induced stress in wheat roots. PODs are a group of enzymes that are also responsible for the plant seedling growth and development. Soluble POD catalyzes the oxidation of structurally diverse phenolic substrates to protect the cells from toxic influence of oxygen radicals (Santos *et al*., 2004). Not only PODs, even the activity of polyphenol oxidases, another group of enzymes involved in stress-amelioration was found to increase significantly. The activity of PPOs increased by ~62% and 120% at 50 and 500 μM Pb-exposure, respectively, compared to control (Table 2). Likewise, Cd, another heavy metal has been reported to increase the activity of PPOs (Lavid *et al.,* 2001).

In summary, the present study concludes that Pb-induced toxicity in emerging wheat seedlings involve the biochemical alterations in terms of macromolecules and

the activities of related enzymes to cope with the Pb-stress.

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