Effect of Heavy Metal Treatment on Molecular Changes in Root Tips of *Lupinus luteus* L.

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Abstract: Heavy metals cause changes either in the pattern of cellular stress protein expression or in the enzyme activity. In the present study, the effect of three different toxic element treatments (heavy metals Pb, Cd and metaloid As) on small heat shock proteins synthesis as well as the superoxide dismutase (SOD) activity in root tips of yellow lupine (*Lupinus luteus* L., varieta Juno) was studied. Solutions with four different concentrations from each salt [$Pb(NO_3)_2$, $Cd(NO_3)_2$ and As_2O_3] were applied to achieve ascending oxidative stress conditions in comparison to unstressed, water treated, control variant. SDS-PAGE of crude protein extracts from treated variants showed enhanced protein signals in 15–17 kDa area comparing to the control one. The increased proteosynthetic activity indicates the possible participation of the low molecular weight proteins in the cell defence reactions. Similar increasing tendency was observed in the case of SOD enzymatic activity. Presence of heavy metals resulted in immediate elevated enzymatic response. Our results showed that methods applied in this study can be used for detection of oxidative stress in plants.

Keywords: oxidative stress; heavy metals; superoxid dismutase; lupine

INTRODUCTION

Typical character of toxic heavy metals is a high environmental persistence. The highest level of metal persistence was observed in soil substrate, what resulted in their uptake by plants through the roots (Tomáš *et al.* 2008; Vollmannová *et al.* 2008). In many cases the input is not substrate – specific, what subsequently leads to huge uptake of toxic metals. One of the biochemical changes occurring in plants subjected to heavy metal treatment is the production of reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydrogen peroxide, singlet oxygen and hydroxyl radicals ($^\bullet$OH) (Cho & Park 2000). The enzymes like superoxide dismutase (SOD), peroxidases (POX) and catalases (CAT) play main role in the scavenging of ROS. Uniqueness of SOD enzyme is given by ability to inactivate the superoxide radical and produce hydrogen peroxide (Gupta *et al.* 1999), which is consequently reduced by other enzymes of cell redox system (POX, CAT). SOD is reported as a primary enzyme in the cell oxidative defending system (Bowler *et al.* 1992) as well as the main signal molecule of oxidative stress. A different defending reaction of a plant to heavy metal treatment was described by Przymusiński and Gwóźdź (1999). They observed that presence of $Pb(NO_3)_2$ during yellow lupine (*Lupinus luteus* L.) germination caused an increased synthesis of stress protein with relative molecular weight 16 kDa. Western blott analysis confirmed that 16 kDa protein is immunologically related to PR-10 proteins (Przymusiński *et al.* 2004).

The aim of our research was to evaluate the possibility of usage of SOD and 16 kDa protein as a reliable marker of oxidative stress caused by toxic element treatment (Pb, Cd, and As).

MATERIAL AND METHODS

Plant material. Seeds of yellow lupine (*Lupinus luteus* L.) were sterilised in 0.2% $HgCl_2$, washed

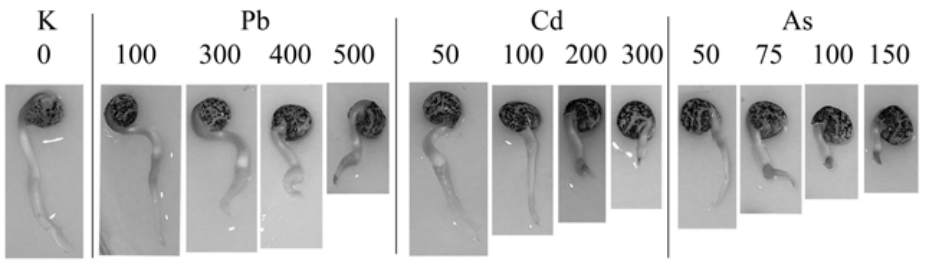

Figure 1. Yellow lupine root growth inhibition after 48 h of heavy metal treatment

in sterilised water and germinated in Petri dishes containing water-moistened filter paper until they reached 6–8 mm in length (48 h). After 48 h of germination, 10 ml of each solution with four different concentrations of toxic element was added. Final concentrations of ions varied from 100 to 500 mg/dm^3 for Pb^{2+}, 50 to 300 mg/dm^3 for Cd^{2+} and 50 to 150 mg/dm^3 for As^{3+}. Seedlings were growing under stress conditions for next 48 h in dark at 23°C. Qualitative and quantitative changes, like root necrotisation, average root length and weight, were studied during the yellow lupine seeds germination.

Protein extraction and gel electrophoresis under denaturing conditions. Proteins were extracted according to Przymusiński and Gwóźdź (1999) from root tips (5 mm) and the total protein concentration was estimated (Bradford 1976). Protein samples (30 µg) were mixed with extraction buffer (2% SDS, 19% glycerol, 5% 2-mercaptoethanol, 1M TRIS-HCl, pH 6,8 and 0.001% pyronine G) and heated at 98°C for 4 min immediately before loading on 4% (w/v) stacking polyacrylamide gel and 16% (w/v) resolving gel. Electrophoresis was running at 80 V (Mini-PROTEAN 3 Biotech, US). Gels were stained in 0.5% Coomassie Brilliant Blue R 250 in ethanol and 10% 3-chloroacetic acid. Protein ladder (Fermentas, Canada) was used as a standard.

Determination of SOD activity. Root tips of yellow lupine were frozen in liquid nitrogen, ground to a fine powder and resuspended in extracting buffer according to Pereira *et al.* method (2002). Total protein concentration was measured (Bradford 1976). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT (Beauchamp & Fridovich 1971). After adding the protein extract and exposition to light (50 µmol/m^2/s^1) for 15 min the photoreaction started. Absorbance at 560 nm was measured on Microplate Reader ELx808IU (Bio-Tek Instruments, US).

RESULTS

Toxic element treatment caused a significant quality reduction as well as inhibition of growth of yellow lupine roots (Figure 1). The tolerance indexes for toxic elements were calculated as a ratio of average length of roots growing in tested solutions and water (Wilkins 1957). Negative effect of toxic elements was proportional to their increasing concentration in solution. Application of As_2O_3 was observed as the most limiting growth factor in the relation to control variant (H_2O) (Figure 2).

The most evident changes in protein signals were detected in 15–17 kDa area (Figure 3). The same proteins were described by Przymusińskі and Gwóźdź (1999) after $Pb(NO_3)_2$ treatment with concentration of 350 mg/dm^3. Authors classified polypeptides of the 16 kDa region as the PR-10 family pathogenesis related proteins. Other studies of PR-10 proteins revealed their ability to react with different metal ions as Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+} (Przymusińskі *et al.* 2004; Souza *et al.* 2008).

The protein extracts from lupine roots were used for detection of SOD activity. Comparing

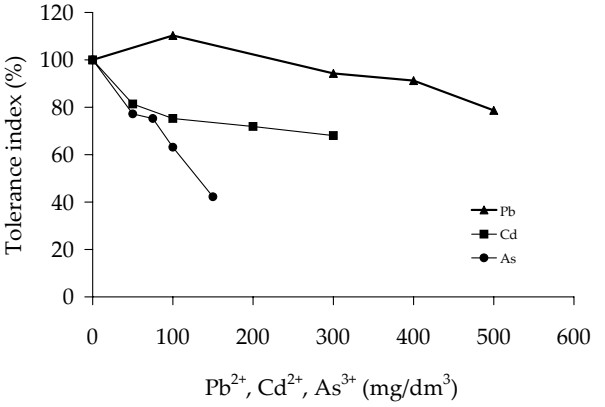

Figure 2. Tolerance indexes presenting proportional (%) changes of root length after application of different heavy metals ($Pb(NO_3)_2$, $Cd(NO_3)_2$, and As_2O_3

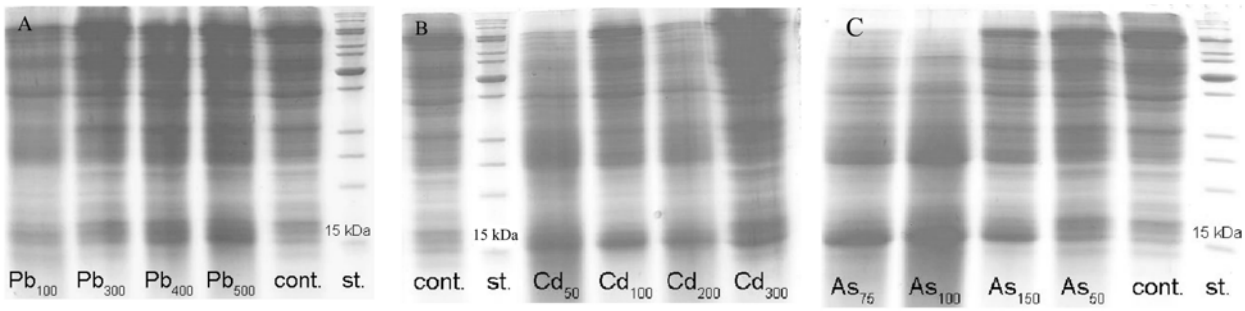

Figure 3. Polypeptide pattern after $Pb(NO_3)_2$ (A), $Cd(NO_3)_2$ (B), As_2O_3 (C) treatment. Pb_{100}–Pb_{500} = concentrations of Pb^{2+}, Cd_{50}–Cd_{300} = concentrations of Cd^{2+}, As_{50}–As_{150} = concentrations of As^{3+}, cont. = control variant (water), st. = protein ladder

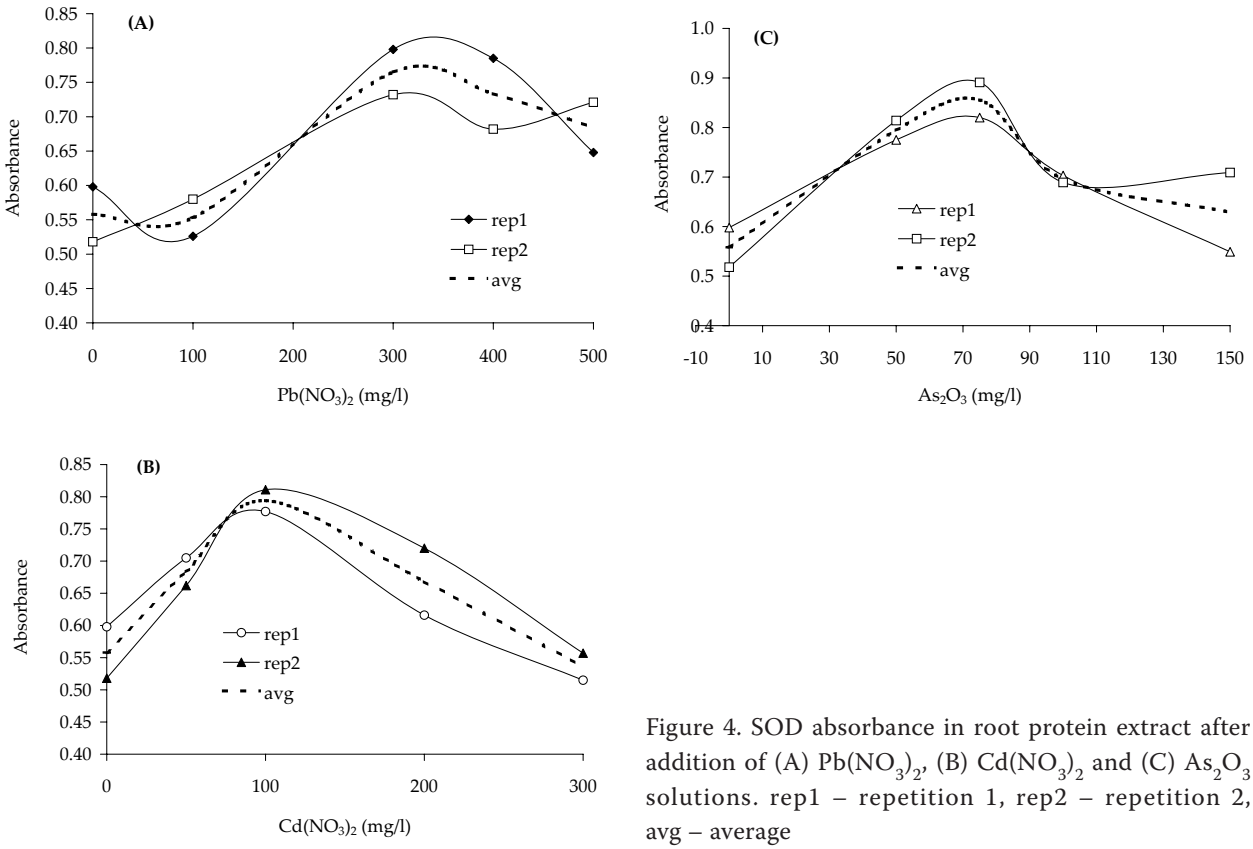

Figure 4. SOD absorbance in root protein extract after addition of (A) $Pb(NO_3)_2$, (B) $Cd(NO_3)_2$ and (C) As_2O_3 solutions. rep1 – repetition 1, rep2 – repetition 2, avg – average

the SOD activity in different variants of the experiments, some similarity in activation progress was observed. First two applied concentrations of each element induced a strong increase of SOD activity (Figure 4). However, further elevation of their concentration had the inverse effect. The decrease can be explained by disturbed oxidation-reduction balance caused by a high level of ROS. The same effect was observed during the exposition of pea roots to lead longer than 48 h (Malecka *et al.* 2001) and bean treated with Cd^{2+} (Somashekaraiah *et al.* 1992).

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