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EFFECTS OF CADMIUM STRESS ON ANTIOXIDANT ENZYMES IN *VIGNA RADIATA* ROOTS

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ABSTRACT: A brief study to analyze oxidative stress and antioxidant response of cadmium stress was carried out. The plant chosen *Vigna radiata* (var. SML-668) was hydroponically grown in 0, 50µM, 100µM and 150µM, cadmium chloride. The roots were taken from hydroponic culture at 6 days of growth for various enzymatic and metabolic esti-mations. Among the antioxidant enzymes, root catalase and ascorbate peroxidase decreased while guaiacol peroxidase and superoxide dismutase increased in response to cadmium. The lipid peroxidation and protein oxidation too increased probably due to production of active oxygen species by heavy metal stress which causes lipid peroxidation. So, Cd was definitely toxic to root system of *V. radiata* plants as indicated by consistent increase in peroxidation and protein oxidation. However, the plant was able to counter this partially by concomitant increase in SOD and guaiacol peroxidase. But, the other two antioxidant enzymes catalase and ascorbate peroxidase could not counter oxidative stress generated by Cd as they were decreased.

INTRODUCTION: Cadmium is a heavy and non-essential element which is released into the environment by power stations, heating systems, metal-working industries or urban traffic. Its high toxicity and large solubility in water has made it an important pollutant. [1] Plant Cd concentrations are between 0.05-0.2ppm, [2] but can be much higher in plants growing in contaminated soils. Heavy metals are generally involved in generation of reactive oxygen species (ROS). [3] As an example, transition metals like Fe^{3+} and Cu^{2+} participate in the well-known Haber-Weiss cycle, producing HO^{-} from O_2^{-} and H_2O_2 [4].

Metals without redox capacity (such as Cd^{2+}, Pb^{2+}, and Hg^{2+}) can enhance the prooxidant status by reducing the antioxidant glutathione (GSH) pool, activating calcium-dependent systems and affecting iron-mediated processes. [1] These heavy metals can also disrupt the photosynthetic electron chain, lead-ing to O_2^{-} and $^{1}O_2$ production [5]. So, hydroponic cultivation of *Vigna radiata* in 50/100/150 µM Cd stresses with lipid and protein peroxidation in cadmium stressed *V. radiata* roots were studied.

MATERIALS AND METHODS:
Collection of experimental material:
The certified seeds of *Vigna radiata* (cv. SML 668) were purchased from local market.

Growth and collection of plant material:
Seeds of *V. radiata* were soaked overnight in minimum DW and surface sterilized next day in 0.1% $HgCl_2$. The pre-soaked and sterilized seeds were then germinated hydroponically in nutrient

solution (Hoagland's solution) containing 8mM KNO_3, 2mM $Ca(NO_3)_2$, 1mM KH_2PO_4, 1mM $MgSO_4$ and micronutrients: 30µM H_3BO_4, 5µM $MnSO_4$, 1µM $CuSO_4$, 1µM $(NH_4)_6Mo_7O_{24}$, 1µM $ZnSO_4$ and 1µM $CoCl_2$ (Dguimi *et al*, 2009).[6]

Preparation of Plant crude extract:
Fresh root tissue was homogenized (1:5, w/v) in ice-cold 0.1M potassium phosphate buffer of pH 7.5 and containing 3mM dithiothreitol, 1mM EDTA and 1% PVP. The mixture was centrifuged at 12000rpm for 15min at 4°C and supernatant used for enzyme assays.

Assays of antioxidant enzymes:
Catalase (CAT) was estimated by the method of Vitoria *et al* (2001).[7] The assay mixture was 0.1ml extract added to 1.9ml of 50mM potassium phosphate buffer (pH 7.0) containing 10mM H_2O_2. The decreases in absorption were noted at 240nm and quantified from extinction coefficient of 0.036 $mM^{-1} cm^{-1}$ and activity expressed as µmol H_2O_2 reduced $min^{-1} g^{-1}$ FW.

Guaiacol Peroxidase (GPX) was estimated with guaiacol as substrate according to the method of Vitoria *et al* (2001).[7] The assay mixture was 0.1ml extract added to 1.9ml of 50mM potassium phosphate buffer (pH 7.0) containing 10mM H_2O_2 and 9mM guaiacol. The formation of tetraguaiacol was monitored by noting increase in absorbance at 470nm and quantified using the extinction coefficient 26.6 $mM^{-1} cm^{-1}$ and activity expressed as micro mol guaiacol oxidized $min^{-1} g^{-1}$ FW.

Activity of Superoxide dismutase was done according to the modified method of Beauchamp and Fridovich (1971) by monitoring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) to purple formazan at 560nm. The 3ml reaction mixture consisted of 2.9ml 50mM potassium phosphate buffer (pH 7.8) containing 10mM methionine, 168µM NBT, 0.025% Triton X-100 and 1.17µM riboflavin, and 0.1ml enzyme. The amount of methionine-mediated formazan formed (A_t) was compared with amount of formazan formed in the absence of enzyme (A_c) and activity was finally reported as Units per min per mg FW. Ascorbate peroxidase was estimated by the method of Mishra *et al* (2006) by monitoring the rate of

ascorbate oxidation (µ=2.8 $mM^{-1} cm^{-1}$). The assay mixture was 0.1ml extract added to 1.9ml 50mM potassium phosphate buffer (pH 7.0) containing 0.1mM H_2O_2, 0.5mM ascorbate and 0.1mM EDTA. The change in absorbance was monitored at 290nm and activity expressed as µmol ascorbate oxidized $min^{-1} g^{-1}$ FW.

Lipid Peroxidation: It was done by method of Zhang *et al* (2010).[8] 1g roots were homogenized in 5ml 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 10 min at 4°C. To 2 ml supernatant, 2 ml of 20% TCA containing 0.67% TBA was added. The mixture was heated at 90°C for 30 min for formation of pink-colored 1:2 adduct between MDA & TBA and then quickly cooled on ice. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant at 532 nm was read, and the value for the non specific absorption at 600nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155$mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW.

Protein oxidation: The protein oxidation was assessed by estimation of carbonyl groups formed during the experiment (Mercier *et al*., 2004). For each sample of homogenate, two equal aliquots of 0.5 ml were taken and proteins in both were precipitated by 10% trichloroacetic acid (w/v, final concentration) and centrifuged at 5000 g for 10 min. Pellet was mixed in 2ml 2N HCl and added 2mg 2,4-dinitrophenylhydrazine (DNPH) to 1ml from it. Both samples were incubated for 1 h at room temperature and proteins of both samples were reprecipitated with 10% TCA (w/v final concentration) by centrifugation at 5000 g for 10 min. The pellets were then washed twice with 1 ml of ethanol: ethyl acetate (1:1) to eliminate traces of DNPH and to solubilise residual lipids. Carbonyl concentration was calculated through extinction coefficient of 21.0 $mM^{-1} cm^{-1}$ at 370 nm for protein hydrazones.

RESULTS: The plant systems face damage due to interference with uptake of many essential minerals, hampering the photosynthesis, causing oxidative stress i.e. rise in ROS (hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide) due to interference in activities of

antioxidant enzymes. However, Cd stress effects germination of seeds also and manifestation of Cd stress is expected to occur as modulation of antioxidant enzymes. Higher plants can uptake Cd, depending on its availability and concentration, from soil or water; rather little is taken up directly from the atmosphere (Clemens, 2006).[9] So, this study was conducted to carry out brief *in vivo* analysis of antioxidant enzymes' behaviour in plant system.

Catalase: This enzyme from *V. radiata* root was suppressed with increase in exogenous Cd level and at all Cd levels (**Fig. 1**). In present study, it may be suggested that CAT activity was probably compensated by other isoperoxidases (Wu *et al*, 2003).
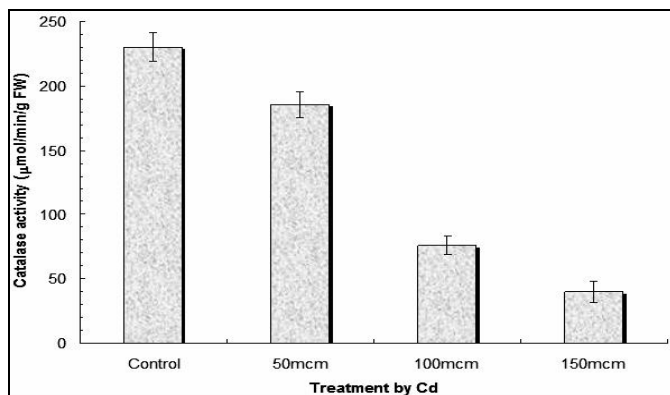

FIG. 1: CHANGES IN ROOT CATALASE ACTIVITY OF *V. RADIATA* SEEDLINGS UNDER VARIOUS REGIMES OF Cd.

Guaiacol peroxidase: This enzyme from *V. radiata* root was stimulated by all the levels of Cd in external medium (**Fig. 2**). This was probably due to overproduction of AOS by Cd stress to reduce the harmful effect of Cd generate AOS.
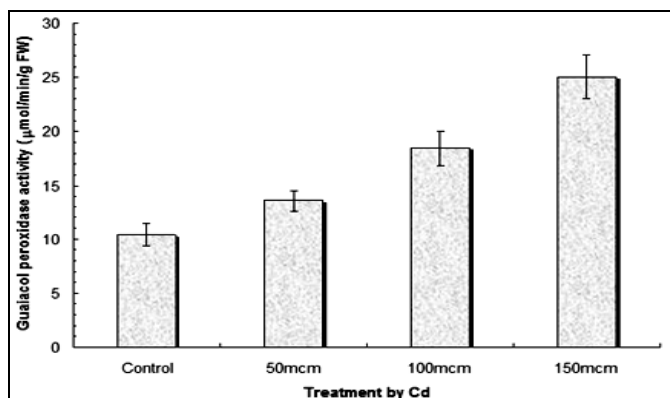

FIG. 2: CHANGES IN ROOT GUAIACOL PEROXIDASE ACTIVITY OF *V. RADIATA* SEEDLINGS UNDER VARIOUS REGIMES OF CADMIUM.

Superoxide dismutase: The superoxide dismutase of *V. radiata* root was stimulated by the Cd till 100μM but suppressed slightly by 150μM Cd in external medium (**Fig. 3**). This was probably due to that Cd has been shown to enhance lipid peroxidation (Halliwell and Gutteridge, 1989), which leads to AOS formation.
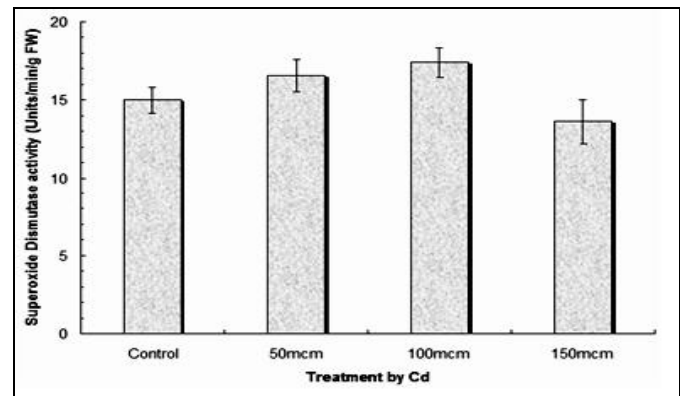

FIG. 3: CHANGES IN ROOT SUPEROXIDE DISMUTASE ACTIVITY OF *V. RADIATA* SEEDLINGS UNDER VARIOUS REGIMES OF CADMIUM

Ascorbate peroxidase:
The ascorbate peroxidase was stimulated by only 50μM Cd but inhibited by the two higher levels (**Fig. 4**). It may be suggested that plant tolerance mechanism involve a system that remove free radicals, preventing the accumulation of O_2*^-, and therefore increasing the requirement of antioxidative peroxidases viz. ascorbate peroxidase.
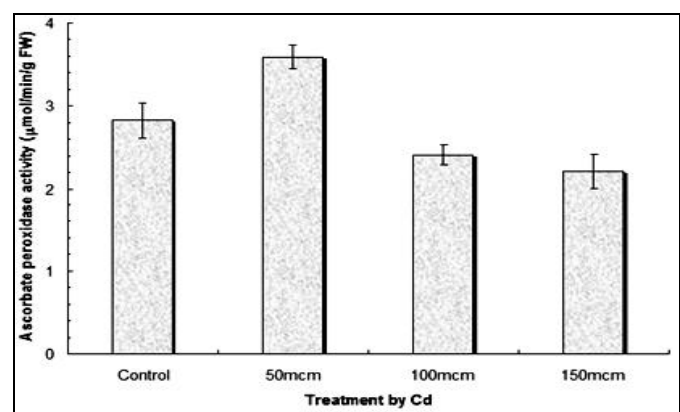

FIG. 4: CHANGES IN ROOT ASCORBATE PEROXIDASE ACTIVITY OF *V. RADIATA* SEEDLINGS UNDER VARIOUS REGIMES OF Cd.

Lipid peroxidation: In present study level of lipid peroxidation continuously increased with increase in exogenous Cd level throughout the studied range (**Table 1**). This was probably due to production of

active oxygen species by heavy metal stress (Greger *et al*., 1995 and Lin *et al*., 2000).[10, 11]

TABLE 1: LEVEL OF LIPID PEROXIDATION CONTINUOUSLY INCREASED WITH INCREASE IN EXOGENOUS Cd LEVEL THROUGHOUT THE STUDIED RANGE

Treatments	Lipid peroxidation (nmoles MDA /g FW)	Protein oxidation (nmoles/mg protein)
Control	56.466±4.31	367.3±17.12
50µM Cd	64.297±5.16	447.47±16.34
100µM Cd	85.86±3.32	618.49±12.17
150µM Cd	87.14±3.15	495.48±17.24

Protein oxidation: In present study, amount of protein oxidation was increased with increasing Cd concentration up to 50 and 100µM followed by reduced protein oxidation at Cd concentration 150µM. But in whole study amount of protein oxidation was much higher over control (**Table 1**).

CONCLUSION: Cd was definitely toxic to root system of *V. radiata* plants as indicated by consistent increase in peroxidation and protein oxidation. However the plant was able to counter it partially by concomitant increase in SOD and guaiacol peroxidase. The other two antioxidant enzymes catalase and ascorbate peroxidase could not counter oxidative stress generated by Cd as they were decreased.

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