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Effect of Cadmium on Lipid Peroxidation and Activities of Antioxidant Enzymes in Moth Bean Callus

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Abstract: Effect of cadmium on lipid peroxidation and some antioxidant enzymes was studied in callus of moth bean after 7, 14 and 21 days of cadmium nitrate ($Cd(NO3)_2$) addition to culture medium. Cd enhanced malondialdehyde (MDA) content in moth bean callus indicating enhanced lipid peroxidation. Superoxide dismutase (SOD) activity also significantly increased following cadmium addition in culture medium. In contrast, activities of catalase (CAT), peroxidase (PO), polyphenoloxidase (PPO), ascorbate peroxidase(APX) and acid phosphatase (acid-P) were significantly reduced. Results suggest that Cd induced oxidation injury in moth bean callus is caused by enhancing lipid peroxidation and inhibiting activities of some enzymes viz., CAT, PO and APX of antioxidant defense mechanism. Enhanced SOD activity indicates that the enzyme served as important component of defense mechanism against metal-induced oxidation injury.

Key words: Antioxidant enzymes, cadmium, lipid peroxidation, *Vigna aconitifolia* L. callus.

Heavy metals exert adverse effects on physiological and biochemical activities of plants including increased permeability of cells due to membrane damage in some plants (Jeana and Choudhary, 1982; Bora *et al.*, 2003). Membrane damage in plants is mainly due to disbalance between production of active oxygen species (AOS) and quenching activity of antioxidants under various environmental stresses (Dhindsa and Matowe, 1981; Zhang and Kirkham, 1996; Shah *et al.*, 2001; Bora *et al.*, 2003).

The study of the role of antioxidants in mechanism of metal tolerance is relatively recent (Shah *et al.*, 2001; Bora *et al.*, 2003). So in the present investigation, attempts have been made to examine mechanism of cadmium injury by assessing extent of membrane damage by measuring lipid peroxidation product MDA and by study of role of some antioxidant enzymes like SOD, CAT, PO and APX which are known to scavenge AOS in plants. In order to study the mechanism of Cd tolerance, callus cultures are considered the most convenient system as it provides more standardized experimental conditions and eliminates interfering process of translocation and organ specific trapping of ions.

Materials and Methods

Seeds of moth bean (*Vigna aconitifolia* (JACOQ) Marechal) cv RMO-40 were surface sterilized with 1% sodium hypochlorite for 5 minutes and then germinated on Murashige and Skoog's (MS) revised medium (Murashige and Skoog,

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1962) in flask. After 8 to 10 days hypocotyl segments were aseptically excised and transferred to MS medium supplemented with 0.5 mg L^{-1} kinetin and 1.5 mg L^{-1} 2,4-D (callus medium) to initiate callus formation. After several subcultures a mass of uniform callus (approx. 100 mg) were transferred to callus medium supplemented with cadmium nitrate 3.08 mg L^{-1} (10 μM) and 7.7 mg L^{-1} (25 μM).

Biochemical parameters were measured 7, 14 and 21 days following callus transfer to medium containing cadmium nitrate. Lipid peroxidation in the callus was measured in terms of MDA, a decomposition product of the peroxidation of polyunsaturated fatty acids, as thiobarbituric acid reactive substances (Heath and Packer, 1968; Dhindsa *et al.*, 1981) from callus extracts in 5% TCA. After centrifugation, the absorbance of the extract was read at 532 nm and the values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using its extinction coefficient (Heath and Packer, 1968).

For assay of PO, APX, PPO, acid-P and protease, 500 mg of callus was extracted in 0.05 M Tris-HCl buffer (pH 7.0) containing 0.001 M EDTA and 0.003 M $MgCl_2$ as described earlier (Bora *et al.*, 1980; 1991). The PO activity was measured as a change in absorbance at 470 nm by incubating enzyme with hydrogen peroxide and guaiacol (Bora *et al.*, 1980). The increase in absorbance at 470 nm was followed for one minute. PPO was assayed as described earlier (Bora *et al.*, 1980). P-nitrophenyl phosphate was used to a substrate for assay acid-P activity (Zink and Veliky, 1979) and the activity was expressed as change in absorbance h^{-1} g^{-1} fr wt. (Bergmeyer, 1974). Protease was assayed by the method of Yomo and Varner (1973). APX and SOD were assayed by the method described by Zhang and Kirkham (1996). Activity of catalase (CAT) was measured by the method of Chance and Maehly (1955) and expressed as change in absorbance h^{-1} g^{-1} fr wt. Experiment was laid out in complete randomized design. All data are means of four replications. Data were analyzed by analysis of variance to determine the significance of treatments.

Results and Discussion

The membrane damage is often related to membrane lipid peroxidation caused by free radicals and hydroperoxides (Hendry *et al.*, 1992). MDA showed greater accumulation in Cd-stressed callus tissue (Table1), indicating free radicals mediated membrane damage in callus under Cd-stress. It has been currently assumed that negative effects of various stresses is partially due to generation of AOS and/or inhibition of the system which defends against them (Mishra and Choudhuri, 1996; Shah *et al.*, 2001). When plants are subjected to stresses the balance between the production of AOS and the quenching activity of antioxidants may be upset and oxidative damage may occur (Dhindsa and Matowe, 1981; Bartosz, 1997). Plants have evolved number of antioxidants to detoxify and eliminate highly reactive species. The antioxidant defence system includes SOD, CAT, PO and APX besides hydrophilic and hydrophobic low molecular mass antioxidants. SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (Scandalios, 1993; Reddy *et al.*, 1998). Hydrogen peroxide is broken down to water by CAT, APX and PO, thereby minimizing

Table 1. Effect of Cd^{2+} on malondialdehyde content (MDA) and superoxide dismutase (SOD) activity in moth bean callus 7, 14 and 21 days after initiation of cultures

Treatments	MDA content (Per cent over control)			SOD Activity (Per cent over control)		
	Days			Days		
	7	14	21	7	14	21
Control	100	100	100	100	100	100
Cd^{2+} 10 µM	145	105	110	105	185	285
Cd^{2+} 25 µM	165	115	155	110	285	95

chances of lipid peroxidation and membrane damage (Bartosz, 1997).

Addition of Cd to medium, increased SOD activity in callus tissue over control, possibly due to detoxification of O_2-radical, generated under the Cd-stress by converting them into H_2O_2 (Table 1). However, the activities of other scavenging enzymes of defence mechanism, like PO and APX were reduced drastically throughout the callus growth following Cd incorporation in medium (Table 2), indicating their inability to detoxify H_2O_2 produced by enhanced activity of SOD in the system, thus resulting in membrane damage. Similarly, PPO activity in Cd-treated callus tissue was low compared to control. Where as enhanced PPO activity has been reported in damaged tissue by biotic stress, and it has been suggested that activation of PPO is non-specific consequence for tissue injury, rather than the cause of injury (Daly, 1972).

Enhanced protease (Mukherjee and Rao, 1993) and acid-P activities have been reported in leaves due to dark induced senescence. In present study, increased protease activity in Cd stress callus tissue may be due to injury to callus tissue. The acid-P activity was not found to correlate with Cd-stress tissue injury as it exhibited lower activity of the enzyme (Table 3).

Results suggest that Cd-stress, not only accelerates generation of AOS in callus tissue of the moth bean as evidenced by accumulation of MDA in treated callus, but also drastically reduced activities of scavenging enzymes (antioxidant enzymes) like CAT, PO, and APX of defense mechanism against AOS, thus resulting in callus tissue injury under Cd-stress.

Table 2. Effect of Cd^{2+} on catalase (CAT), peroxidase (PO), polyphenloxidase (PPO) and ascorbate peroxidase (APX) in moth bean callus

Treatments	Days after callus transfer											
	CAT (Δ_{240} h^{-1} g^{-1} f.wt.)			PO (Δ_{470} h^{-1} g^{-1} f.wt.)			PPO (Δ_{420} h^{-1} g^{-1} f.wt.)			APX (Δ_{290} h^{-1} g^{-1} f.wt.)		
	7	14	21	7	14	21	7	14	21	7	14	21
Control	2.14	1.64	0.70	84.4	6.69	22.8	30.1	26.8	23.3	19.4	14.0	8.52
Cd^{2+} 10 µM	1.56	1.21	0.22	71.2	4.46	22.6	27.3	22.3	19.2	0.54	9.8	1.67
Cd^{2+} 25 µM	1.31	0.93	0.13	37.2	2.39	7.5	28.2	20.0	16.5	1.94	7.7	0.79
CD 1%	0.25	0.25	0.25	9.3	9.3	9.3	2.0	2.0	2.0	1.3	1.3	1.3

Table 3. Effect of Cd^{2+} on protease (Pro) and acid phosphatase (acid-P) activity in moth bean callus 7, 14 and 21 days after initiation of cultures

Treatments	Days after callus transfer					
	Protease (Δ_{280} h^{-1} g^{-1} f.wt.)			Acid-P (Δ_{410} h^{-1} g^{-1} f.wt.)		
	7	14	21	7	14	21
Control	61.7	110.9	124.8	73.7	121.8	96.2
Cd^{2+} 10 µM	75.9	121.0	128.3	79.8	91.3	81.0
Cd^{2+} 25 µM	114.7	132.1	136.3	51.3	46.3	40.4
CD 1%	5.7	5.7	5.7	15.6	15.6	15.6

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