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Original Research Paper

Cadmium-Induced Changes in Antioxidative Enzyme Activities and Content of Leaf Pigments in *Cajanus cajan* (L.)

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ABSTRACT

Experiment was conducted to investigate the effects of different concentrations of cadmium (10 ppm, 20 ppm and 30 ppm) on antioxidant enzyme activities and leaf pigment content of *Cajanus cajan.* Content of chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and total carotenoids in primary leaves of *Cajanus cajan* was decreased with increase in cadmium concentration. Chlorophyll-*b* was more sensitive than chlorophyll-*a* to cadmium stress. Activities of enzymes such as CAT and POD were enhanced with increase in cadmium treatment. More increase was observed in leaves as compared to root and stem.

INTRODUCTION

Metal pollution is continuously increasing due to anthropogenic activities, which interferes with the environment and make the conditions toxic for living organisms. Cadmium enters into food chains, and thus become harmful to humans (Stolt et al. 2006). High cadmium contents in plants inhibit the growth, leaf pigments and induce even death (Hassan et al. 2005, Burzynski & Zurek 2007, Ebbs & Uchil 2008, He et al. 2008).

Plants adopt different strategies to reduce cadmium induced oxidative damage. One of them is the increase of activities of antioxidative enzymes (Gratao et al. 2006). The antioxidant system of plants tends to eliminate or reduce cell injury by reactive species (ROS) (Salin 1987, Foyer et al. 1994). The enzymatic antioxidant system is a protective mechanism that operates with the sequential and simultaneous action of various enzymes including POD, CAT and superoxide dismutase (SOD). Hydrogen peroxide (H_2O_2) is eliminated by various antioxidant enzymes such as CAT, which are located in peroxisomes and mitochondria (Adam et al. 1995, Willekens et al. 1997). POD participates in lignin biosynthesis, IAA degradation and conversion of H_2O_2 to water, while MDA formation is considered to be the general indicator of lipid peroxidation (Salin 1987, Somashkaraiah et al. 1992, Chaoui et al. 1997). Cadmium also induces oxidative stress in plant cells. Cadmium can either inhibit or stimulate the activities of several antioxidative enzymes before any visible symptom of toxicity appears (Sandalio et al. 2001). Heavy metals are known to interfere with chlorophyll synthesis either through the direct inhibition of an enzymatic step or through the induced deficiency of an essential nutrient (Van Assche & Clijters 1990).

Pigeon pea (*Cajanus cajan*) is an important grain legume commonly grown and consumed in tropical and subtropical regions of the world. India accounts for over 80% of the world supply of pigeon pea. The chemical composition of *C. cajan* was found to contain protein (22-27 %), crude fibres (7-10 %) and lysine (7.59 %). *C. cajan* is a good source of dietary minerals such as calcium, phosphorus, magnesium, iron, sulphur and potassium. Also *C. cajan* is a good source of soluble vitamins, especially thiamin, riboflavin, niacin and choline (Singh 1977).

MATERIALS AND METHODS

The germination was carried out in Petri dishes. Seeds were surface sterilized with H_2O_2 for the prevention of surface fungal/bacterial contamination. Different ppm solutions were prepared in pure distilled water in laboratory by using Cd $(NO_3)_2$ (cadmium nitrate). Pure distilled water was used as control for the study. Ten seeds were placed on cotton in each Petri dish and 40 mL solution of each concentration was supplied once for seed germination. Distilled water was applied every alternate day after this treatment. The Petri dishes were monitored daily for fungal and other inspections. The studies were carried out in control, 10ppm, 20ppm and 30ppm of cadmium concentrations. Enzyme activities and leaf pigments were estimated on 10^{th} and 20^{th} day after seed sowing. Enzyme activities were estimated in different organs of the test plant.

Enzyme assays: Plants were harvested at the experimental period and thoroughly washed under running tap water. The leaves, roots and stems were collected for the preparation of extracts used for the enzyme assays. Crude extract was prepared by grinding 1g of leaves in 10 mL distilled water using ceramic mortar and pestle. Filtration was made using Whatman No. 1 filter paper and the filtrate was used for enzyme analyses (De Biasi et al. 2003).

CAT activity was measured according to the method of Matsumura et al. (2002) in a reaction mixture containing 1 mL of 5 mM potassium phosphate (pH 7.0), 1 mL of 45 mM H_2O_2 and 1 mL of the crude extract. The activity was determined by the decrease of absorbance at 240 nm due to H_2O_2 consumption using spectrophotometer. Activity was expressed in units/g fresh weight.

POD activity was determined according to the method of Chanda & Singh (1997). The reaction mixture contained 1 mL of the extract, 1 mL of 1 mM H_2O_2, 1 mL of 4 mM guaiacol and 1 mL 8 mM potassium phosphate buffer (pH 6.5). The change in absorbance at 470 mM due to the oxidation of guaiacol to form tetraguaiacol in the presence of H_2O_2 was measured. The peroxidase activity was expressed in units/g fresh weight.

For pigment determination, 500 mg of dry leaf samples were homogenized in 20 mL of 80 % acetone using mortar and pestle and centrifuged at 6000 rpm for 15 minutes. Finally, the supernatant was made up to 20 mL and optical densities (O.D.) were measured at 480 and 510 nm wavelengths for carotenoids and 645 and 663 nm for chlorophyll on a UV-VIS spectrophotometer. The amount of chlorophyll-*a* and *b*, and carotenoids were calculated by using the formulae given by Machlachlan & Zalik (1963) and Duxbury & Yentsch (1956). Total chlorophyll was calculated by adding the amounts of chlorophyll-*a* and chlorophyll-*b*.

$$\text{Chl-}a\text{ (mg/g F.W.)} = \frac{[(12.3 \times D_{663}) - (0.86 \times D_{645})] \times V}{d \times 1000 \times W}$$

$$\text{Chl-}b\text{ (mg/g F.W.)} = \frac{[(19.3 \times D_{645}) - (3.6 \times D_{663})] \times V}{d \times 1000 \times W}$$

$$\text{Caratenoids (mg/g F.W.)} = \frac{[7.6 \times D_{480} - 1.49 \times D_{510}] \times V}{d \times 1000 \times W}$$

Where, V = Volume of extract (mL)
d = Length of light path
W= Fresh weight of sample (g)

RESULTS AND DISCUSSION

Activities of antioxidative enzymes such as CAT and POD were assayed in leaves, root and stem of *Cajanus cajan* grown under the influence of different cadmium concentrations. The activities of CAT (Fig. 1) and POD (Fig. 2) were enhanced with increasing cadmium concentration.

The results presented in this study showed that heavy metals increased the activity of CAT and POD in test plant. It is possible that heavy metal stress reduce the capacity of the plants to assimilate carbon; this would trigger an increase in photosynthetic electron flux to molecular oxygen, resulting in the increased production of CAT and POD. Since, these reactive oxygen species are damaging to lipids, proteins and pigments, they are rapidly scavenged by antioxidant enzymes. There are evidences that increased levels of these scavenging enzymes may play a role in limiting the degree of photodamage to plants (Rao & Sresty 2000, Schutzendubel et al. 2001).

It is well known that CAT and POD play an important role in preventing oxidative stress by catalysing the reduction of hydrogen peroxide (Weckx & Clijsters 1996). Plants adopt different strategies to reduce cadmium induced oxidative damage. One of them is the increase of activities of antioxidative enzymes (Gratao et al. 2006).

Marked increase in CAT and POD activity was found in different organs of the plant on exposure of 10ppm cadmium and above that producing noticeably higher amount of CAT and POD than control. The plants with 30ppm cadmium treatment showed notably higher CAT and POD activity levels than all other treatments.

Devi & Prasad (1998) found that CAT and POD activities increased on heavy metal treatment, suggesting that excess heavy metal may increase the production of hydrogen peroxide (H_2O_2). Hydrogen peroxide is a necessary substrate for the cell wall stiffening process catalysed by cell wall peroxidase, which is considered to be one of the mechanisms resulting in growth inhibition. CAT is an enzyme involved in antioxidant defence that eliminates peroxide. Oxidative stress is the phenomenon implicated as one of the main causes of cellular damage in all organisms exposed to a wide variety of stress conditions (Foyer et al. 1994). In many plants, the toxicity of cadmium has been related to the increase of lipid peroxidation and alteration of antioxidant systems (Shaw 1995).

The primary leaves of *Cajanus cajan* were affected by cadmium, which resulted in a decline of chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid content (Table 1 and Table 2). Presence of 10 ppm of cadmium resulted in a significant decrease of pigments. Beyond that concentration, decrease of pigments content was observed with elevated concentration level of cadmium. When *Cajanus cajan* was exposed to 30 ppm cadmium, the amount of pigments reached

Table 1: Effects of cadmium on leaf pigments (mg/g fresh weight) of *Cajanus cajan* on 10th day of growth.

Content	Control	Concentration of cadmium		
		10 ppm	20 ppm	30 ppm
Chlorophyll-*a*	0.9294±0.0085	0.7821±0.0192 (15.84)	0.6877±0.0227 (26.00)	0.6021±0.0244 (35.21)
Chlorophyll-*b*	0.5686±0.0092	0.4639±0.0177 (18.41)	0.3512±0.0222 (38.23)	0.3039±0.0178 (46.55)
Carotenoids	0.1964±0.0072	0.1776±0.0046 (9.57)	0.1703±0.0052 (13.29)	0.1506±0.0045 (23.31)
Total chlorophyll	1.4980±0.0177	1.2460±0.0369 (17.12)	1.0389±0.0449 (32.11)	0.9060±0.0422 (40.88)

Table 2: Effects of cadmium on leaf pigments (mg/g fresh weight) of *Cajanus cajan* on 20th day of growth.

Content	Control	Concentration of cadmium		
		10 ppm	20 ppm	30 ppm
Chlorophyll-a	0.9558±0.0062	0.7620±0.0207 (20.27)	0.6603±0.0231 (30.91)	0.5671±0.0212 (40.66)
Chlorophyll-b	0.6121±0.0108	0.4112±0.0181 (32.82)	0.3080±0.0201 (49.68)	0.2585±0.0166 (57.76)
Carotenoid	0.1836±0.0083	0.1607±0.0076 (12.47)	0.1508±0.0063 (17.86)	0.1365±0.0056 (25.65)
Total chlorophyll	1.5679.0170	1.1732±0.0388 (26.54)	0.9683±0.0432 (40.29)	0.8256±0.0422 (49.21)

All the values are means ± S.D.; Values in parantheses are percentage decreased, compared to control

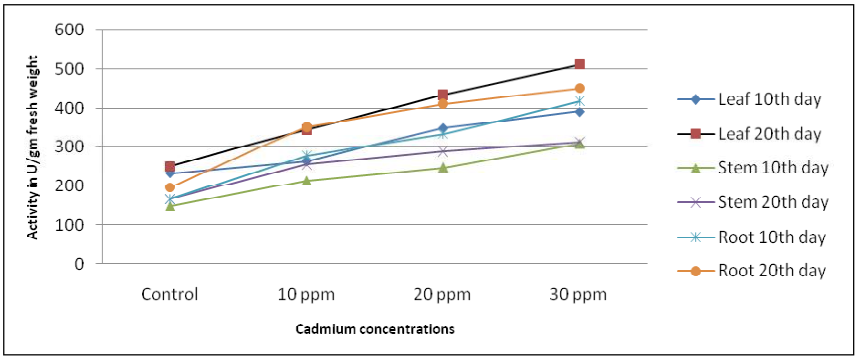

Fig. 1: Effects of cadmium on CAT activities of *Cajanus cajan.* .

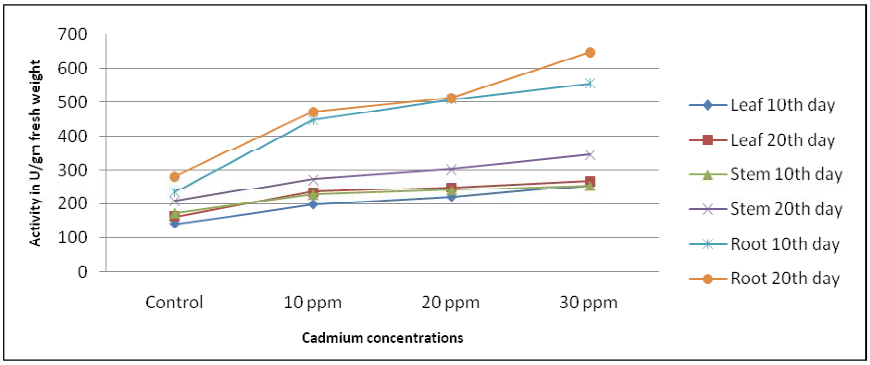

Fig. 1: Effects of cadmium on POD activities of *Cajanus cajan.* .

a minimum value. The result suggests that chlorophyll-*b* is more sensitive than chlorophyll-*a* to cadmium stress. Greater reduction in chlorophyll-*b* has also been reported in *Oryza sativa* and some mangrove species under heavy metal stress (Chen et al. 2008). The decrease in the chlorophyll content, which is related to heavy metal stress, may be the result of inhibition by heavy metals of the enzymes which are responsible for chlorophyll biosynthesis (Stobart et al. 1985). The mechanism of heavy metals on photosynthetic pigments may owe to three reasons:

1. Heavy metal inhibits uptake and transportation of other metal elements such as Mn, Zn and Fe by antagonistic

effects, and therefore, the fronds lose the capacity of synthesis of pigments (Gardea-Torresdey et al. 2004, Lin & Wu 1994).

2. Heavy metals are known for direct inhibition of an enzymatic step (Van Assche & Clijters 1990, Sharma & Agarwal 2005).

The toxic effects of cadmium on *Cajanus cajan* have been discussed in this paper. Our results demonstrated that cadmium has adverse effects on chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid content. While antioxidant enzyme activities like CAT and POD were increased.

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