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Effect of Cd on antioxidative system in *Phaesiolus mungo* and its interaction with certain micronutrients

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Abstract: In this study, the effect of cadmium (Cd) and its interaction with phosphorus (P) and copper (Cu) on lipid peroxidation, electrolye leakage percentage, catalase, peroxidase, pigment, fresh weight and dry weight in black gram (*Vigna mungo* var. PU35) were investigated. The experiment was conducted in the wire house condition. Clay pots with total area of 310 cm^2 per pot containing soil and compost into 3:1 ratio were used for growing the seed of black gram. The plants were treated with solution containing six Cd level as 3 $CdSO_4.7H_2O$ (0, 0.1, 0.2, 0.4, 1.0 and 2.0 mM L^{-1}) for 30 days. At the lowest Cd level the concentration of malondialdehyde were decreased, electrolye leakage percentage increased at higher concentration, whereas the content of carotenoids, fresh weight and dry weight were increased at lowest concentration of Cd. The activity of catalase were increased at all Cd level, while the activity of peroxidase decreases at lower level of Cd while increased at higher level of Cd. The results evidence the importance of enzymatic and non-enzymatic antioxidant system in response to cadmium toxicity in black gram.

Key words: *Vigna mungo*, Peroxidation, Electrolye leakage percentage, Catalase, Peroxidase, Pigment, Copper, Phosphorus, Cadmium

Introduction

Cadmium (Cd) is one of the most important metals in terms of food-chain contamination, because it is readily taken up by the cells of different plant species (Gomes-Junior *et al*., 2006a,b; Liu *et al*., 2007). The measuring contamination and consequential accumulation of heavy metals in the soil by anthropogenic activities, in particular the disposal of sewage sludge can be very serious in agricultural term if crops re grown and cultivated in such a polluted environment. Growth, yield, fruit quality and several physiological responses to sewage sludge were investigated in plant and in some cases in vitro culture. Among heavy metal, Cd which is not a nutrient for plants, is toxic and can accumulate in different human tissues and organ and preferentially in root of higher plant.

Certain heavy metals such as copper and iron can be toxic through their participation in redox cycler like Fenton and/or Haber-Weiss reactions. In contrast, cadmium is non-redox metal unable to perform single electron transfer reactions, and does not produce reactive oxygen species (ROS) such as super oxide anion (O_2^-), singles oxygen (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), but generates oxidative stress by interfering with antioxidative defense system (Benavides *et al*., 2005; Gratao *et al*., 2005).

In plants ROS is produced continuously as by products of various metabolic pathways that are localized in different cellular compartments (Gratao *et al*., 2005), but under stressful conditions, their formation might be excess of antioxidant scavenging capacity, thus creating oxidative stress by reaction and damage to all biomolecules, especially protein, amino acid etc. In addition, one of the most damaging effects of oxygen cytocalic species and their products in cells is the peroxidation of membrane lipid and ion leakage (Grtao *et al*., 2005; Liu *et al*., 2007).

Cadmium inhibits the photosynthetic process (Van Assche and Clijters, 1985), this effect is related to disorders in chlorophyll biosynthesis (Stobart *et al*., 1985; Abdel-Basset *et al*., 1995). It is also connected with changes in the composition of fatty acids and with decreased content of acylipids and proteins in thylakoid membrane (Krupa, 1988) Cd may accelerate ageing of the photosynthetic apparatus (Skorzynska *et al*., 1991; Krupa, 1988).

To control the level of ROS and to protect the cells, plant passes low molecular weight antioxidants- ascorbic acid, reduced glutathione, carotenoids, toco-pherols and antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase and catalase that scavenge the ROS (Gratao *et al*., 2005). Catalase and several peroxidases scavenge the H_2O_2 produced in different physiological activities and in the reaction catalyzed by the SOD. Black gram is an important crop plant because their seed are used in the formation of different food product that is used in our daily life and also due to insufficient information available on Cd toxicity in this species.

Materials and Methods

In this study, the effect of cadmium (Cd) and its interaction with phosphorus (P) and copper (Cu) on lipid peroxidation, electrolye leakage percentage, catalase, peroxidase, pigment, fresh weight and dry weight in black gram (*Vigna mungo* var. PU35) were investigated. The experiment was conducted in the wire house condition. Clay pots with total area of 310 cm^2 per pot containing soil and compost into 3:1 ratio were used for growing the seed of black gram. The plants were treated with solution containing six Cd level as 3 $CdSO_4.7H_2O$ (0, 0.1, 0.2, 0.4, 1.0 and 2.0 mM L^{-1}) for 30 days. The combination of 1.00mM Cd+40 mM P, 1.00 mM Cd+10 mM Cu, 2.0 mM Cd+40 mM P and 2.0 mM Cd+10 mM Cu were also applied for interactive study in separate pots.

Catalase activity: Activity of enzyme catalase was measured by the modified method of Bisht (1972). The reaction was initiated with 1.0ml enzyme extract and the content was mixed thoroughly. The reaction was allowed to proceed for 5 minute and then stopped by the addition of 5 ml $5NH_2SO_4$. The reaction mixture was treated against $0.1NKMnO_4$. Enzyme activity was expressed as mmoles H_2O_2 decomposed per 100 mg fresh weight.

Peroxidase activity: Activity of enzyme peroxidase was measured by the modified method of Luck (1963). The reaction was carried out in 15 ml capacity tube in presence of 5ml buffer solution (0.1M pH-7),

1 ml 0.01% H_2O_2, 0.9 ml distilled water, 1ml phenyl diamine 0.5%, $5NH_2SO_4$ and 0.1ml enzyme extract. Activity of peroxidase was expressed as difference in optical density per 100 mg fresh weight.

Chlorophyll: Chlorophyll was estimated by the method of Arnon (1949). Leaves were plucked and washed with distilled water then blotted. 100 mg leaves were taken and crushed in 10 ml chilled acetone (85% v/v). Extract was centrifuged at 2000 rpm for 10 min. for the obsorbance of supernatant was read at 663 and 645 nm using spectrophotometer.

Carotenoid: Carotenoid content was estimated by the method of Duxbury and Yentsch 1956. The leaves were taken and washed weighed 100 mg, crussed in 10 ml acetone (85% v/v). For absorbance of supernatent was read at 480 and 510 nm using spectrophotometer.

Electrolyte leakage percentage: Sullivann and Ross (1979) method was used for electrolyte leakage percentage. Twenty leaf discs were taken in boiling tube containing 20 ml DW and electrical conductivity was measured (Eca). The Ecb and Ecc also measured at 50 and 100æ%C respectively.

Lipid peroxidation: The level of lipid peroxidation in plant tissue was measured in terms of malonaldehyde content, a product of lipid peroxidation determined by the thiobarbituric acid reaction by Heath and Packer (1968).

Results

The effect of Cd on cell membrane integrity was determined by evaluating MDA content and ELP of plant tissues. Compared to control plants, a significant change (8.01 decrease) in MDA content was noticed in plants supplied with lowest level of Cd, while, at highest concentration of Cd supply (2.0 mM L^{-1}) a significant change (15.21% increase) in MDA content was noticed, ELP was increased at all the concentration, it was noticed (48.28% increase) in ELP in plants supplied with highest Cd (2.0 mM L^{-1}) supply as compared to control plants. Plant grown with lowest (0.1 mM L^{-1}) showed an increase in total chlorophyll, Chl a, Chl b and carotenoid contents, We observed that total chlorophyll content was enhanced by 59.5% in the plants treated with solution of Cd of 0.1 mM L^{-1} concentration, while the synthesis of carotenoid decreased by 59.62% in the plants supplied with the solution of Cd of 2.0 mM L^{-1} concentration as compared to control plants. Plant treated with higher concentration of Cd, while these plants are separately recovered with phosphorus and copper, give good results in sense of pigment synthesis. Plant treated with 1.0 mM L^{-1} of Cd and recovered with 40 ppm of phosphorus shows an 7.25% increase in total chlorophyll synthesis, as compared to plant treated with Cd alone of same concentration. Rest of the results of pigment analysis is given in tables. Estimation of catalase, fresh weight and dry weight of plants treated with Cd, shows a decrease in fresh weight and dry weight as compared to control plants. Plants treated with highest concentration of Cd (2.0 mM L^{-1}) shows a 70.92% and 73.90% decrease in fresh weight and dry weight respectively.

It was noticed, a gradual increase in catalase activity from lower concentration of Cd (0.1 mM L^{-1}) to higher concentration of Cd (2.0 mM L^{-1}) treated plants as compared to control plants. It was observed 28.25% increase in catalase activity in plant treated with 2.0 mM L^{-1} concentration of Cd. The results of activity of catalase in the plants treated with different concentration of Cd while interacting with phosphorus and copper are given in Tables. It was also noticed, a gradual decrease in peroxidase activity from lower concentration of Cd(0.1mML^{-1}) to concentration of Cd(0.4 MmL^{-1}) treated plants as compared to control plants . plants treated with Cd of (1.0 mML^{-1}) and (2.0 mML^{-1}) concentrations shows a increase in peroxidase activity by 15.22% and 28.26% respectively as compared to control plants. Phosphorus shows good recovery than the copper. Plants treated with Cd of (1 mM) concentration while recovered with 40 ppm of phosphorus shows a decrease in peroxidase activity by 28.26% as compared to respected plants treated only with Cd. Plants treated with 10 ppm copper and (2.0 mML^{-1}) of Cd shows a decrease in peroxidase activity by 20.34% as compared to plants treated only with Cd of 2.0 mML^{-1}.

Discussion

The environmental degradation, promoted mainly by anthropogenic action, has imposed strong pressure on the quality of ecosystems. The pollution of soil and water by a wide range of contaminants for both plants and animals has become a matter of great concern to researchers. In this sense, the elevated levels of heavy metals such as in the environment are a reality today. Cd occurs naturally at a low concentration in the soil, but its level has been steadily increasing due to mining and smelting, dispersal of sewage, sludge and the use of Cd rich phosphate fertilizers (Wagner, 1993; Liu *et al*., 2007).

The present study shows that plants of black gram presented a significant decrease in fresh weight and dry weight at all Cd treatments. Cd is known to inhibit plant growth (Aidid and Okamato, 1993). Cd growth inhibition could be due to the inhibition of the cell division and elongation rate of cells that mainly occurs by an irresistible inhibition of proton pump responsible for the process (Aidid and Okamato, 1993; Liu *et al*., 2003/4).

Oxidative stress is a response that results from increased levels of ROS in cells exposed to heavy metals (Beauchamp and Fridovich, 1971). Under Cd treatment, an increase in MDA content and ion leakage percentage indicated the oxidative stress in plant leaves. MDA content, however was lower in plants recovered with phosphorus and copper as compared to plants treated with same concentration of the Cd. Ion leakage percentage increasing at higher concentration of Cd while it was decreased in the plant recovered with phosphorus and copper.

In present study the MDA levels decreased upon addition of 0.1, 0.2 mM Cd, indicating lower lipid peroxidation. We suggest that the decrease in MDA concentration in the whole plant supplied with (0.1, 0.2) mM L^{-1} of Cd may be due to a decrease in unsaturated fatty acid and concentration relative to saturated fatty acid, which has also been reported in cucumber under stressful conditions (Kramer *et al*., 1991).

Interestingly, in present study the ELP was only increased upon addition of Cd at the highest level. Increased ELP has been reported by other investigators working with other plant specie exposed to a range of Cd concentrations (Mishra *et al*., 2006; Ekmekgi *et al*., 2007; Liu *et al*., 2007). In order to scavenge ROS and to avoid oxidative damage, plants possess several antioxidant enzymes. Superoxide dismutase the first enzyme in the detoxifying process converts superoxide radicals to H_2O_2 at a very fast rate (Gratao *et al*., 2005). Although H_2O_2 takes part in several important functions in plant cells (Foyer and Noctor, 2005; Phang *et al*., 2002), control of its

Table - 2: Effect of different concentration of Cd and its interaction with P & Cu on various morphological and biochemical parameters in Black gram

Parameter	Treatments									
	0.0 mM Cd (Control)	**0.1 mM Cd**	**0.2 mM Cd**	**0.4 mM Cd**	**1.0 mM Cd**	**2.0 mM Cd**	**1.0 mM Cd +40 mM P**	**1.0 mM Cd +10 mM Cu**	**2.0 mM Cd + 40 mM P**	**2.0 mM Cd + 10 Cu**
Plant height (cm)	63.75±2.54	60.00±3.45 (-05.88)	57.00±3.58 (-10.59)	53.48±2.66* (-16.11)	50.75±2.11* (-20.39)	42.00±2.16* (-34.12)	53.75±2.41* (+05.58)	57.57±4.68 (+11.85)	47.05±1.15* (+10.64)	48.41±1.85* (+13.24)
Fresh weight (g)	14.55±0.64	13.73±0.86 (-05.63)	10.43±0.78 (-28.31)	09.59±0.61* (-34.08)	06.51±0.18* (-55.25)	04.23±0.34* (-70.92)	07.02±0.21* (+07.26)	07.13±0.32* (+08.69)	05.25±0.17* (+19.25)	05.10±0.24* (+17.05)
Dry weight (g)	02.00±0.15	01.96±0.11 (-02.00)	01.41±0.22 (-29.50)	01.38±0.15 (-31.00)	00.82±0.09* (-59.00)	00.54±0.04* (-73.00)	00.68±0.09* (00.00)	01.42±0.15 (+41.42)	00.79±0.08* (+31.64)	00.82±0.04* (+34.14)
Moisture (%)	86±3.64	86±3.89 (00.00)	86±4.51 (00.00)	86±2.67 (00.00)	97±3.98 (+11.34)	90±3.88 (+04.44)	90±2.67 (-07.20)	80±1.54 (-17.53)	85±1.35 (-05.56)	84±5.56 (-06.67)
Total chlorophyll (mg g^{-1} FW)	02.42±0.11	03.86±0.14* (+59.50)	02.57±0.05 (+06.19)	01.82±0.03 (-24.79)	01.38±0.05* (-42.98)	01.08±0.06* (-55.37)	01.48±0.15 (+07.25)	01.57±0.09 (+13.77)	01.28±0.06* (+18.52)	01.41±0.07 (+30.56)
Chlorophyll 'a' (mg g^{-1} FW)	01.56±0.05	02.19±0.05 (+40.38)	01.52±0.08 (-02.56)	01.42±0.05 (-08.97)	01.08±0.02 (-30.77)	00.71±0.04* (-54.49)	00.93±0.01 (00.00)	01.22±0.02 (+12.96)	00.98±0.04 (+38.03)	01.01±0.03 (+42.25)
Chlorophyll 'b' (mg g^{-1} FW)	00.86±0.00	01.68±0.02* (+93.43)	01.02±0.04 (+17.32)	00.40±0.00* (-54.21)	00.30±0.00* (-65.74)	00.27±0.00* (-69.21)	00.25±0.00* (00.00)	00.35±0.00* (+16.67)	00.30±0.00* (+20.00)	00.40±0.01* (+60.00)
Cartenoids (mg g^{-1} FW)	00.52±0.01	00.55±0.01 (+05.77)	00.50±0.01 (-03.85)	00.35±0.00 (-32.69)	00.26±0.00* (-50.00)	00.21±0.00* (-59.62)	00.26±0.00* (00.00)	00.29±0.01 (+11.54)	00.26±0.0*1 (+23.81)	00.30±0.00* (+42.86)
Catalase activity (H_2O_2 hydrolysed/100mg FW)	640±12	700±15.14* (+09.38)	745±20.45* (+16.41)	760±19.15* (+18.75)	770±14.51* (+20.31)	850±21.51* (+32.81)	520±9.69* (-32.47)	560±4.87* (-27.27)	600±6.45 (-29.41)	560±5.59* (-34.12)
Peroxidase Activity (Δ OD /100mg FW)	09.20±1.11	06.00±0.25* (-34.78)	5.0±0.54* (-45.65)	04.60±0.25* (-50.00)	10.60±0.98 (+15.22)	11.80±1.05 (+28.26)	07.60±0.56* (-28.30)	08.80±0.87 (-16.98)	08.40±0.45 (-28.81)	09.40±0.67 (-20.34)
Electrolyte leakage (%)	87±1.59	90±2.57 (+03.45)	98±2.65 (+12.64)	104±2.96* (+19.54)	112±2.64* (+28.74)	129±5.69* (+48.28)	104±2.54* (-07.14)	102±3.64* (-08.93)	112±2.54* (00.00)	115±1.95* (-02.68)
Lipid peroxidation (100mg FW)	17.36±1.08	15.97±0.68 (-08.01)	16.43±0.94 (-05.36)	17.21±1.21 (-00.86)	18.37±1.65 (+05.82)	20.00±1.68* (+15.21)	15.66±0.98 (-14.75)	20.62±1.56* (+12.25)	15.34±1.94 (-23.31)	15.97±1.51 (-20.15)

Values are mean of three replicates ± Standard Error and values given in parenthesis shows Damage & recovery in % over control, * = values are significant at p=<0.05 level

buildup is essential to prevent oxidative damage to membranes and proteins. In fact, the increase in CAT activity as found here in, which can be associated with H_2O_2 scavenging, was also observed by Vitoria *et al*. (2001) in *Raphanus sativus* and by Gomes-Junior *et al*. (2006a,b) in *coffea arabica* under Cd stressful conditions. This increase suggests a compensatory mechanism of defense against oxidative stress caused by toxic metal concentrations and can be explained by increase in its substrate to maintain the level of H_2O_2 as an adaptive mechanism of the plants (Cargnelutti *et al*., 2006).

In the present study, the activity of peroxidase activity decreases at lower concentration while its activity increasing at higher concentration of Cd treatments. Such a decrease has also been reported in some Cd treated plants (Zhang *et al*., 2002; Gomes-Junior *et al*., 2006). The reduction in APX activity may be due to GSH depletion and a subsequent reduction in the ascorbate-glutathione cycles (Gomes-Junior *et al*., 2006). The reduction in GSH could be caused by an increased rate of phytochelatin synthesis induced by Cd ions. Further more, the decreased activity of peroxidase was apparently compensated by the increases activity of other H_2O_2 degrading enzymes like CAT (Mishra *et al*., 2006).

Cd reduces Chl biosynthesis by inhibiting the biosynthesis of 5-amino levolinic Cd (ALA), a precursor of chl, and activity of the enzymes Pchlide reductase (Stobart *et al*., 1985) and ALA dehydratase (Padmaja *et al*., 1990), and stimulating the activity of chlorophyllase (Abdel-Basset *et al*., 1995). The inhibition of ALA synthesis may be at the site of availability of glutamate for ALA synthesis (Parekh *et al*., 1990). Weigel and Jager (1980) reported a severe inhibition of glutamate dehydrogenase activity by Cd in vitro in *Phaseolus vulgaris* although the effect was not observed in vivo.

We showed that MDA concentration decreased upon addition of the lowest Cd concentration whereas ELP increased in plants exposed to highest Cd supply, demonstrating that plasma membrane structure was affected by Cd in the substrate. Also Cd strongly interfered with photosynthesis of the plant because the synthesis of total Chl, Chl a, Chl b and carotenoids were decreased at higher concentration of Cd.

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