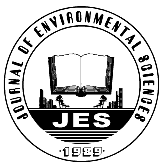

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Phytotoxicity of cadmium on protein, proline and antioxidant enzyme activities in growing *Arachis hypogaea* L. seedlings

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Abstract

Phytotoxicity of cadmium on growing *Arachis hypogaea* L. seedlings was studied. Seeds were exposed to 25, 50, and 100 μmol/L $CdCl_2$ concentrations, for a period of 10, 15, 20 and 25 d. The extent of damage to chlorophyll, protein, proline, nitrate and nitrite reductase, antioxidant enzyme activity in leaves and roots were evaluated after 10 d of cadmium stress. The higher concentration of cadmium (100 μmol/L) resulted (leaves and roots) total chlorophyll 91.01%, protein 79.51%, 83.61%, nitrate reductase 79.39%, 80.72% and nitrite reductase 77.07%, 75.88% activity decreased with increase in cadmium concentrations and exposure periods. Cadmium caused significant changes in the activity of antioxidative enzymes. Contrastingly Cd treated plant tissues showed an increase in proline 159.87%, 239.6%, gluthion reductase (GR) 337.72%, 306.14%, superoxide disumutase (SOD) 688.56%, 381.72%, ascorbate peroxidase (APX) 226.47%, 252.14%, peroxidase (POD) 72.19%, 60.29% and catalase (CAT) 228.96%, 214.74% as compared to control. Cadmium stress caused a significant increase in the rate of SOD activity in leaves and roots of plant species. Results show the crop *A. hypogaea* is highly sensitive even at very low cadmium concentrations.

Key words: toxicological effects; cadmium; protein; proline; antioxidant enzymes

Introduction

Metal toxicities have received widespread attention as large amounts are released into the environment and affect living organisms. Heavy metal intoxication, especially by lead, cadmium, arsenic, and mercury, constitute serious threat to human health (Wenneberg, 1994). However, anthropogenic inputs associated with agricultural practices, mineral exploration, industrial processes and solid waste management are important sources to heavy metal contamination of terrestrial ecosystems (Alumaa *et al*., 2002). Vegetable species thriving in metal-polluted soils absorb elements (Baker, 1981). Accumulation of heavy metals and metalloids in agricultural soils has become important due to food safety issues, potential health risks and detrimental effects on soil ecosystems.

Cd imparts toxicity to the environment with organ toxicity and a long elimination half-life (10–30 years) (Jarup *et al*., 1998). Cadmium occurs in small amounts with an average of 0.2 mg/kg in the geosphere (Lindsay, 1979). Diet is the main source of exposure from contaminated water and crops grown on Cd polluted soil (Thornton, 1992) and tobacco smoke (Lauwerys *et al*., 1994). Exposure to Cd can also result from combustion of fossil fuels, phosphatic fertilizers and as a by-product of industrial processes (Schaller and Angerer, 1992). Epidemiological studies of cadmium on industrial workers or inhabitants in Cd polluted areas have shown adverse renal effects (Buchet *et al*., 1990).

Cadmium is easily translocated from plant roots to above ground tissues (Yang *et al*., 1998), and potentially threatens human health. Cadmium in plants interferes with physiological processes, resulting in declined productivity (Florijn and Van Beusichem, 1993). Cadmium can harness photosynthetic activity, chlorophyll content, plant growth and induce oxidative stress (Zhou and Huang, 2001; Yi and Ching, 2003; Zhou *et al*., 2003).

Cd stress leads to protein degradation through amino acid metabolism resulting in decreased plant growth. Proline levels increase with cadmium stress and osmotic adjustment is associated with increase in proline and polyamine contents of plant cells (Rai and Raizada, 1988; Gowrinathan and Rao, 1992). Inhibitive effect of cadmium on activity of enzymes such as nitrate reductase and nitrite reductase is reported by Boussama *et al*. (1999a, 1999b).

Organisms with aerobic metabolism face constant risk from reactive oxygen species (ROS), including the superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2), which are inevitably generated via number of metabolic pathways (Kanazawa *et al*., 2000; Becana *et al*., 2000). Chloroplast, mitochondrial and plasma membrane linked electron transport invariably generate ROS as byproducts (Becana *et al*., 2000). Their presence causes

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oxidative damage to the biomolecules such as lipids, proteins, nucleic acids (Kanazawa *et al*., 2000). It is now known that a variety of abiotic stresses including drought, salinity, extreme temperatures, high irradiance, UV light, nutrient deficiency, air pollutants, etc. directly or indirectly cause molecular damage to plants through the formation of ROS (Lin and Kao, 2000). To scavenge ROS and avoid oxidative damage plants posses the antioxidative enzymes superoxide dismutase (SOD), catalase and peroxidase (Kanazawa *et al*., 2000). The enzyme SOD dismutates (O_2^-) to H_2O_2 and oxygen. Catalases are synthesized in a tissue specific and age dependent manner and scavenge H_2O_2 generated during the photorespiration and β-oxidation of fatty acids (Lin and Kao, 2000). Peroxidases, which are located in cytosol, vacuole, cell wall as well as in extra cellular space and use guaiacol as electron donor, utilise H_2O_2 in the oxidation of various inorganic and organic substrates (Asada *et al*., 1994). The production of ROS must be carefully regulated to avoid unwanted cellular cytotoxicity and oxidative damage (Halliwell and Gutteridge, 1990).

The crop gains significance due to its high commercial and nutrient value and in addition it is also used as fodder especially in Andhra Pradesh, South India. In certain areas of this region, it is noticed that the Cd levels are substantially high which could hamper the plant growth and yield. Therefore this study examines, by pot culture, the influence of the heavy metal Cd on *Arachis hypogaea* by determining chlorophyll, carotenoids, protein, proline, nitrate and nitrite reductase activities and behaviour of antioxidant enzymes in leaf and root.

1 Materials and methods

Arachis hypogaea seeds were obtained from Nagarjuna Ranga agricultural regional research station Tirupati, Andhra Pradesh, India. Seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10 min and rinsed with double distilled water. Seeds were sown in earth pots (30 cm × 25 cm, diameter and deep) containing red soil and farmyard manure in 3:1 proportion. The pots were kept under natural photoradiation and each pot contained 10 seedlings. The seeds were initially treated with three different concentrations ($CdCl_2$ solutions 0 (control), 25, 50, 100 μmol/L) in separate pots, till the maximum growth of the crop was observed and observations were recorded after 10, 15, 20, 25 d of treatment (triplicates). The solutions used for the treatments were 30 ml for each pot and three different chosen concentrations. Totally the solutions were added 13 times during the experimental period. The soil moisture ranged from 4% to 8% and the moisture was maintained in greenhouse conditions. Cd content was below detectable limit, soil pH was 7.58 and soil organic carbon (SOC) was 0.31%. The seeds were treated with three different Cd concentrations once every two days with above solutions.

1.1 Analysis of chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, protein and proline

The plants were uprooted carefully, leaves and roots were washed with distilled water and then grinded using mortar and pestle for physiological and biochemical studies. Chlorophyll content in the leaves (100 mg) of treated and control plants were extracted in 80% chilled acetone and estimated by the method of Arnon (1949) and determined the absorbance of the solution at 645, 663, and 652 nm against the solvent (80%) blank, using spectrophotometer (UV-Vis double beam spectrophotometer 118, Systronics, India). Chlorophyll content was expressed as mg/g fw (fresh weight).

Protein content in the roots and leaves (500 mg) were extracted in with buffers used for enzymes assay, grind well the samples with a pestle and mortar in 5–10 ml of buffer and centrifuge, the supernatant for protein was measured at 660 nm by the method of Lowry *et al*. (1951) using bovine serum albumin as the standard protein. Protein content was expressed as mg/g fw.

Proline was determined according to the procedure described by Bates *et al*. (1973). Leaves and roots (500 mg) were extracted with 3% aqueous 5-sulphosalicylic acid, centrifuged at 5000 r/min. The sample of the supernatant was used for the proline assay and measured at 520 nm. Proline content was expressed as μg/g fw.

1.2 Activity of enzymes

1.2.1 Nitrate reductase activity

The nitrate reductase (NR) activity was plant material leaves and root (100 mg) as ground in a chilled mortar at 4°C with 0.1 mol/L potassium phosphate buffer, pH 7.4 (8 ml/g fw) containing 1 mmol/L EDTA, 7.5 mmol/L cysteine, 2.5% (w/v) casein. The homogenate was centrifuged (for 15 min at 4°C) and the supernatant was assayed for NADH-NR activity as determined by method of Wray and Filner (1970). The supernatant was measured spectrophotometrically at 540 nm. NR activity was expressed as μmol NO_2/(g fw·h).

1.2.2 Nitrite reductase activity

The nitrite reductase (NiR) extraction was undertaken in the same conditions as those described for nitrate reductase. Nitrite reductase activity was measured as the reduction in the amount of nitrite in the assay mixture by incubating 0.1 ml of supernatant for 30 min at 30°C with 100 mmol/L of potassium phosphate buffer (pH 7.4), 15 mmol/L $NaNO_2$ and 5 mmol/L methyl viologen as electron donor (final pH 7.4 and total volume 1.0 ml). The absorbance of the supernatant was determined spectrophotometrically at 540 nm by the method of Losada and Paneque (1971). NiR activity was expressed as μmol NO_2/(g fw·h).

1.2.3 Glutathione reductase

Glutathione reductase was assayed according to Schaedle and Bassham (1977). Fresh leaves and root samples (200 mg) were homogenized using chilled mortar and pestle in 5 ml of 50 mmol/L Tris-HCl buffer (pH 7.6). The homogenate was centrifuged for 30 min at 4°C and the supernatant after dialysis was used for enzyme assay. The

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supernatant was measured at 340 nm in a spectrophotometer. Glutathione reductase was expressed as μmol NADPH oxidized/(mg protein·min).

1.2.4 Superoxide dismutase

The superoxide dismutase was assayed according to the method of Mishra and Fridovich (1972). About 200 mg leaves and root samples from freshly uprooted plants were extracted using chilled mortar and pestle in 5 ml of 100 mmol/L K-phosphate buffer (pH 7.8), containing 0.1 mmol/L EDTA, 0.1% (v/v) Triton X-100 and 2% (w/v) of soluble polyvinyl pyrrolidone (PVP). The extracts were filtered through muslin cloth and centrifuged for 10 min at 4°C. Supernatant was dialyzed in cellophane membrane tubings against the cold extraction buffer for 4 h with 3–4 changes of the buffer and after dialysis was used for enzyme assay. Supernatant was recorded at 470 nm in a spectrophotometer. Superoxide dismutase was expressed as U/(μg protein·min).

1.2.5 Ascorbate peroxidase

About 200 mg leaves and root samples were homogenized in 5 ml of 50 mmol/L K-phosphate buffer (pH 7.8) containing 1% PVP, 1 mmol/L ascorbic acid and 1 mmol/L Phenylmethylsulfonyl fluoride (PMSF) as described by Moran *et al*. (1994). After centrifugation for 10 min at 4°C, the supernatant was dialyzed against the same extraction buffer and it served as enzyme. Ascorbate peroxidase was assayed according to Nakano and Asada (1981). Reaction mixture in a total volume of 1 ml contained 50 mmol/L K-phosphate buffer (pH 7.0), 0.2 mmol/L ascorbic acid, 0.2 mmol/L EDTA, 20 mmol/L H_2O_2 and enzyme. Supernatant was measured at 290 nm in a spectrophotometer. Ascorbate peroxidase was expressed as μmol/(g fw·min).

1.2.6 Peroxidase

Peroxidase in fresh leaves and root samples (100 mg) was homogenized in 50 mmol/L Tris-HCl buffer, pH 7.0, the supernatant solution centrifuge for 20 min and peroxidase activity was assayed by the method of Kar and Mishra (1976). The reaction mixture contained 100 mmol/L Tris-buffer (pH 7.0), 10 mmol/L pyrogallol and 5 mmol/L H_2O_2. The supernatant was measured at 425 nm in a spectrophotometer. Peroxidase activity was expressed as U/(g protein·min).

1.2.7 Catalase

The activity of catalase was assayed according to Beers and Sizer (1952). Fresh samples leaves and root (200 mg) were homogenized in 5 ml of 50 mmol/L Tris/NaOH buffer (pH 8.0) containing 0.5 mmol/L EDTA, 2% (w/v) PVP and 0.5% (v/v) Triton X-100. The homogenate was centrifuged for 10 min at 4°C and after dialysis supernatant was used for enzyme assay. Supernatant was measured at 240 nm in a spectrophotometer. The activity of catalase was expressed as μmol H_2O_2 oxidised/(mg protein·min).

1.2.8 Statistics

Experimental results presented are the mean values of the experiments done in triplicates. The data was statistically analyzed using a two tailed *T*-test (SPSS 14.0) to compare paired means between different cadmium treatments and exposure periods at 5% and 1% level of probability.

2 Results

Total chlorophyll including chlorophyll a and b (Table 1) decreased from the day 10 to 25 and with increased Cd concentrations (25, 50, 100 μmol/L). At 25 μmol/L and during the day 10–25, the percentage changes for total chlorophyll, chlorophyll *a* and *b* varied from 13.28% to 69.44%, 18.48% to 71.12% and 2.3% to 65.85% respectively. At 50 μmol/L and during the day 10–25, the percentage changes were 29.52% to 79.33%, 37.5% to 81.99% and 12.64% to 72.36% respectively. At 100 μmol/L and during the day 10–25, the percentage change was 42.07% to 91.01%, 42.39% to 91.93% and 41.38% to 88.62% respectively.

Protein in leaves and roots (Table 2) is shown in decreasing trend with increasing concentrations (25, 50, 100 μmol/L) during the day 10–25. At 25 μmol/L and during the day 10–25, the percentage change for protein in leaves and roots were 4.38% to 42.45% and 4.38% to 39.38%

Table 1 Effect of cadmium on chlorophyll contents (mg/g fw) of *Arachis hypogaea* L. at different concentrations and exposure periods

$CdCl_2$ conc. (μmol/L)	Exposure period (d)			
	10	15	20	25
Chlorophyll-*a*				
0	1.84±0.25	2.06±0.15*	2.64±0.58	3.22±0.35
25	1.50±0.12*	1.34±0.10*	1.05±0.37**	0.93±0.19*
50	1.15±0.16*	1.0±0.26*	0.76±0.21*	0.58±0.13**
100	1.06±0.32*	0.81±0.15*	0.62±0.54*	0.26±0.29*
Chlorophyll-*b*				
0	0.87±0.76	0.92±0.15	1.08±0.25	1.23±0.50
25	0.85±0.45*	0.61±0.51*	0.5±0.18*	0.43±0.57*
50	0.76±0.25*	0.55±0.21**	0.47±0.15*	0.34±0.19*
100	0.51±0.15**	0.40±0.11*	0.23±0.15*	0.14±0.25*
Total chlorophyll				
0	2.71±0.03	2.98±0.06	3.72±0.41	4.45±0.85
25	2.35±0.05*	1.95±0.6*	1.55±0.20**	1.36±0.15*
50	1.91±0.03*	1.55±0.41*	1.23±0.35*	0.92±0.39**
100	1.57±0.45*	1.21±0.05*	0.85±1.02**	0.40±0.07*

Values are means of three replicates ±SE. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

Table 2 Effect of cadmium on protein content (mg/g fw) of *Arachis hypogaea* L. at different concentrations and exposure periods

$CdCl_2$ conc. (μmol/L)	Exposure period (d)			
	10	15	20	25
Leaf				
0	12.1±0.11*	13.74±0.20	15.83±0.70	17.62±0.35
25	11.57±0.13	11.11±0.81*	10.87±0.28*	10.14±0.03*
50	10.39±0.17 *	9.95±0.02*	8.73±0.11**	7.62±0.58**
100	8.08±0.34**	6.49±0.21*	4.24±0.61	3.61±0.10**
Root				
0	8.91±0.19	9.48±1.01	10.02±1.06	12.08±1.06
25	8.52±0.65**	8.15±1.30**	7.98±1.07**	7.25±1.08
50	7.84±0.81*	6.50±0.18	5.59±1.06	4.89±1.40*
100	5.48±0.43	4.84±1.04*	4.22±1.65*	1.98±1.12*

Values are means of three replicates ±SE. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

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respectively. At 50 μmol/L the percentage change was 14.13% to 56.75% and 12.01% to 59.52% respectively, and at 100 μmol/L the percentage change in leaves and roots was 33.22% to 79.51% and 38.5% to 83.61% respectively.

A decrease in nitrate and nitrite reductase activity (Fig.1) was observed in leaves and roots during the day 10–25 with increased cadmium application (25, 50, 100 μmol/L). At 25–100 μmol/L and during the day 10–25, the percentage changes for nitrate reductase in leaves and roots were 0.94% to 79.39% and 5.68% to 80.72% respectively. The percentage change is less on 10th day leaves at 25 μmol/L of cadmium exposure. Under similar conditions, the percentage change for nitrite reductase in leaves and roots was 5.53% to 77.07% and 1.2% to 75.88% respectively.

Proline in plant tissues (leaves, roots) increased from the day 10 to the day 25 with increasing molar concentrations (Table 3). At 25 μmol/L the percentage change for leaves was 25.81% to 63.42% and for root 41.67% to 100.85%. At 50 μmol/L the percentage change was 75.36% to 104.86% and 109.71% to 151.3%, respectively. At 100 μmol/L the percentage change was 111.2% to 159.87% and 153.66% to 239.6% for leaves and roots, respectively.

Proline in plant tissues (leaves, roots) increased from the day 10 to the day 25 with increasing molar concentrations (Table 3). At 25 μmol/L the percentage change for leaves was 25.81% to 63.42% and for root 41.67% to 100.85%. At 50 μmol/L the percentage change was 75.36% to 104.86% and 109.71% to 151.3%, respectively. At 100 μmol/L the percentage change was 111.2% to 159.87% and 153.66% to 239.6% for leaves and roots, respectively.

The activity of gluthion reductase (GR) (Fig.1) in leaves and roots increased under similar conditions of the experiment maintained for chlorophyll, proteins, SOD etc. during the day 10–25, at 25 μmol/L the percentage change for GR activity in leaves and roots was 19.32% to 57.35% and 7.77% to 92.41%, respectively. At 50 μmol/L the percentage change was 45.99% to 156.2% and 84.83% to 156.47% respectively. At 100 μmol/L the percentage change values were 154.79% to 377.72% and 169.27% to 306.14% in leaves and roots, respectively.

As cadmium application increased from 25, 50, 100 μmol/L, the SOD in leaves and roots (Fig.2) showed increasing trend during the day 10–25. At 25 μmol/L the percentage change in SOD varied from 53.56% to 289.74% in leaves and 44.18% to 142.48% in roots. At 50 μmol/L the percentage changes was 190.43% to 412.19% and 135.71% to 151.57% respectively. At 100 μmol/L the percentage change was 375.41% to 688.56% and 211.5% to 381.72%, respectively.

The activity of ascorbate peroxidase (APX) increased (Fig.2) in leaves and roots during the day 10–25 with increase in Cd concentrations (25, 50, 100 μmol/L). At 25, 50,100 μmol/L and during the day 10–25, the percentage change was 19.38% to 74.66%, 54.52% to 173.45%, 107.24% to 226.47% respectively for leaves and similarly the percentage change for roots at various concentrations were 0.83% to 48.93%, 20.84% to 137.56% and 162.12% to 252.14%, respectively.

Increased activity of peroxidase (POD) was observed

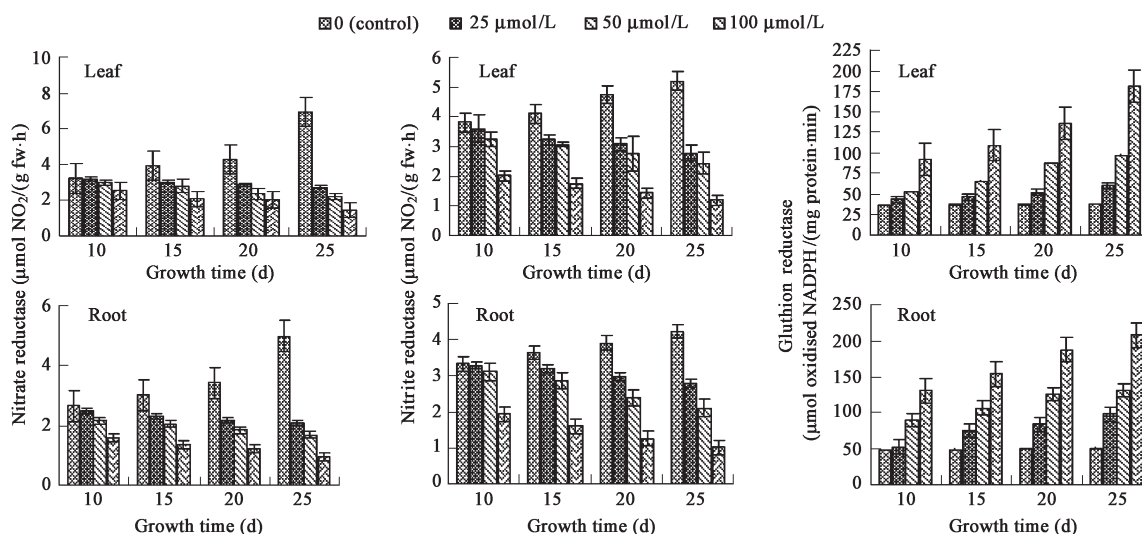

Fig. 1 Effect of $CdCl_2$ on nitrate, nitrite reductase (μmol NO_2/(g fw·h)), and gluthion reductase (μmol NADPH oxidized/(mg protein·min)) in leafs and roots of *Arachis hypogaea*. Values are mean±SE and bars indicate standard errors.

Table 3 Effect of cadmium on proline content (μg/g fw) of *Arachis hypogaea* L. at different concentrations and exposure periods

$CdCl_2$ conc. (μmol/L)	Exposure period (d)				Exposure period (d)			
	10	15	20	25	10	15	20	25
	Leaf				Root			
0	254.37±1.03	256.75±0.18	258.9±1.04	269.43±1.05	195.11±1.01	196.12±1.02	197.94±1.32	195.41±1.44
25	320.03±0.13*	367.29±1.55	402.64±1.25*	440.29±1.38	276.42±1.14*	321.58±1.05*	368.11±1.01*	392.48±1.11*
50	446.06±1.07	491.84±1.91	526.65±1.29**	551.96±1.78*	409.16±1.11*	439.41±1.24**	476.67±1.39	491.07±1.25**
100	537.22±1.18	579.61±1.28*	624.25±1.26	700.16±1.27*	494.92±1.19	549.55±1.28	613.85±1.18*	663.61±1.12

Values are means of three replicates ±SE. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

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(Fig.2) in leaves and roots during the day 10–25 with increasing cadmium molar concentrations (25, 50, 100 μmol/L). At 25 μmol/L the percentage changes was 16.67% to 28.06% for leaves and 12.34% to 19.86% for roots, respectively. At 50 and 100 μmol/L the percentage change in POD activity was 37.37% to 55.61%, 26.51% to 37.56% respectively for leaves and 48.12% to 72.19%, 36.74% to 60.29% respectively for roots.

During the day 10–25 and with increase in cadmium concentrations (25, 50, 100 μmol/L) the activity of catalase (CAT) in (Fig.3) leaves and roots showed an increasing trend. At 25, 50, 100 μmol/L and during the day 10–25, the percentage change was 11.88% to 65.02%, 6.16% to 65.39%, 64.86% to 143.92% respectively for leaves and similarly the percentage change for roots at various concentrations were 59.23% to 128.32%, 142.74% to 228.96% and 129.13% to 214.74% respectively.

3 Discussion

Cadmium causes many morphological, physiological and biochemical changes in growing plants. Decrease in the chlorophyll is the primary bioindicator of Cd phytotoxicity. In our studies with increase in cadmium concentrations, the total chlorophyll including chlorophyll *a* and *b* has decreased. The strong inhibition of chlorophyll content in wheat leaves under Cd and Ni stress are in accordance with the finding of Sheoran *et al*. (1990). Two possible mechanisms of cadmium toxicity on photosynthesis have been proposed to explain the decrease in chlorophyll pigments. Cd can alter both chlorophyll biosynthesis by inhibiting protochlorophyllide reductase and the photosynthetic electron transport by inhibiting the water-splitting enzyme located at the oxidizing site of photosystem II (Van Assche and Clijsters, 1990).

The present study reveals that the protein content in the leaves and roots decrease with increasing cadmium concentrations for all growth periods. Our studies reveal that Cd toxicity is more in root than in leaf. The percentage changes are more both in roots and leaves at 100 μmol/L Cd concentrations on the day 25. The decrease in protein is more pronounced in root than in leaves which may be due to degradation of proteins (Davies, 1987).

Generally, it is assumed that in higher plants, assimilation of ammonia derived from nitrate reduction is mediated by the GS/GOGAT (glutamate synthase) cycle (Tischner,

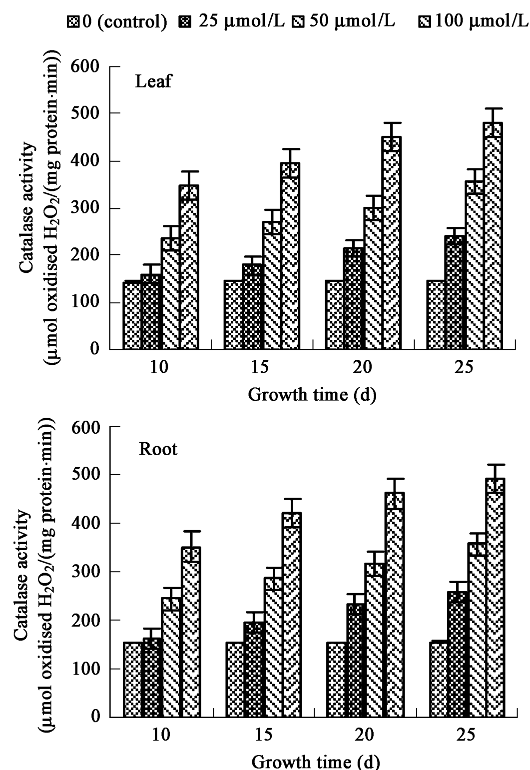

Fig. 3 Effect of $CdCl_2$ on catalase activity (μmol H_2O_2 oxidised/(mg protein·min)) in leafs and roots of *Arachis hypogaea*. Values are means±SE and bars indicate standard errors.

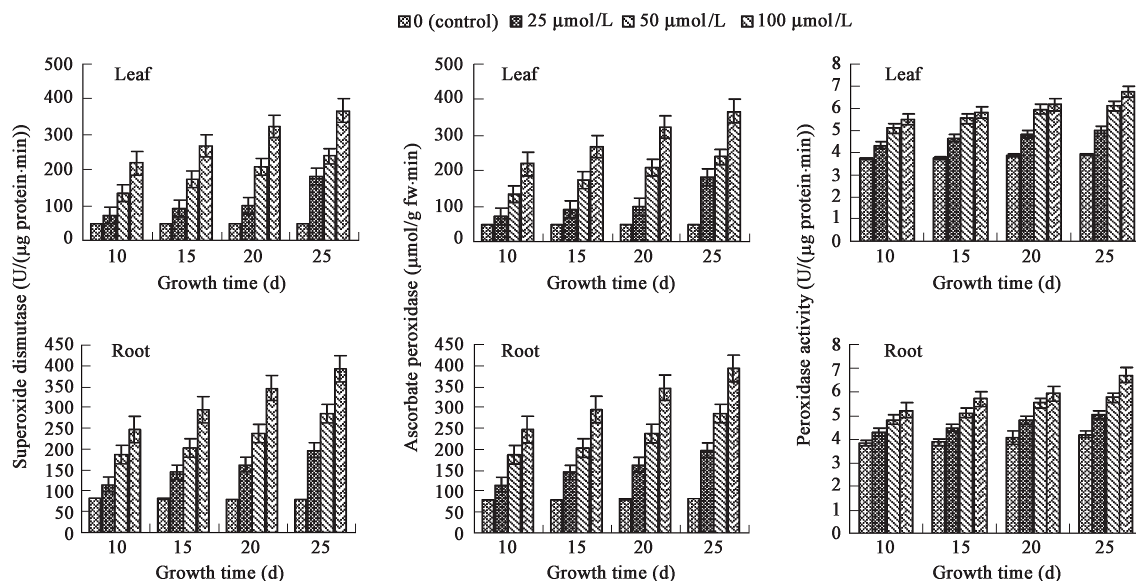

Fig. 2 Effect of $CdCl_2$ on SOD (U/(μg protein·min)), APX (μmol/(g fw·min)), and POD (U/(μg protein·min)) in leafs and roots of *Arachis hypogaea*. Values are mean±SE and bars indicate standard errors.

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2000). We have shown that the treatment of plants by Cd leads to inhibitory effects on NR and NiR activities, the effects are significantly greater in leaves than in roots. Similar effects have been observed by Boussama *et al.* (1999a) and Gouia *et al.* (2000).

Proline increased significantly in leaves and roots under all applied Cd concentrations. Proline levels are greater in leaves than in roots. Balestrasse *et al.* (2005) has reported that proline levels increased in roots of soya bean plants with cadmium stress. Proline accumulation has also been proposed as a mechanism of storage of excess nitrogen (Rhodes *et al.*, 1999). Accumulation of free Proline in response to heavy metal exposure seems to be widespread among plants (Costa and Morel, 1994). It has been suggested that proline accumulation in plants under Cd stress is due to the decrease of the plant water potential and the functional significance of this accumulation could be related to the water balance (Schat *et al.*, 1997).

Antioxidant enzymes and certain metabolites play an important role in adaptation and ultimate survival of plants during periods of stress. In fact, activities of antioxidative enzymes are inducible by oxidative stress (Foyer *et al.*, 1994), which reflects a general strategy required to overcome stress.

Superoxide dismutase is an essential component of plants antioxidative defense system as it dismutates two O_2^- to water and oxygen (Cakmak and Horst, 1991). Our results show that leaves exhibit greater enhancement in the activity of SOD in leaves compared to roots in *A. hypogaea*. With increase in the levels of Cd in the growth medium, SOD activity has been reported to be stimulated under a variety of stressful conditions including Cu, Al, Mn, Fe and Zn toxicity (Prasad *et al.*, 1999; Cakmak and Horst, 1991). The increase in SOD activity in response to stresses is possibly attributed to the de-novo synthesis of the enzymic protein (Cakmak and Horst, 1991). Increased SOD activity in transgenic plants has been shown to confer increased protection from oxidative damage (Allen *et al.*, 1997). Hence, it is postulated that overall activity of SOD enzymes is of more relevance in metal stress studies for the maintenance of the overall defense system of plants subjected to oxidative damage (Slooten *et al.*, 1995).

The APX activity increased in leaves but less compared to roots at the applied Cd levels. At 100 μmol/L Cd concentrations APX activity was more in root than leaves. The induction of APX activity in plants was also reported in *Ceretophyllum demersum* under copper stress (Devi and Prasad, 1998), Cu treated *Phaseolus vulgaris* (Gupta *et al.*, 1999) and Cd treated green plants of barley (Hegedus *et al.*, 2001).

In our results GR activity has increased in leaves and roots with Cd concentrations. The GR activity was more pronounced in roots than in leaves. Leaves showed high GR activity at 100 μmol/L on the day 25. Kuo and Kao (2004) reported that GR activity increased on the day 2 and day 3 in rice leaves. The enzymes of Halliwell-Asada pathway ascorbate-glutathione cycle such as ascorbate peroxidase and glutathione reductase also play a significant role in scavenging H_2O_2 mainly in chloroplasts and in maintaining the redox status of the cell (Foyer *et al.*, 1997).

Our results showed that the POD and CAT activities increased in both leaves and root tissues with increased concentrations and exposure periods. POD activity was more in leaves compared to roots at 100 μmol/L. CAT activities was more in roots than leaves. The percentage change at 100 μmol/L Cd concentrations was more in leaves on the day 25. Oxidative stress caused by Cd in plants led to increased expression and activities of antioxidant enzymes such as POD and CAT (Skorzynska-Polit *et al.*, 2003/4). In roots the increased POD activity with Cd sensitivity of the pea genotypes supported previous suggestions to POD activity as a biomarker for metal toxicity in plants (Radotic *et al.*, 2000; Metwally *et al.*, 2005). Shah *et al.* (2001) have reported that the activity of CAT increased in rice seedlings during early days of growth with maximum on the day 15 and Cd level of 100 μmol/L showed increased CAT activity in both root and shoots. Catalase is one of the key enzymes in the removal of toxic peroxides. This is mostly universal oxidoreductase that scavenges H_2O_2 via a two electron transfer producing O_2 and H_2O (Lin and Kao, 2000). An increase in the activity of catalase has been reported in certain plant species exposed to toxic concentrations of heavy metals Cu, Pb, Zn (Prasad *et al.*, 1999; De Vos and Schat, 1991).

These results suggest that Cd induced physiological, biochemical changes in plants. The activity of antioxidative enzymes could serve as important components of antioxidative defence mechanism against oxidative injury.

4 Conclusions

The data presented in this paper have demonstrated that the Cd treatment in growing seedlings of *A. hypogaea* impairs the chlorophyll, proteins, proline, NR, NiR and enzymatic antioxidant defense system in leaf and root tissues. It seems that the Cd treatment resulted in considerable impairment of the antioxidant defense system and led to the oxidative stress in the tissues of the crop. 100 μmol/L of cadmium treatment has proved to be critical and vulnerable to the growing seedlings of *A. hypogaea*.

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