RESEARCH ARTICLE

Changes induced by Cu^{2+} and Cr^{6+} metal stress in polyamines, auxins, abscisic acid titers and antioxidative enzymes activities of radish seedlings

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ABSTRACT

The present study determined the effects of copper and chromium metals on the endogenous titers of polyamines, auxins, abscisic acid and antioxidative enzyme activities in *Raphanus sativus* L. cv. Pusa chetki seedlings. Among polyamines, putrescine and spermidine contents were enhanced by Cu^{2+} metal to 62.44 and 402.8 μg g^{-1} f.w. respectively over control. Spermine which was not observed in control was recorded in highest concentration (1287.9 μg g^{-1} f.w.) in Cu^{2+} metal stressed seedlings. On the other hand Cr^{6+} metal treated seedlings showed reduced contents of putrescine (1.43 μg g^{-1} f.w.), cadaverine (0.09 μg g^{-1} f.w.), spermidine (277.99 μg g^{-1} f.w.) and spermine (2.29 μg g^{-1} f.w.) when compared to control and Cu^{2+} metal treated seedlings. Significant decline in free and bound IAA concentration were found under Cu^{2+} and Cr^{6+} metal stress when compared to control. Naphthalene acetic acid not recorded in control seedlings was detected in seedlings treated with Cu^{2+} and Cr^{6+} metal. Activities of guaiacol peroxidase and catalase were reduced significantly under Cu^{2+} and Cr^{6+} metal stress in comparison to control. Superoxide dismutase activity enhanced significantly under Cr^{6+} rather than Cu^{2+} metal treatment. In addition the phytotoxicity of Cu^{2+} and Cr^{6+} metal on seedling growth was also determined. The data suggest that Cr^{6+} is more phytotoxic than exposure to Cu^{2+} metal.

Key words: catalase, chromium, copper, guaiacol peroxidase, polyamines (PAs), superoxide dismutase

INTRODUCTION

Heavy metals are major environmental contaminants occurring in soils and other habitats. Toxic levels of some of them (Cd, Cu, Pb, Hg and Cr) could appear in natural and agricultural areas as a result of human activities. Among these metals, copper is widely distributed in nature due to multifarious use for industrial and agronomic purposes. Soil contamination with Cu^{2+} has become an important environmental threat to human life through its penetrance to the food chain (Chary et al., 2008). It is required in small quantities for normal plant growth and development (Mengel and Kirkby, 1987), but leads to phytotoxicity at higher concentrations (Berenguer et al., 2008). Copper toxicity is mediated by the formation of free radicals (Luna et al., 1994) and by the catalysis of the Haber-Weiss reaction (Halliwel and Gutteridge, 1984). These radicals are highly toxic and can oxidize biological macromolecules such as nucleic acids, proteins and lipids, thereby disturbing the cell stability and membrane permeability (Schutzendubel and Polle, 2002). Similarly, Chatterjee et al. (2006) also documented oxidative damages and changes in radish physiology under copper stress. On the other hand, reactive oxygen species

(ROS) producing capacity of Cr^{3+} or Cr^{6+} is mainly attributed to its redox character. Excess of Cr^{3+} or Cr^{6+} metal induces the production of ROS such as H_2O_2 to form $OH^{\bullet}$, O_2^- directly by interacting with glutathione, NADPH and H_2O_2 (Shanker et al., 2005) and thus causes oxidative damage to biomolecules of immense importance like DNA, proteins and pigments. It also stimulates lipid peroxidation (Shanker et al., 2005).

Plants have strong defense system consisting of antioxidative enzymes, like catalase (Willekens et al., 1997), peroxidase (Csiszar et al., 2008), glutathione reductase (Romero-Puertas et al., 2006) and superoxide dismutase (Alscher et al., 2002), ascorbate peroxidase (Davletova et al., 2005) and antioxidants (vitamins, NAD, FAD, ascorbic acid). The phytohormones like auxins (Park et al., 2007), cytokinins (Zhang and Ervin, 2008), abscisic acid (Staneloni et al., 2008), ethylene (Tamaoki et al., 2008), salicylates (Flors et al., 2008), jasmonic acid (Maksymiec et al., 2005), brassinosteriods (Haubrick and Assmann, 2006; Clouse, 2008) and polyamines (Tassoni et al., 2008) are also known to confer resistance in plants against various abiotic and biotic stresses.

Auxins are growth regulators required for normal plant growth and development. Recent studies also indicate their stress protective property in plants. They are now widely used to confer resistance in plants against salinity (Iqbal and Ashraf, 2007), drought, chilling stress (Posymk et al., 2009), heat stress and heavy metals stress (Khan and Chaudhary, 2006; Dimpka et al., 2008).

Active involvement of ABA in physiological processes such as stomatal closure, embryo morphogenesis, synthesis of storage proteins, lipids and seed germination is well understood. ABA plays an important role in stress protection by triggering plant responses to adverse environmental stimuli. Exogenous application of ABA has been reported to enhance chilling stress tolerance (Zhou et al., 2005). ABA induced adaptation to salt stress in suspension cultures of tobacco (*Nicotiana tabacum* L. Cv. Wisconsin) has been also reported by Etehadnia et al. (2008).

Another class of nitrogen-rich metabolites known as polyamines [such as putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm)] are low molecular weight organic cations, ubiquitously distributed in all living organisms (Groppa and Benavides, 2008). They are actively involved in the regulation of a large number of physiological processes like embryogenesis, cell division, morphogenesis and development (Minocha and Minocha, 2005; Groppa and Benavides, 2008; Kumar et al., 2008). They are now accepted as an important component of plant stress responses (Alcazar et al., 2006; Tassoni et al., 2008). Changes in the endogenous titers of polyamines have been associated with the retardation of senescence, and various environmental stresses like osmotic, salinity (Pirintsos et al., 2004; Tang et al., 2005; Shevyakova et al., 2006; Liu et al., 2007; Wen et al., 2008) and heavy metal stress (Shevyakova et al., 2006; Liu and Moriguchi, 2007; Zapata et al., 2008). They have the capacity for scavenging free radicals and reactive oxygen species (Ha et al., 1998), thus exerting a strong antioxidant function during various types of stress (Groppa and Benavides, 2008).

Raphanus sativus L. plants are facing variety of abiotic stresses. The prominent stress among these is heavy metal stress. Literature survey revealed scanty information on the comparative account of Cu^{2+} and Cr^{6+} metal stress on the endogenous levels of polyamines, auxins, and ABA and antioxidant system of *Raphanus sativus*. So keeping this in mind the present investigation was designed to find out the effects of Cu^{2+} and Cr^{6+} metal stress on the biosynthesis of polyamines, auxins and ABA in this economically and medicinally important root vegetable. In addition to this, antioxidant system (antioxidative enzyme activities, lipid peroxidation and proline content), and seedling growth were also assessed under metal stress.

MATERIAL AND METHODS

Plant material and treatments: Seeds of *Raphanus sativus* L. cv. 'Pusa chetki' used in the present investigation were procured from Punjab Agriculture University, Ludhiana, India. Seeds were surface sterilized with 0.01% sodium hypochlorite and then rinsed with 3-4 times with distilled water. Seeds were grown in autoclaved Petriplates lined with *Whatman No*.1 filter paper at 25 ± 2^{o}C with a photoperiod of 16 h under fluorescent white light (175 μmol/m^2/s) in a controlled environmental growth chamber maintained at 70-80% relative humidity. Seeds were subjected to IC_{50} concentrations of 0.2 mM $CuSO_4.5H_2O$ (Cu^{2+} ions) and 1.2 mM K_2CrO_4 (Cr^{6+} ions) stress, whereas control seedlings were raised in distilled water alone. Seedlings were harvested on the 7th day (3-3.5 cm hypocotyls length) for the analysis of PAs (putrescine, cadaverine, spermidine and spermine), auxins (Indole acetic acid, Naphthalene acetic acid

and Indole butyric acid) ABA and antioxidative enzyme activities and seedling growth.

Isolation and characterization of polyamines: Polyamines were isolated and characterized by following the method proposed by Fontaniella et al. (2001). About 2g of seedlings were extracted with 5% perchloric acid (PCA) (v/v) in a pre-chilled pestle and mortar at 4° C. Homogenized extracts were centrifuged at 15,000 r.p.m. for 25 min at 4° C. Supernatant used as a source of PAs was dried in vacuum at 45° C and residue was redissolved in 5% PCA. Impurties like polyphenolics were removed by stirring the residue with polyvinylpolypyrrolidone (PVPP) (50 mg/ml). Dansylation of the testing samples was carried out in derivatization vials. Samples after dansylation were cleaned according to Seiler and Knodgen (1979). They were finally dissolved in 1 ml of methanol (HPLC grade) for HPLC analysis.

HPLC analysis of polyamines: Analysis of PAs was carried out on Waters (515) Chromatograph HPLC equipped with Waters (717) Plus Autosampler and Photodiode Array Detector (2996).For the assessment of endogenous PAs, samples were injected in to 20 μl injector loop in RP-C18 Column (15 cm $\times$ 4 mm i.d.), 5 μm particle size, reversed phased column at 30° C using Methanol: Water linear gradient from 50:50 (v/v) to 80:20 (v/v) for 30 min. The last proportion was maintained at 1 ml/min. The detection of PAs was carried out by measuring the fluorescence intensity at 254 nm of samples and making their comparisons with the peaks and retention times of standard PAs (Put, Cad, Spd and Spm). The standard PAs were prepared at a concentration of 1mM as described earlier.

Isolation and characterization of auxins and ABA: Assessment of endogenous auxins and ABA content in the seedlings subjected to Cu^{2+} and Cr^{6+} stress was done by the method of Nagar and Sood (2003, 2006). 2 g of seedlings harvested on the 7th day were homogenized with 80% methanol (20 ml/g) containing butylated hydroxytoulene (BHT) (100 mg/l). Homogenates were kept overnight at 4° C in the dark, filtered, and re-extracted (4$\times$) with 80% methanol. Resulting solution was frozen at -20° C, thawed and centrifuged at 9000 r.p.m for 30 min at 4° C to remove suspended impurities. Supernatants were dried in *vacuo* and taken up in 0.1 M potassium phosphate buffer (pH 8.1) and then applied to polyvinylpyrrolidone (PVP) column. Eluate obtained was evaporated to dryness, taken up in water and pH adjusted to 2.5 with 1 N HCl and then partitioned (4$\times$) with diethyl ether. Combined ether phases were evaporated to dryness in *vacuo* and the residue was dissolved in methanol for the estimation of free auxins. The remaining aqueous phase was hydrolyzed at pH 11.0 for 1 h at 60° C. The hydrolysate was cooled and acidified with 1 N HCl upto pH 3 and finally partitioned against diethyl ether. The combined ether phases were evaporated in *vacuo*, and taken up in methanol (HPLC grade) for the estimation of bound auxins.

HPLC analysis of auxins and ABA: For the determination of endogenous auxins and ABA concentrations, methanolic extracts ready for HPLC analysis were filtered through 0.45 μM Millipore filters. Elution was carried out with Methanol (40%) in 30 mM acetic acid (HPLC grade) at a flow rate of 1 ml/min. The solvents were filtered and degassed. Column eluants were passed through UV detector (2996 PDA detector) at 280 nm and auxins (IAA, NAA and IBA) and ABA were characterized and quantified by making comparisons of retention times, fluorescence intensity and peak heights of extracted auxins with reference to their standards (IAA, NAA, IBA, and ABA, Sigma Aldrich Ltd., India), prepared at a concentration of 1 mM.

Determination of protein contents and antioxidative enzyme activities.

Preparation of plant extracts: For the estimation of protein content and activities of superoxide dismutase, catalase and guaiacol peroxidase, and glutathione reductase, 2 g of plant tissue was homogenized in pre-chilled pestle and mortar with 6ml of 100 mM potassium phosphate buffer (pH 7.0) under ice-cold conditions. The homogenate was centrifuged at 15000$\times g$ at 5° C for 20 min and the supernatants were used for determining protein content and enzyme activities.

Protein estimation: The protein content was determined by following the method proposed by Lowry et al. (1951).

Guaiacol peroxidase (GPOX) (EC1.11.1.7) activity: The GPOX activity was determined by the method of Putter (1974). The reaction mixture consisted of 3 ml of phosphate buffer, 50 μl guaiacol solution, 100 μl of enzyme sample and 30 μl of H_2O_2 solution. The rate of formation of guaiacol dehydrogenation product (GDHP) was determined spectrophometrically at 436 nm.

Catalase (CAT) (EC 1.11.1.6) activity: The activity was determined as per the method of Aebi (1983). The reaction

mixture consisted of 300 μl of enzyme extract taken in the test cuvette and to this 1.5 ml of phosphate buffer and 1.2 ml of H_2O_2 solution was added. This was followed by decrease in absorbance per min at 240 nm.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity: The SOD activity was determined by the method of Kono (1976). In this method, reaction mixture containing 1.9 ml of sodium carbonate buffer, 750 μl NBT and 150 μl Triton X-100 were taken in a test cuvette and reaction was started by adding 150 μl hydroxylamine hydrochloride. After 2 min, 70 μl of enzyme extract was added. The percentage inhibition in the reduction rate of NBT was recorded with increase in absorbance at 540 nm.

Glutathione reductase (GR) (EC 1.6.4.2) activity: The GR activity was determined by following the Foyer and Halliwell method (*1976*) as described earlier.

Seedling growth: The effects of Cu^{2+} and Cr^{6+} metal stress on 7 days old *Raphanus* seedlings were determined. The growth was assessed in the terms of shoot length, root length and fresh biomass which were assessed for the metal treated and untreated control seedlings.

Statistical analysis: Three repetitions were designed for each experiment and data was expressed as mean values ± SE. One way analysis of variance (ANOVA) was carried and data was presented at significance of $p \leq 0.05$.

RESULTS

Endogenous contents of Polyamines: Putrescine (Put) content in 7d old untreated control seedlings was 36.64 μg g^{-1}f.w., whereas under Cu^{2+} metal stress an increase in Put content was observed (62.44 μg g^{-1} f.w.) in comparison to control. Cadaverine (Cad) synthesis showed varied pattern (Table 1) in its synthesis in comparison to other PAs. Significant decrease in Cad level was found in Cu^{2+} metal stressed seedlings (6.71 μg g^{-1} f.w) (Table1) when compared to control ones (57.22 μg g^{-1} f.w). Enhanced content of spermidine (Spd) level was observed in seedlings treated with Cu^{2+} metal (402.8 μg g^{-1} f.w.) when compared to Spd content found in control seedlings (178.4 μg g^{-1} f.w.). The synthesis of spermine (Spm) revealed drastic changes in its production under Cu^{2+} metal stress. A radical increase in Spm content (1287.9 μg g^{-1} f.w.) was found in seedlings exposed to Cu^{2+} metal stress whereas no Spm was detected in control seedlings (Table1).

Cr^{6+} metal treatment results in selective enhancement of the PAs levels, in comparison to control. Significant reduction in Put content (1.43 μg/g f.w.) was observed in Cr^{6+} metal stressed seedlings in comparison to control (36.64 μg g^{-1}f.w.) Cad concentration (0.090 μg/g f.w.) was observed to decrease in Cr^{6+} metal treated seedlings. Significantly higher Spd content (277.99 μg/g f.w.) was found in seedlings under Cr^{6+} metal stress. The Spm content which was not detected in over control seedlings was recorded in seedlings exposed to Cr^{6+} metal treatment (2.29 μg/g f.w.) (Table1).

Table 1. Endogenous levels of polyamines (μg/g f.w.) in 7d old radish seedlings grown under 0.2 mM $CuSO_4 \cdot 5H_2O$ (Cu^{2+}) and 1.2 mM K_2CrO_4 (Cr^{6+}) metal stress.

S.No.	Control	0.2 mM $CuSO_4 \cdot 5H_2O$	1.2 mM K_2CrO_4
Put	36.64 ± 2.20^b	62.44 ± 3.90^a	1.43 ± 0.33^a
Cad	57.22 ± 4.03^b	6.71 ± 0.75^a	0.090 ± 0.01^a
Spd	178.4 ± 9.21^b	402.8 ± 6.96^a	277.99 ± 23.39^a
Spm	n.d.	1287.9 ± 17.22^a	2.29 ± 0.68^a

Superscripts letters indicate statistically significant differences between control and metal treatment at $p \leq 0.05$). The data are mean values ± SE of three independent replicates.

Endogenous content of auxins: The exposure of seedlings to both Cu^{2+} and Cr^{6+} metal stress led to significant changes in the levels of auxins. Free IAA (2.56 μg/g f.w.) and bound IAA content (1.5 μg/g f.w.) observed in Cu^{2+} metal stressed seedlings was much lower than free IAA and bound IAA (7.29 μg/g f.w. and 2.07 μg/g f.w., respectively) found in control seedlings. No free and bound NAA was recorded in untreated control seedlings. However, higher contents of free NAA (35.01 μg/g f.w.) and bound NAA (22.37 μg/g f.w.) were detected in Cu^{2+} metal stressed seedlings. Free IBA (6.96 μg/g f.w.) and bound IBA (5.02 μg/g f.w.) content recorded in Cu^{2+} metal treated seedlings was higher than free IBA and bound IBA (1.01 μg/g f.w and 0.94 μg/g f.w., respectively) found in control seedlings.

Significant reduction in both free IAA (0.65 μg/g f.w.) and bound IAA (0.53 μg/g f.w.) was recorded in Cr^{6+} metal stressed seedlings in comparison to free IAA and bound IAA contents found in control seedlings. Cr^{6+} metal stress was observed to initiate synthesis of NAA, which was not detected in control seedlings. The concentration of both free NAA (7.20 μg/g f.w.) and bound NAA (5.77 μg/g f.w.) detected in Cr^{6+} metal stressed seedlings was higher than under Cu^{2+} stress. Similarly the concentration of free IBA

(7.41 μg/g f.w.) and bound IBA (5.80 μg/g f.w.) observed in Cr^{6+} metal stressed seedlings was higher than IBA content found in control seedlings.

Table 2. Endogenous contents (μg/g f.w.) of free and bound auxins (IAA, NAA and IBA) and abscisic acid (ABA) in radish seedlings grown under 0.2 mM $CuSO_4 5H_2O$ (Cu^{2+}) and 1.2 mM K_2CrO_4 (Cr^{6+}) metal stress.

S.No.	Control	0.2 mM $CuSO_4 5H_2O$	1.2 mM K_2CrO_4
Free IAA	7.29±0.69[b]	2.56±0.21[a]	0.65±0.21[a]
Bound IAA	2.07±0.57	1.5±0.20[a]	0.53±0.20
Free NAA	n.d.	35.01±3.43[a]	7.20±0.34
Bound NAA	n.d.	22.37±1.11[a]	5.77±0.21
Free IBA	1.01±0.98[b]	6.96±0.63[a]	7.41±0.24[a]
Bound IBA	0.94±0.09[b]	5.02±0.26[a]	5.80±0.18[a]
Free ABA	5.79±0.76	10.67±0.95	13.51±0.03[a]
Bound ABA	4.60±0.05[b]	10.94±0.87	11.22±0.58[a]

Superscript letters indicate statistically significant differences between control and metal treatment at $p \leq 0.05$. The data are mean values ± SE of three independent replicates.

Endogenous contents of ABA: Significant increase in both free and bound forms of ABA were observed under Cu^{2+} and Cr^{6+} metal stress when compared to control. However, maximum rise in free ABA (13.51 μg/g f.w.) and bound (11.22 μg/g f.w.) was recorded under Cr^{6+} metal stress when compared to control and Cu^{2+} metal stressed seedlings.

Antioxidative enzyme activities: Copper stress has a drastic effect on radish physiology. The GPOX activity was reduced significantly from (0.270 U/mg protein f.w.) in control seedlings to (0.066 U/mg protein f.w.) in seedlings undergone Cu^{2+} metal treatment (Table 3). On the other hand CAT activity showed a decline from 0.171 U/mg protein f.w. (control) to 0.076 U/mg protein f.w. (Cu^{2+}-metal treatment) (Table 3). GR activity was also severely affected by Cu^{2+} metal stress, with decrease in activity (2.2 U/mg protein f.w.) in Cu^{2+} metal treatment, when compared to 10.5 U/mg protein f.w. in untreated control seedlings (Table 3). The SOD activity recorded enhancement from 3.56 U/mg protein f.w. to 8.65 U/mg protein f.w. in control and Cu^{2+}-metal treated seedlings respectively (Table 3). A significant reduction in protein content from 20.78 mg/g f.w. (control) to 9.79 mg/f.w. (Cu^{2+} metal stress) has been also observed (Table 3).

Chromium (Cr^{6+}) metal stress exhibits more phytotoxicity than Cu^{2+} metal in terms of antioxidative enzyme activity. A significant decrease in GPOX activity (0.0514 U/mg protein f.w.) in Cr^{6+} metal stressed seedlings when compared to GPOX activity (0.270 U/mg protein f.w.) found in control seedlings (Table 3). Similarly, a reduction in CAT activity under Cr^{6+} metal stress (0.053 U/mg protein f.w.) was observed in comparison to control. Drastic decrease in GR activity (1.21 U/mg protein f.w.) was found in Cr^{6+} metal stressed seedlings in compassion to 10.5 U/mg protein f.w. in control seedlings. SOD activity showed significant rise (12.4 U/mg protein f.w.) in comparison to control seedlings (Table 3). A significant reduction in protein content (7.62 mg/g f.w.) was observed in Cr^{6+} metal stressed seedlings in comparison to protein content (20.78 mg/g f.w.) recorded in control seedlings (Table 3).

Table 3. Effects of EBL treatments on antioxidative enzyme activity (U/mg protein g^{-1} f.w.) growth parameters of 7d old radish seedlings grown under 0.2 mM $CuSO_4 5H_2O$ (Cu^{2+}) and 1.2 mM K_2CrO_4 (Cr^{6+}) metal stress.

S.No.	GPOX	CAT	SOD	GR	Protein (mg/g FW)
Control	0.270±0.02[b]	0.171±0.011[b]	3.56±0.706[b]	10.5±0.98[b]	20.78±1.22[b]
0.2 mM Cu	0.066±0.0052[a]	0.076±0.010[a]	8.65±1.02[a]	2.2±0.103[a]	9.79±1.23[a]
1.2 mM Cr	0.0514±0.0082[a]	0.053±0.006[a]	12.4±0.710[a]	1.21±0.092[a]	7.62±0.657[a,b]

Superscript letters indicate statically significant differences between control and metal treatment at $p \leq 0.05$. The data are mean values ± SE of three independent replicates.

Seedling growth: Cu^{2+} metal stress significantly reduced shoot length (1.5 cm) and root length (3.24 cm) of *Raphanus* seedlings in comparison to shoot (3.53 cm) and root length (6.50 cm) observed in control seedlings (Table 4). Decrease in fresh weight (0.150 g) was observed in Cu^{2+} metal stressed seedlings when compared to fresh weight (0.240 g) recorded in control seedlings. Similarly, Cr^{6+} metal stress reduced shoot length (2.23 cm) and root length (3.16 cm) in comparison to control seedlings. Moreover decrease in fresh weight (0.108 g) was more than Cu^{2+} metal induced decrease in fresh weight when compared to control seedlings.

Table 4. Effects of EBL treatments on growth parameters of 7 d old radish seedlings grown under 0.2 mM $CuSO_4 5H_2O$ (Cu^{2+}) and 1.2 mM K_2CrO_4 (Cr^{6+}) metal stress.

S.No.	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control	6.50±0.099[b]	3.53±0.186[b]	0.240±0.005[b]
0.2 mM Cu	3.24±0.071[a]	1.5±0.121[a]	0.150±0.005[a]
1.2 mM Cr	3.16±0.128[a]	2.23±0.203[a]	0.108±0.010[a]

Superscript letters indicate statistically significant differences between control and metal treatment at $p \leq 0.05$). The data are mean values ± SE of three independent replicates.

DISCUSSION

Heavy metal stress leads to oxidative damage caused by the production of free radicals and ROS (Halliwel and Gutteridge, 1984). Both Cu^{2+} and Cr^{6+} metal induces oxidative stress in plant metabolism, ultimately resulting into the release of free radicals or oxidants (Luna et al., 1994). To counteract ill effects of ROS plants have evolved defense mechanisms consisting of antioxidants, antioxidative enzymes and phytohormones. The Cu^{2+} and Cr^{6+} metal phytotoxicity was observed in the present investigation in the form of reduced shoot and root growth. The phytotoxicity of Cu^{2+} metal in reducing shoot length was more than Cr^{6+} metal, which in turn showed its more toxicity on root length. Decrease in fresh weight of the seedlings was more pronounced under Cr^{6+} metal stress than Cu^{2+} metal stress. Decrease in shoot length and root length under metal stress may be due to improper chlorophyll synthesis and blockage of root hairs, which resulted in impaired uptake of nutrients and water (Barcelo et al., 1986; Shanker et al., 2005). Moreover impaired shoot and root system might be unable to undergo normal photosynthesis, cell division and cell elongation thus resulting in a visible reduction in fresh weight of the seedlings under Cu^{2+} and Cr^{6+} metal stress in comparison to untreated control seedlings (Table 4). The present investigation also revealed selective synthesis of high molecular weight PAs like Spm and Spd (Table 1) on account of more amine groups than low molecular weight Cad and Put (Table1) which has lesser number of amine groups clearly indicating faster scavenging of oxidants or the free radicals generated under Cu^{2+} and Cr^{6+} metal stress. Shevyakova et al. (2006) also recorded enhanced synthesis of Spd and Spm than Put and Cad in *Mesembryanthemum crystalinum* under salinity stress. The strong antioxidant character of high molecular weight PAs than low molecular weight PAs had also been recorded by Ha et al. (1998).

The reduction in Cad content has also been reported to induce oxidative burst which led to enhanced activity of superoxide dismutase (Vladimir et al., 2009). Our findings are in agreement with these observations that reduction in Cad concentration acts as stress response for inducing the enhanced activity of SOD (Vladimir et al., 2009). The enhanced synthesis of Put than Cad under Cu^{2+} metal stress indicated its effectiveness in oxidative stress management than Cad. However Cr^{6+} metal stress reduced Put content than control value, which in turn suggests selective induction of PAs biosynthesis under Cr^{6+} metal stress. These results clearly suggest that Cu^{2+} and Cr^{6+} metal stress has been very selective in the induction of polyamine synthesis. This has also signified the extraordinary ability of Spd and Spm in metal stress management than other PAs.

The active involvement of auxins in plant stress management is recently explored. Partial restoration of cambial activity in *Luffa cylindrica* L. under mercury stress on application of auxins was reported by Khan and Chaudhary (2006). Similarly, Dimpka et al. (2008) recorded the phytoremediation role of auxins and siderophores in *Streptomyces spp.* against heavy metals (Cu, Ni and Cd). Significant changes in auxins content under Cu^{2+} and Cr^{6+} metal stress revealed their significant involvement in metal stress management. Such that reduced synthesis of free and bound IAA under Cu^{2+} and Cr^{6+} metal stress could be correlated with reduced shoot and root growth and decreased fresh weight. On the other hand enhanced synthesis of free and bound NAA and IBA under Cu^{2+} and Cr^{6+} metal stress indicated there active involvement in metal stress management than IAA. Moreover Cr^{6+} metal induced synthesis of free and bound NAA and IBA more effectively than Cu^{2+} metal stress (Table 2). Similarly increased synthesis of free and bound ABA under Cu^{2+} and Cr^{6+} stress also showed their active involvement in metal stress tolerance (Table 2). Our findings are in agreement with Khan and Choudhary (2006) and Kurepin et al. (2008) who also showed increase in the contents of auxins and ABA in *Luffa cylindrica* and *Brassica napus* respectively.

Decrease in the activities of GPOX, CAT and GR under Cu^{2+} and Cr^{6+} metal stress were observed (Table 3). However significant enhancement in the activity of SOD recorded under Cu^{2+} and Cr^{6+} metal stress was observed to be accompanied by increase in the endogenous titers high molecular weight

PAs (Put, Spd and Spm). Whereas reduction in Cad content observed under Cu^{2+} and Cr^{6+} metal stress might be able to enhance synthesis and activity of SOD. More recently, Vladimir et al. (2009) observed that burst of Cad pool might act as a signal for initiating the enhanced activity of SOD, which may be able to protect the plants against oxidative stress induced by Cu^{2+} and Cr^{6+} metal stress along with PAs and auxins in a better way.

CONCLUSION

The present investigation revealed pronounced phytotoxicity of Cr^{6+} over Cu^{2+} stress in terms of reduced shoot and root growth, and fresh weight. The synthesis of PAs was more influenced by Cu^{2+} metal than Cr^{6+} metal. Moreover, Cu^{2+} was able to induce the synthesis of high molecular weight PAs (Spd and Spm) more effectively than Cr^{6+} metal. The synthesis of free and bound forms of IAA, NAA and IBA were observed to be significantly altered under Cr^{6+} metal stress than Cu^{2+} metal stress. Therefore the results obtained suggest that Cr^{6+} metal stress mainly targeted seedling growth and synthesis of auxins while Cu^{2+} metal showed its phytotoxicity in terms of enhanced synthesis of PAs.

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