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Biochemical Changes under Chromium Stress on Germinating Seedlings of *Vigna radiata*

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Abstract

Hexavalant chromium is considered the most toxic form because of its high solubility in water. Cr is known to induce production of elevated concentration of reactive oxygen species (ROS) resulted in macromolecule damage. Plants are having unique mechanisms to overcome ROS induced damage by accumulation of proline, ascorbate and glutathione and increasing the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidaes (APX), peroxidise (POX). In the present investigation effects of chromium on seed germination of Mung bean (*Vigna radiata* 'Gujarat Mung-4') were studied. Seeds were treated with different Cr concentrations (50, 100, 150 and 200 µM) for seven days. On 7th day root and shoot length was measured and activities of antioxidant enzyme SOD, APX, POX, CAT and GR were checked along with protein, proline and lipid peroxidation. It was observed that there is gradual decrease in shoot and root length with respect to the increase in Cr concentration. Level of lipid peroxidation significantly increased along with proline and antioxidant enzyme activity at higher Cr concentration. Lipid peroxidation is an indication of membrane damage due to elevated production of reactive oxygen species (ROS). To combat oxidative damage by ROS antioxidant enzyme activity increased significantly, which indicates that antioxidant enzymes (SOD, CAT, APX and GR) play a crucial role during Cr stress during germination of *V. radiata.*

Keywords: catalase, chromium, glutathione reductase, lipid peroxidation, MDA, peroxidise, proline, superoxide dismutase

Abbreviations: APX - Ascorbate peroxides; CAT - Catalase; Cr^{+6} - Hexavalent chromium; GR - Glutathione reductase; MDA - Malondialdehyde; POX - Peroxidasel; ROS - Reactive oxygen species; SOD - Superoxide dismutase

Introduction

Agricultural land contamination with heavy metals is a serious problem, which has gathered considerable public attention in recent decades. Some metals are required for plant growth and development but some of them are very toxic at elevated concentration. Sources of heavy metal contamination include anthropogenic activities, like use of pesticides, agriculture waste and industrial waste. Hexavalant chromium is considered the most toxic form because of its high solubility in water, which usually occurs amalgamated with oxygen as chromate or dichromate (Samantaray *et al.,* 1998, Caldelas *et al.*, 2012; Hayat *et al.*, 2012). Cr is known to induce production of elevated concentration of ROS resulted in macromolecule damage. Plants have evolved mechanisms to overcome ROS induced damage by accumulation of proline, ascorbate and glutathione and increasing the activities of SOD, CAT, GR, POX and APX (Samantaray *et al.,* 1998; Nataraj *et al.,* 2009, Thounaojam *et al.,* 2012; Tripathi *et al.* 2012;). Antioxidants are the compounds, which act as osmotic buffers; beside this, they scavenge ROS (Nataraj and Paramar, 2008, Nataraj and Roshan, 2008, Nataraj *et al.*, 2009). Soluble protein content of germinating seedling is an important indicator of metabolic changes under various stress conditions and predicts physiological status of seedlings (Nataraj and Roshan, 2008, Martins *et al.*, 2013). Mung bean is an important staple food of the world population having high nutritional quality among pulses.

The present investigation was undertaken to test the effects of chromium on growth, induction of oxidative stress and antioxidative responses in Mung bean cultivar 'Gujarat Mung-4.'

Materials and methods

Plant material, growth conditions and treatment

The present study was carried out on Mung (*Vigna radiata*) 'Gujarat Mung-4', which was collected from research centre of Junagadh Agriculture University (JAU), Junagadh. The seeds were surface sterilized with 0.1% $HgCl_2$ (w/v) for one minute. Solutions of different heavy metal concentrations (50, 100, 150, 200 µM) were prepared, using $K_2Cr_2O_7$ and distilled water. Six seeds were placed on Petri plates over Whatman no^{-1} filter paper and treated with 5 ml solution of different chromium (Cr) concentrations. For every treatment three replicates, each with six seeds, were maintained. The fresh solution was added after 48 hours to the above described Petri plates. Seven day old seedlings were selected for bio-chemical analysis.

Total soluble protein, proline and lipid peroxidation

Protein content was determined as described by Lowry *et al*. (1951). Proline content was estimated following the procedure of Bates *et al.* (1973). The extent of lipid peroxidation in terms of malondialdehyde (MDA) formation was measured according to the method of Esterbauer and Cheeseman (1990).

Determination of enzymatic antioxidants

After 7 days of treatment, 0.50 g fresh seedlings were homogenized with 8 ml 50 mM phosphate buffer solution (pH 7.8) in an ice bath and then centrifuged at 10,000 x g for 15 min at 4 °C. The supernatant was designated as crude enzyme extract and stored at 4 °C for further enzyme assay.

Superoxide dismutase (EC 1.15.1.1)

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue-tetrazolium (NBT) according to Beauchamp and Fridovich (1971).

Catalase (EC 1.11.1.6)

Catalase content was measured by the method of Aebi (1984). The assay mixture contains 3 ml of 50 mM potassium-phosphate buffer (pH 7.0), 0.4ml of 15mM H_2O_2 and 0.04ml of crude enzyme extract. The activity of catalase (CAT) was measured monitoring H_2O_2 decomposition at 240 nm.

Glutathione reductase (EC 1.6.4.2)

GR activity was assayed according to Smith et al. (1988). The reaction mixture consisted of 100µl NADPH (2.4 mM), 100 µl oxidised glutathione (GSSG, 10 mM) and 2.7 ml 25 mM potassium-phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of NADPH was determined by its absorbance at 340 nm.

Peroxidase (EC 1.11.1.7)

Peroxidase content was determined using the method of Chance and Maehly (1955). The amount of purpurogalline formed was determined by reading the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 1 ml 2.5N H_2SO_4.

Ascorbate peroxidase (EC 1.11.1.11)

The activity of ascorbate-peroxidase (APX) was measured according to the method of Nakano and Asada (1981). The reagent mixture consisted of 3 ml of 50 mM phosphate buffer (pH-7.0). 0.5 ml 0.1 mM EDTA, 0.5 ml 0.5 mM ascorbate, 0.5 ml 0.1 mM H_2O_2 and 0.5 ml plant extract.

***Statistical analysis*:** Presented data represent the mean values with standard deviation (S.D.). All results were subjected to one-way analysis of variance (ANOVA) using the statistical software package (Graph pad PRISM 3.0). The significance of differences between exposed and control plants was tested by Tukey's multiple comparison tests. The difference was considered significant at p levels lower than 0.05 ($p < 0.05$).

Results and discussions

The effect of Cr on shoot length and root length is presented in Fig. 1 and Fig. 2. Shoot and root length significantly decreased with respect to increase in Cr concentration. Similar responses of inhibitory activity of Cr on root and shoot length was reported in rice (Tripathi *et al.*, 2012). This reduction could be due to the accumulation of high concentration of Cr in roots and/or a nonexistence of any defined Cr translocation mechanism, thereby enhancing the Cr sequestration in the tissue thus, inhibiting root development (Diwan *et al.*, 2010; Lu *et al.*, 2004).

As shown in Fig. 3, total soluble protein content increased with the increasing Cr^{+6} concentrations in the

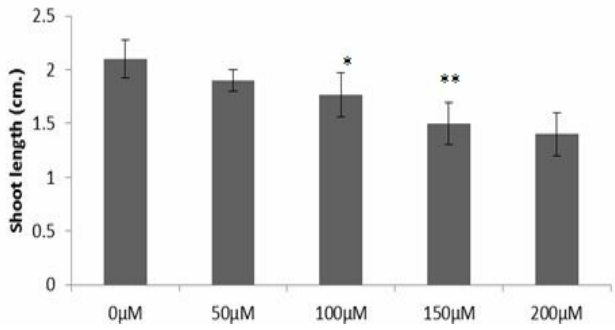

Fig. 1. Effect of different Cr concentrations on shoot length in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p<0.05 ** p<0.01 and *** p<0.001

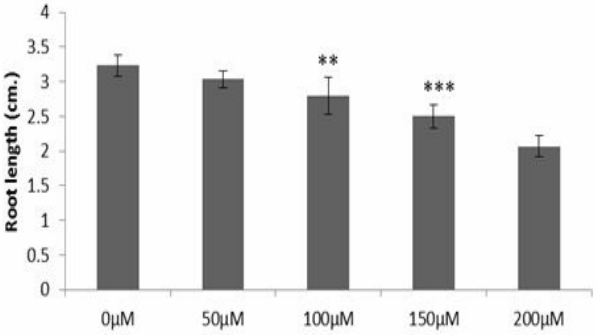

Fig. 2. *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p<0.05 ** p<0.01 and *** p<0.001

treatment. At lower concentration 50 µM of Cr^{+6} exposures a statistically non-significant increase of total soluble protein was observed. A significant increase in protein content found in seeds treated with 150 µM and 200 µM Cr^{+6} ($p<0.001$). Similar observations were reported in *Brassica juncea* under cadmium stress *(Seth et al.*, 2008, Szollosi *et al*. 2009).in 100 µM to ($p<0.001$) 200 µM Cr^{+6} treated seeds. Proline accumulation in response to heavy metal stress was reported in several plants (Nataraj and Paramar, 2008; Nataraj *et al*. 2009, Yilmaz and Parlak, 2011).

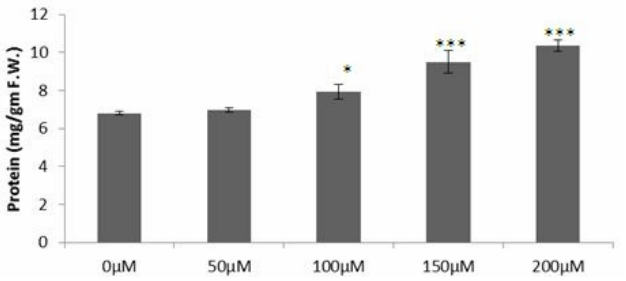

Fig. 3. Effect of different Cr concentrations on total soluble protein content in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * $p<0.05$ ** $p<0.01$ and *** $p<0.001$ $p<0.001$

Lipid peroxidation is measured in terms of MDA content. MDA content increased in all the Cr treated germinating seeds (Fig. 4.). A non-significant increase was found during the 50 µM treatment, while a significant increase ($p<0.001$) in MDA content was found at 150 and 200 µM Cr^{+6}. As MDA content is a product of lipid peroxidation, the elevated concentration of MDA content clearly reflects membrane damage due peroxidation of membrane's lipid content in the presence of ROS. MDA level is regarded as a biochemical indicator for injury mediated by ROS (Ozdener *et al.*, 2011). Our results indicate that excessive heavy metal increases oxidative stress as it is evident from increased lipid peroxidation. It is in accordance with Ozdener *et al.* (2011) and Tripathi *et al*. (2012).

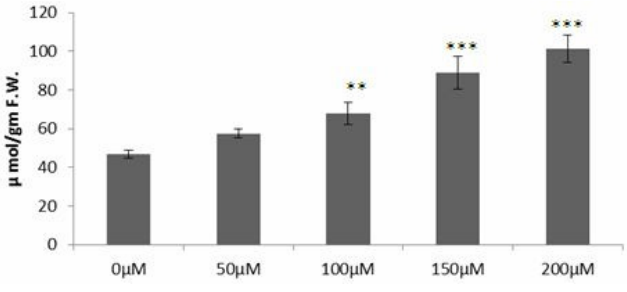

Fig. 4. Effect of different Cr concentrations on MDA content in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * $p<0.05$ ** $p<0.01$ and *** $p<0.001$.

The amounts of proline accumulation in *V. radiata.* 'Gujarat Mung-4 under Cr^{+6} stress are shown in Fig. 5. The amount of proline increased significantly ($p<0.05$)

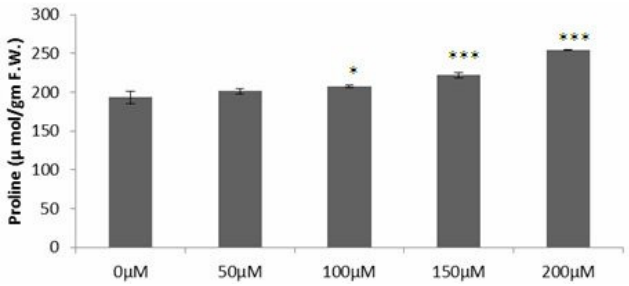

Fig. 5. Effect of different Cr concentrations on proline content in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * $p<0.05$ ** $p<0.01$ and *** $p<0.001$

SOD, CAT, GR, POX and APX are important components in preventing oxidative stress in plants by scavenging free radicals and peroxides with the elevation of their activities when exposed to heavy metal stress (Shanker *et al*., 2005). Antioxidant enzyme activities after exposure to Cr^{+6} are shown in Fig. 6 to 10. In the present investigation, it was found that SOD, GR and POX activity increased significantly at ($p<0.001$) 100,150 and 200 µM, whereas CAT and APX activity significantly increased ($p<0.001$) at 50 µM in comparison with 200 µM in *V. radiata* 'Gujarat Mung-4'. Enhanced activities of these enzymes suggest the active involvement of SOD in the removal of superoxide radical and H_2O_2 by CAT and GR. Similar reports were presented earlier due to metal treated germinating seeds of raya and fenugreek (Nataraj and Parmar, 2008; Nataraj and Roshan, 2008; Nataraj *et al*., 2009).

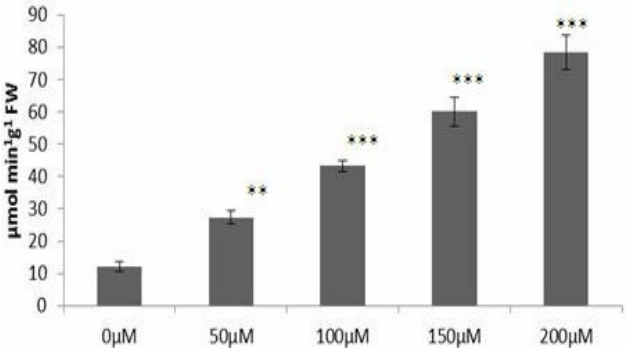

Fig. 6. Effect of different Cr concentrations on superoxide dismutase activity in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly Fig.2. Effect of different Cr concentrations on root length in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * $p<0.05$ ** $p<0.01$ and *** $p<0.001$.

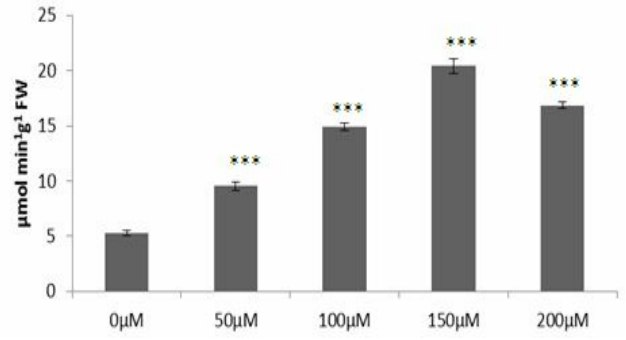

Fig. 7 Effect of different Cr concentrations on catalase activity in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p<0.05 ** p<0.01 and *** p<0.001.

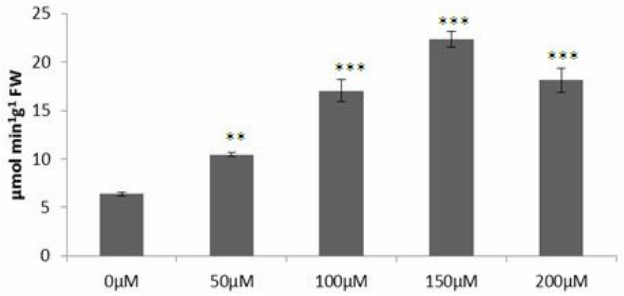

Fig. 9. Effect of different Cr concentrations on peroxidase activity in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p < 0.05 ** p<0.01 and *** p<0.001.

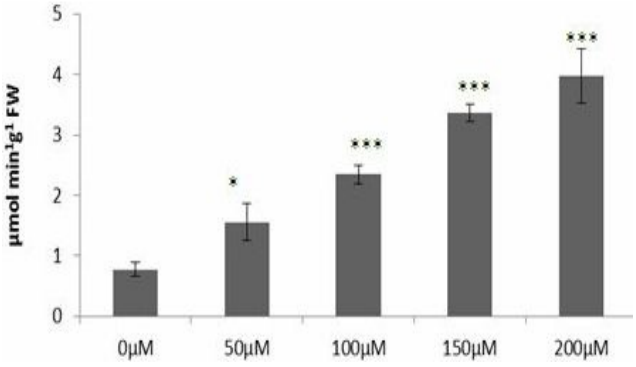

Fig. 8 Effect of different Cr concentrations on glutathione reductase activity in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p<0.05 ** p<0.01 and *** p<0.001.

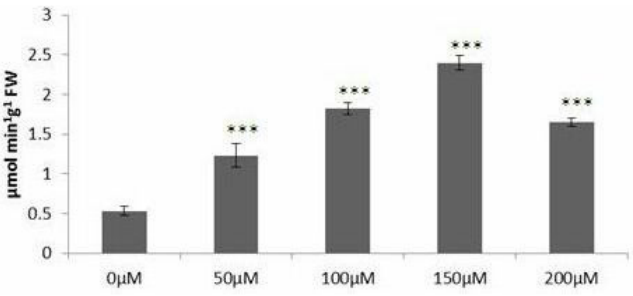

Fig. 10. Effect of different Cr concentrations on ascorbate peroxidase activity in *Vigna radiata* 'Gujarat Mung-4'. Values represent the mean ± S.D. (n=3) asterisk * indicates mean value significantly different from control at * p<0.05 ** p<0.01 and *** p<0.001.

Conclusions

In conclusion, root/shoot length of *V. radiata* was significantly affected by Cr concentration. Exposure of *V. radiata* to Cr, resulted in increasing the level of lipid peroxidation which is an indication of formation of elevated level of reactive oxygen species. To combat oxidative damage by ROS, antioxidative enzyme (SOD, CAT, GR, APX and POX) activity increases, this suggests antioxidative defence mechanism of *V. radiata* seedlings under Cr stress.

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References

Aebi H (1984). Catalase *in vitro*. Methods Enzymol 105:121-176.

Bates LS, Walrden RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276-287.

Caldelas C, Araus JL, Febrero A, Bort J (2012). Accumulation and toxic effects of chromium and zinc in *Iris pseudacorus* L. Acta Physiol Planta 34:1217-1228.

Chance B, Maehly A (1955). Assay of catalases and peroxidases. Methods in Enzymology 2:764-775.

Diwan H, Khan I, Ahmad A, Iqbal M. (2010). Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. Plant Growth Regul 61:97-107.

Esterbauer H, Cheeseman KH (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods in Enzymology 186:407-421.

Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN, Ahmad A (2012). Physiological changes induced by chromium stress in plants: an overview. Protoplasma 249:599-611.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin Phenol reagent. J Biol Chem 193:265-275.

Lu X, Kruatrachue M, Pokethitiyook P, Homyok K (2004). Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. Sci Asia 30:93-103.

Martins N, Gonçalves S, Romano A (2013). Aluminum inhibits root growth and induces hydrogen peroxide accumulation in *Plantago algarbiensis* and *P. almogravensis* seedlings. Protoplasma 250(6):1295-302.

Nakano Y, Asada K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant Cell Physiol 22:867-880.

Nataraj M, Parmar A, Kher MM. (2009). Impact of chromium during early germination of fenugreek in interaction with copper, zinc lead and cadmium. PRAJÑA-J Pure App Sci 17:40-47.

Nataraj M, Parmar S (2008). Biochemical response during the germination of raya and fenugreek seeds under heavy metal stress. J Cell Tiss Rese 8:1589-1594.

Nataraj M, Roshan A (2008). Impact of chromium on Raya and Fenugreek a biochemical study. Adv Biol Sci 723-728.

Ozdener Y, Aydin BK, Fatma Aygün S, Yürekli F (2011). Effect of hexavalent chromium on the growth and physiological and biochemical parameters on *Brassica oleracea* L. var. *acephala* DC. Acta Biol Hun 62:463-476.

Samantaray S, Rout GR, Das P (1998). Role of chromium on plant growth and metabolism. Acta Physio Plant 20:201-212.

Seth CS, Kumar Chaturvedi P, Misra V (2008). The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotoxicol Environml Saf 71: 76-85.

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005). Chromium toxicity in plants. Environ Intern 31:739-753.

Smith IK, Vierheller TL, Thorne CA (1988). Assay of glutathione reductase in crude tissue homogenates using 5, 5′-dithiobis (2-nitrobenzoic acid). Anal Biochem 175:408-413.

Szollosi R, Varga IS, Erdei L, Mihalik E (2009). Cadmium-induced oxidative stress and antioxidative mechanisms in germinating Indian mustard (*Brassica juncea* L.) seeds. Ecotoxicol Environ Saf 72:1337-1342.

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma GD, Sahoo L, Panda SK (2012). Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Biochem 53:33-39.

Tripathi DK, Singh VP, Kumar D, Chauhan DK (2012). Impact of exogenous silicon addition on chromium uptake, growth, mineral elements, oxidative stress, antioxidant capacity, and leaf and root structures in rice seedlings exposed to hexavalent chromium. Acta Physiol Plant 34:279-289.

Yilmaz DD, Parlak KU (2011). Changes in proline accumulation and antioxidative enzyme activities in *Groenlandia densa* under cadmium stress. Ecological Indicators 11:417-423.