Growth and Biochemical Variations in *Macrotyloma uniflorum* Var. Madhu Under Chromium Stress

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Received: 16-01-2019 Accepted: 01-08-2019

ABSTRACT

The discharge of contaminated mining and industrial residues has become a major cause of environmental pollution. Seed is highly protective against external metal stresses. But they are highly sensitive during vegetative developmental process. In this study, germination was conducted in *Macrotyloma uniflorum* var. Madhu, in order to find out the effect of chromium toxicity on germination, growth and biochemical variations. The seeds were germinated in five different concentrations of chromium (25μM, 50μM, 100μM, 150μM and 200 μM) solution. The hydroponically grown plants revealed the toxic phenotypic expression (root length, shoot length, seedling length, shoot vigor index, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight) of plant after 3 growth stage (15 d, 30 d and 45 d). Similarly biochemical characteristics as well as antioxidant activities (Chlorophyll, carotenoid, carbohydrate, reducing sugar, catalase, proline content and peroxidase) were studied to see the toxic effect of chromium.

Key words: Chromium, GPX, *Macrotyloma uniflorum*, SOD.

INTRODUCTION

The term heavy metal usually includes metals and metalloids with atomic density greater than 5 g/cm³, or 5 times or more, greater than water (Jarup, 2003). Among these chromium (Cr) is known to be the 7th most important and abundant metal on the earth’s crust (Katz and Salem, 1994). Though chromium (Cr) exists in different oxidation states, the two oxidation states [trivalent (III) and hexavalent (VI)] of chromium are more stable and prevalent in aquatic and terrestrial ecosystems. However, hexavalent Cr found in association with oxygen as chromate (CrO₄²⁻) or dichromate (CrO₇²⁻) anions are the most toxic forms.

Crops grown on soil toxicated with hexavalent chromium usually take up and assemble this metal in their aerial and sub-aerial parts (Jarup, 2003). Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Once crop up take these hexavalent forms of Cr, it adversely affects the physiological processes in crops. As reported previously, the toxic effects of hexavalent Cr include inhibition of plant growth, chlorosis, biochemical lesions, less enzyme activities, decrease in yield etc (Panda and Patra, 2000; Arun et al., 2005; Mohanty et al., 2011). Most often these increased levels of hexavalent Cr in crop organs initiate production of reactive oxygen species (ROS) like H₂O₂, O₂⁻, OH etc. These ROS induce oxidative stress in plants ultimately destroying DNA, proteins and pigments as well as activate lipid peroxidation process (Reddy et al., 2005).

The present study is focused on growth and biochemical variations occurring in *Macrotyloma uniflorum* (Lam) Verdc (horse gram) under the influence of hexavalent chromium. *Macrotyloma uniflorum* is one of the wild pulses of Fabaceae family and has been used as most important pulse in daily diet of poor and malnourished people. This is why it is also known as “poor man’s crop” particularly in drought prone areas. Further adding medicinal value to the crop, the seeds are generally used to treat kidney stones, diabetes and heart diseases. Previous reports suggest that, these seeds contain antiurolithiatic activities which could prevent stone formation in kidneys (Atodariya et al., 2013). It also has an important role as antioxidant as it can tolerate heavy metal contamination and therefore can be useful as a phytoremediating plant. Hence, the present study progressed to assess a general picture of the variations in growth and biochemical properties of *Macrotyloma uniflorum* var. Madhu under Cr (VI) stress.

MATERIALS AND METHODS

Seed germination assay: Certified seeds of *Macrotyloma uniflorum* var. Madhu were procured from Orissa University of Agriculture and Technology, Bhubaneswar and were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes and then thoroughly washed with tap water and distilled water for several times. After the preliminary disinfection seeds were germinated in petriplates over saturated paper towel and supplemented with different concentrations of Cr⁶⁺(K₂Cr₂O₇) solution. The experiment
was established in 3 replications of 10 seeds in triplicate. The petriplates were placed inside BOD incubator at 25±2 °C in two days. After two days the number of seeds germinated from each concentration was recorded. Germination percentage and germination indices of seeds were determined by formula

\[
\text{Germination (\%) =} \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds taken}} \times 100
\]

**Seedling growth and growth parameter study:** The 3 days old seedling was transferred to Hoagland cup containing half-strength sterile Hoagland’s nutrient solution after Epstein (1972) as described by Taiz and Zeiger (2002) and supplemented with different doses of Cr (VI) stress (25, 50, 100 150 and 200 µM). *Macrotyloma uniflorum* seedlings supplemented with different concentrations of Cr (VI) (25μM to 200 μM) were taken for the study of various growth parameters for 15d, 30d and 45d respectively. The plants grown on half-strength Hoagland medium without chromium solution served as control.

**Phenotypic growth of Macrotyloma uniflorum var. Madhu:** Shoot length (cm) was measured from the base of primary leaf to base of hypocotyls. Ten normal seedlings were selected from each treatment along with their replicates were selected after final count of the germination test. The root length (cm) was measured from the tip of the of primary root to base of hypocotyls. The mean shoot and root length(cm) was calculated by adding the shoot and root lengths of already selected ten normal seedlings. Seedling vigor index is calculated by the formula as suggested by Abdual Baki and Anderson (1973) and expressed in number.

\[
\text{Seedling vigor index (SVI) = Germination percentage} \times \text{Seedling length (cm)}
\]

The fresh weight of shoot and root samples were measured in weighing balance and measured in grams per plant. After that the shoots and roots were put in butter paper pockets and kept in an oven maintained at 100 ± 2°C for 24 hrs for root and shoot dried weight. The weight of dried seedlings was recorded and mean dry weight was expressed in milligrams (Pantola et al., 2017).

**Biochemical properties Macrotyloma uniflorum var. Madhu under Cr stress:** The total chlorophyll and carotenoid content were analyzed and calculated by the method of Porra (2002) by using 10 ml of 80% cold acetone. The absorbance of Chlorophyll-a, Chlorophyll-b and carotenoid was recorded at 663.6 nm, 646.6 nm and 470 nm respectively (Mathan et al., 2014).

The protein content were extracted and quantified by the method of Lowry et al. (1951) taking Bovine Serum Albumin as standard. Proline content was estimated by Bates et al. (1973) and the absorbance is measured at 520nm. Soluble carbohydrate was estimated by the anthrone method described by Sadasivam and Manickam (2008). Reducing sugar was estimated by using Nelson-Somogyi method and the absorbance was taken at 525nm. Catalase (CAT; EC 1.11.1.6) and Guaiacol peroxidase activity (POX; EC 1.11.1.1) enzyme assay and activity were measured as per the method (Mathan et al., 2014) with little modifications.

**Statistical analysis:** Data were statistically analyzed by using analysis of variance (ANOVA). The values are expressed as mean ± standard error (S.E.) of three replications. Differences of data from different concentrations and control were considered significant at p<0.05 level.

**RESULTS AND DISCUSSION**

**Germination studies:** Seeds of *Macrotyloma uniflorum* germinated well under all concentrations of chromium (Fig 1A, B). Seed germination at all different concentrations

![Fig 1](image-url)
showed variation as compared to control. The least number of seeds germinated at 200 µM treated plants after 15d of germination. Decline in seed germination ranges up to 19% with supplementation of 200 µM of Cr (VI) (Fig 1C).

Effect of hexavalent Cr on germination parameters of Macrotyloma uniflorum: The effect of Cr (VI) on root length, shoot length, seedling length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight was presented in (Table 1). Phytoxic symptoms were observed at ≥100 µM of Cr (VI). Root length and shoot length showed a decline in its elongation and morphology with increasing chromium concentration up to 50 µM with increasing number of days of exposure. In 15 days old Macrotyloma uniflorum, there was marked difference in elongation and morphology of root and shoot growth in different concentrations of Cr (VI). There is a decline in shoot length at higher concentration of 100 µM Cr (VI), where as in control it was observed to be more elongated. The severity of root length inhibition was observed in similar decreasing trend at 100 µM, 150 µM and 200 µM (Table 1) respectively when compared with control. Reduction in shoot length was observed in treatment with Cr (VI) in Macrotyloma uniflorum. Root and shoot length progressively reduced with 100µM, 150µM and 200 µM of Cr (VI) supplementation with number of days.

Effect of Cr (VI) on biochemical properties of Macrotyloma uniflorum: The chlorophyll content from Macrotyloma uniflorum at different concentrations of chromium stress (from 25 µM to 200 µM) at 3 growth stage i.e. 15, 30 and 45 days old is presented in Fig 2A. Total chlorophyll content seen to decrease with increasing Cr (VI) concentration i.e. 6.82 mg g⁻¹ FW (25 µM) after 15 days of germination to 4.54 mg g⁻¹ FW (200 µM) respectively. Decrease in the total chlorophyll levels was noted after 45 days of germination with increasing Cr (VI) with respect to control (Fig 2A). Carotenoid content tend to increase as a mean to counter act the Cr (VI) stress effects on total chlorophyll content (Fig 2B). Macrotyloma uniflorum seedlings experienced a general inhibition of soluble carbohydrate content (Fig 2C) from 28.39 mg/g FW (25 µM) to 21.11 mg/g FW (200 µM) in 15 days. Whereas in 30 days there was a sharp decline from 24.47 mg/g FW (25 µM) to 15.53 mg/g FW (200 µM) to 23.64 mg/g FW (25 µM) to 13.59 mg/g FW (200 µM) respectively in 45 day old Macrotyloma uniflorum. There was a gradual decrease of reducing sugar in leaves with rise in Cr (VI) level (Fig 2D). In 15 days old Macrotyloma uniflorum the reducing sugar content decreased from 23.37 mg g⁻¹ FW (25 µM) to 16.23 mg g⁻¹ FW (200 µM) to 20.12 mg g⁻¹ FW (25 µM) to 12.45 mg g⁻¹ FW (200 µM) in 45 days old plant.

Total protein concentration of chromium treated seedlings was determined by Lowry method and is given in Fig 3A. In Madhu variety the protein content decreased with...
increasing level of Cr (VI) in 200 μM. Whereas in 30 days of germination at higher Cr (VI) concentrations the protein content decreased from 29.65 mg g\(^{-1}\) FW (50 μM) to 22.17 mg g\(^{-1}\) FW (200 μM) as compared to control. Reduction in protein content follows the same trend 22.31 mg g\(^{-1}\) FW (50 μM) to 15.8 mg g\(^{-1}\) FW (200 μM) respectively in 45 days of germination.

Treatment of different hexavalent chromium concentrations (25 μM, 50 μM, 100 μM, 150 μM, 200 μM) as well as the control pots showed marked variations in the catalase activity of 30 days and 45 days old *Macrotyloma uniflorum* seedlings grown under Cr (VI) stress. In *Macrotyloma uniflorum* the highest catalase activity was observed in 25 μM after 30 days treatment and 45 days treatment of Cr (VI). 200 μM of Cr (VI) treatment shows the least catalase activity. The catalase activity decreased with increasing number of days 30 days and 45 days and increase in the concentration of Cr (VI). The order of catalase activity in the seedlings of *Macrotyloma uniflorum* treated with different chromium concentration were Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) and Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) respectively after 30 and 45 days.

The catalase activity decreased from 1.09 μl/gm FW to 0.74 μl/gm FW (200 μM). The POX level showed an increasing trend with Cr (VI) treatment from 18 μl/gm FW to 32 μl/gm FW (200 μM) in 45 day old *Macrotyloma uniflorum* (Fig 3B).

The results pertaining to proline content is presented in Fig 3C. The proline content increased from 6.112 mg/g FW (25 μM) to 11.329 mg/g FW (200 μM) in 15 days old *Macrotyloma uniflorum*. The level of proline gradually increased from 9.231 mg/g FW (25 μM) to 18.993 mg/g FW (200 μM) in 30 days. Further rise in the proline content from 12.65 mg/g FW (25 μM) to 21.34 mg/g FW (200 μM) respectively is noticed in 45 day old *Macrotyloma uniflorum* (Fig 3C).

 Peroxidase activity increased significantly with respect to the higher levels of Cr (VI) treatments in all the tested varieties. The activity was increased with the increasing dose of Cr (VI) treatment. In *Macrotyloma uniflorum*, the maximum peroxidase activity was observed in Cr (VI) (200 μM) treatments after 30 days and after 45 days seedlings. The order of peroxidase activity in the seedlings of *Macrotyloma uniflorum* treated with different chromium concentration were Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) and Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) respectively after 30 and 45 days (Fig 3D).

SOD activity increased significantly with increased concentrations of Cr (VI) treatments in all the tested varieties. The activity was increased with the increasing dose of Cr (VI) treatment. In *Macrotyloma uniflorum*, the maximum SOD activity was observed in Cr (VI) (200 μM) treatments after 30 days and after 45 days seedlings. The order of SOD activity in the seedlings of *Macrotyloma uniflorum* treated with different chromium concentration were Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) and Control > Cr (VI)(25 μM) > Cr (VI)(50 μM) > Cr (VI)(100 μM) > Cr (VI)(150 μM) > Cr (VI)(200 μM) respectively after 30 and 45 days.
Soil hexavalent chromium when enters plants exhibits ruinous effects on the various aspects of plant growth and metabolism. Germination of seeds is the preliminary process occurring to a crop plant. Hence tolerance to chromium can be an indicative measure for plants to tolerate this type of abiotic stress (Peralta et al., 2001). According to Zeid (2001) reduction in seed germination under Cr stress could be attributed to reduction of activities of amylase enzymes and on the subsequent transport of sugars to the embryo axes. High level of Cr also could able to inhibit root cell division/root elongation which in turn results in decreased root growth (Barcelo et al., 1986).

The present study was conceptualized with the legume crop *Macrotyloma uniflorum* to evaluate the Cr toxicity in relation to germination and plant growth. All the studied traits were negatively affected with the increasing doses of chromium. Plant dry matter production was critically affected by Cr (VI) concentrations (> 100 µM). Nutrient deficiency in shoot can be observed through the poor growth of plants which ultimately can be attributed to the adverse uptake of hexavalent Cr. According to Clijsters and Van Assche (1985) Plant metabolic process which includes photosynthesis, electron transport, photophosphorylation and enzyme activities badly decreased by the intake of Cr. Protoporphyrin-a is the major component containing iron which is required for the development of chlorophyll pigment. It was evident that high level of Cr uptake can reduce the incorporation of iron into the protoporphyrin molecules, ultimately reducing the amounts of chlorophyll pigments in leaves (Krouma and Abdelly, 2003). Plants also can activate some chemical substances such as superoxide dismutase (SOD), catalase etc to detox the heavy metal (Prasad 1998; Shanker et al., 2003). Hydrophilic antioxidant

![Fig 3: Antioxidant parameters of plants after different concentrations (25µM, 50µM, 100µM, 150µM and 200µM) of of chromium stress at different growth stage (15 d, 30 d and 45 d).](image)

(A) Protein content (B) Catalase activity (C) Proline content (D) Peroxidase activity (E) SOD activity.

with different chromium concentration were Control > Cr (VI)(25 µM) > Cr (VI)(50 µM) > Cr (VI)(100 µM) > Cr (VI)(150 µM) > Cr (VI)(200 µM) and Control > Cr (VI)(25 µM) > Cr (VI)(50 µM) > Cr (VI)(100 µM) > Cr (VI)(150 µM) > Cr (VI)(200 µM) respectively after 30 and 45 days (Fig 3E).
proline accumulation suggested *Macrotyloma uniflorum* convey an efficient antioxidative response due to Cr toxicity. Higher concentrations of proline are considered as a profound method to regulate the redox potential, scavenge the hydroxyl radicals, provide osmoprotection and also the major protection against different macromolecules. Plant cells with proline can perform non-enzymatic free radical detoxifications of heavy metals (Mohanty and Patra, 2012). This study with the crop *Macrotyloma uniflorum* in hydroponics provides a promising beginning for revising and comparing the level of chromium toxicity. This elaborated the capability of the legume crop *Macrotyloma uniflorum* against the toxic effect of hexavalent Cr and their tolerance mechanisms.

Plants grown in soil with higher level of hexavalent Cr can develop variety of tolerance mechanisms which can be defined as the potential of that particular plant to survive in the toxic soil. This potential of the plants can be attributed to the interaction between the plant genotype and its environment.

**CONCLUSION**

Chromium stress in this crop triggered a defense mechanism against oxidative stress as evident from the response to different anti-oxidative enzymes activity. In concurrence, the increase in the levels of SOD and POX activity in this crop suggests an effective scavenging mechanism towards removing $\text{H}_2\text{O}_2$ caused by Cr stress. A better anti-oxidative capacity may be an adaptive trait against chromium treated soils and can confer a better sustainability against oxidative stress injury. The response of *Macrotyloma uniflorum* can be used to evaluate heavy metal tolerance potential and can be considered for reclamation of Cr contaminated soils. The above investigation revealed the changes in the metabolic pattern as induced by chromium in *Macrotyloma uniflorum*. Future studies on accumulation of hexavalent Cr with different varieties of *Macrotyloma uniflorum* are essential. The potential of different cultivars of *Macrotyloma uniflorum* on its metabolism under Cr stress needs to be checked. After harvest the Cr accumulated plants and its parts should be disposed with some suitable techniques which can minimize further uptake in the subsequent crop.

**ACKNOWLEDGEMENT**

The authors are thankful to the financial support provided by UGC-DRS-SAPIII and DST-FIST scheme to Department of Botany, Utkal University Bhubaneswar, India.

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