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An *in vitro* hydroponic study on Physiological and Biochemical responses of Indian wild Rice to varying doses of Hexavalent Chromium

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Abstract

Indian wild rice (Oryza nivara) grown as a weed in most of the cultivated paddy fields is used as an experimental tool in current investigation and studied for its various physiological and biochemical alterations in response to hexavalent chromium. After exposure to varying concentrations of toxic hexavalent chromium, the two cultivars of Oryza nivara i.e. IC-283169 and IC-336684 showed significant changes in its morphobiometric and physiological parameters. The present hydroponic study exhibited deleterious effects on germination, plant height and biomass of 14 days treated seedlings. IC-336684 cultivar of wild rice showed significant reduction in root-shoot length as well as dry and fresh biomass of shoot beyond a treatment dose of 50 µM Cr^{+6} *as compared to IC-283169 cultivar. Growth parameters, chlorophyll and carotenoid content showed 50% reduction with treatment dose of 25 µM* Cr^{+6}*. More than 80% reduction in all the above parameters were observed at 100 µM* Cr^{+6} *treatments. Present preliminary study screens the tolerance and sensitivity of wild rice cultivars to toxic doses of* Cr^{+6}*. The aim of this article is to give an overview of the impact of varying doses of hexavalent chromium on two cultivars of Indian wild rice and removal of these toxic contaminants from soil by potent application of weeds like Indian wild rice.*

Keywords: Chromium, chlorophyll, antioxidative enzymes, protein, hydroponically.

Introduction

Plants are exposed to various types of biotic and abiotic stresses. Heavy metal stress is considered as one of the emerging abiotic environmental stress issues now-a-days. Various natural and manmade activities are the causative factors for increased heavy metal content in the environment. Discharge of heavy metals from various anthropogenic activities leads to pollution in soil and water and pose threat to mankind[1]. Irrigation through wastewater, applications of sludge, unsafe disposal of solid wastes, exhaust from automobiles and effluent discharge from industrial activities are listed as some common manmade sources of heavy metals in the environment.

Extensive use of chromium (Cr) in various activities like electroplating, tanning, textile dyeing and as a biocide in power plant cooling water create environmental disturbances due to its toxic potentiality and high persistence in nature. Cr has an electronic configuration of [Ar] $3d^5 4s^1$ and exist in two most common and stable forms i.e, the trivalent Cr(III) and the hexavalent Cr(VI) form. The toxic effect of Cr (VI) on various plants has been reported[2, 3]. Presence of excess amount of chromium in soil and irrigated water beyond the tolerance limit will cause harm to crop growth and yield[4]. Some of the phytotoxic effects induced by Cr(VI) in plants are delayed seed germination, reduced seedling growth, less pigment content, nutrient content and enzyme activities of various[5-10]. Toxicity of Cr also affects human being and cattles when it entered through food chain, causing bronchitis and cancer [11-13].

Oryza nivara Sharma et Shastry, an annual short (usually <2 m) seasonal grass found growing in swampy areas, at edge of pond and tanks, beside streams, in ditches, in or around rice fields is a wild progenitor of the cultivated rice *Oryza sativa* L. This research program was envisaged to assess the phytotoxic effects and accumulation of Cr^{6+} in different plant parts that may be transferred to humans through food chain, particularly in the contaminated areas.

Material and Methods

Plant Material: Dry seeds of two varieties of Indian wild rice (*Oryza nivara* Sharma et Shastry; Accesion No. IC-283169 and IC-336684) were collected from Central Rice Research Institute (CRRI), Cuttack.

Germination study: Pre-treated uniform sized seeds were placed in sterilized petriplates over saturated tissue paper for germination under varying concentrations (0 µM as Control, 10µM, 25 µM, 50µM, 75µM and 100µM) of Cr [source: $K_2Cr_2O_7$] in different petriplates [Pre-treatment of seeds includes surface sterilization with 0.1% mercuric chloride ($HgCl_2$)] at 25º C in darkness for two days. Germinated seeds with 2 mm radicle in different treatment petripalted were

analysed for calculating Germination percentage using following formula[14-15].
% of Germination = No. of seeds germinated/ Total no. of seeds taken X 100

Seedling growth and growth parameter study: Two-days-germinated seeds were grown in well aerated hydroponic culture vessels containing Hoagland's nutrient solution (half strength) as control and Hoagland's solution supplemented with varying concentrations of Cr for seedling growth. The seedlings were grown under white light provided by white fluorescent tubes (36 W Philips TLD) with a photon flux density of 52 µ /m2s (PAR) with a 12h photo period inside the growth chamber for. 7 sand 14 days. After 7 and 14 days growth the Indian wild rice seedlings were analysed for various growth parameters like root length, shoot length, fresh matter and dry matter and a comparison was made between different Cr treatment vessels along with control vessels. For study of dry biomass the seedlings were kept in an oven at 80º C for a period of 3 days or more (till constant weight was attained).

Analysis of Chlorophyll Content: Fresh leaves of 7 and 14 days treated seedlings were grinded in 10 ml of 80% cold acetone and kept in dark in refrigerator for 48 hours at 4^0C for chlorophyll extraction study with further spectrophotometric analysis of chlorophyll content using the method of Porra (2002). The leaf samples from different culture pots were. The absorbance value of extracted liquid was recorded at 663.6 nm, 646.6 nm and 470 nm for Chlorophyll-a, Chlorophyll-b and carotenoid respectively[15].

Estimation of Proline: Plant material (0.5) was grinded in 10ml of 3% sulfo- salicylic acid then the homogenized mixture was centrifuged at 3000 rpm for 10 minutes. Then to the 2ml of supernatant 2ml of acid Ninhydrin reagent and 2ml of Glacial acid was added and boiled in water bath at 100˚C. 4ml of toluene was added to it and allowed to cyclomixer. The upper toluene layer containing chromophore was collected carefully with help of micropipette and the absorbance was measured at 520nm[15].

Estimation of catalase activity: For catalase assay 2 ml of sodium phosphate buffer and 0.5 ml of 12 mM of H_2O_2 was added to 0.5 ml of the plant leaf extract and the O.D was taken at 240 nm. Activity of Catalase enzymes of seedlings grown under different Cr treatments were assayed with measurement of the initial rate of disappearance of H_2O_2. [Extinction coefficient for H_2O_2 is 40mM^{-}1 cm^{-1} at 240nm][15-16].

Estimation of peroxidase activity: For assay of peroxidase activity, 2.8 ml of potassium phosphate buffer (pH = 7.0), 50µl of 10 mM H_2O_2 and 50µl of guaiacol was added to diluted leaf extract. The mixture was mixed properly and absorbance value was recorded at 436 nm. Activity was calculated using the extinction coefficient (25.5 mM^{-1} cm^{-1} at 436 nm) for tetraguaiacol[15-16].

Results and Discussion

Maximum inhibition of seed germination was observed at 100 µM Cr^{6+} treatments for both the varieties of *Oryza nivara* (IC-283169 and IC-336684). The inhibition in seed germination was recorded as 62 % and 58% in IC-283169 and IC-336684 cultivars respectively (figure-1a and 1b)

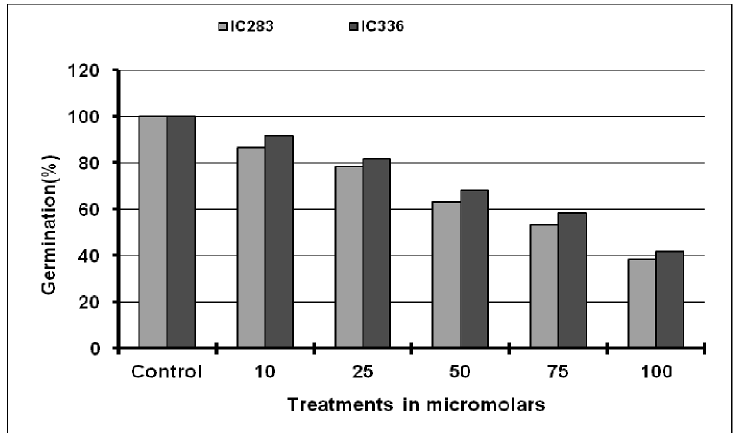

Figure-1
Bar graph showing the effects of Hexavalent Chromium on seed germination of two varieties of seven days and fourteen days grown *Oryza nivara*

Changes in growth parameters: Notable changes have recorded in different growth parameters of 7 and 14 days grown cultivars of Indian wild rice seedlings (figure-2a and 2b and figure-3a and 3b) grown under different treatment concentrations of Cr^{6+}. Shoot length decreased markedly with increased dose of Cr^{6+} treatment from 10µM to 100µM. The decreasing trend in growth parameters corroborates the findings of other researchers in different plants[16-17] with response to Cr stress. Root length and shoot length of IC-336684 cultivar of *Oryza nivara* seedlings showed high sensitivity to Cr toxicity in comparison to IC-283169 cultivar of *Oryza nivara* seedlings as evident from their reduced values at 100µM treatment of Cr^{6+}. Root and shoot length values of IC-336684 cultivar of *Oryza nivara* in response to varying concentration of Cr showed 50 % of that of IC-283169 cultivar. Fresh weight values of roots and shoots were gradually decreased with increased in concentrations of Cr^{+6} treatments in nutrient solution. Parallel growth trend values were found for fresh biomass (figure-4a, 4b and figure-5a and 5b) and dry biomass production (figure-6a and 6b and figure-7a and 7b). The two cultivars of Indian wild rice seedlings were examined for their tolerance and sensitivity towards different concentrations of Cr^{+6}.

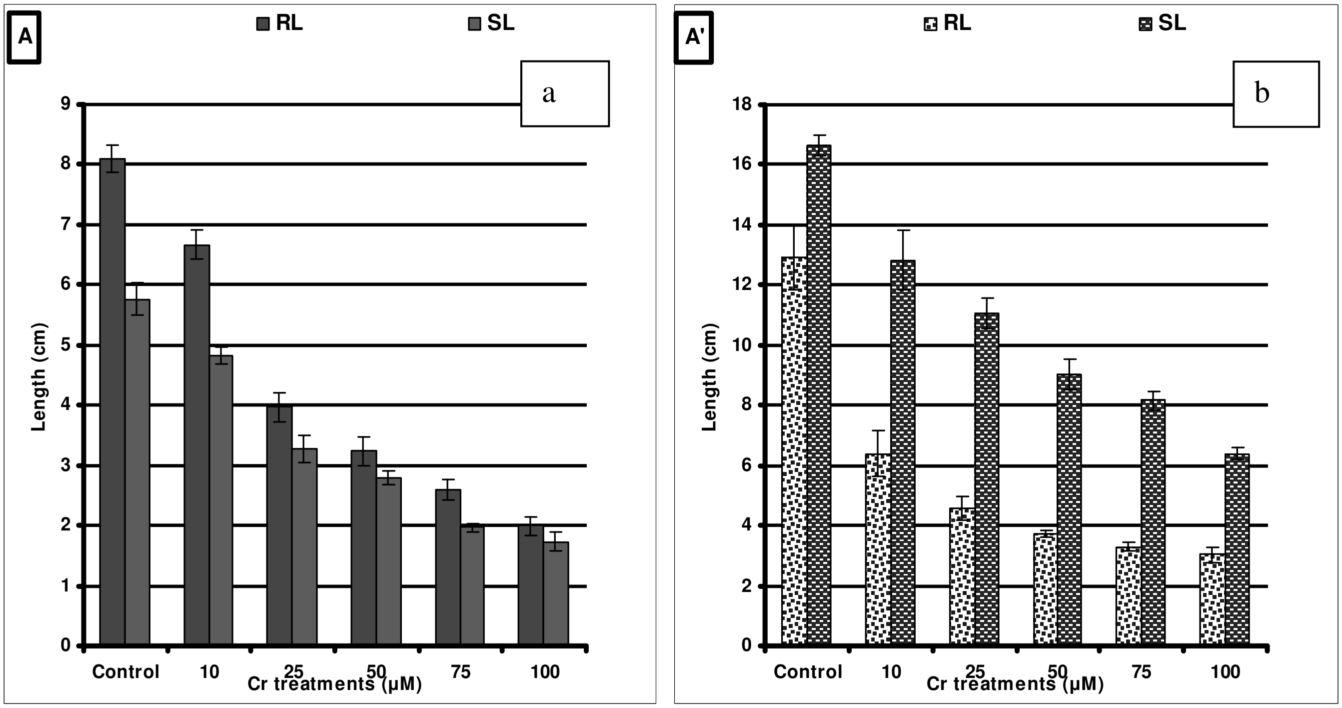

Figure-2a and b

Effect of different concentrations of Cr^{6+} on shoot and root length of 7 and 14 days old IC-283169 variety of *Oryza nivara* seedlings. [NB: A-7 days grown; A'- 14 days grown]

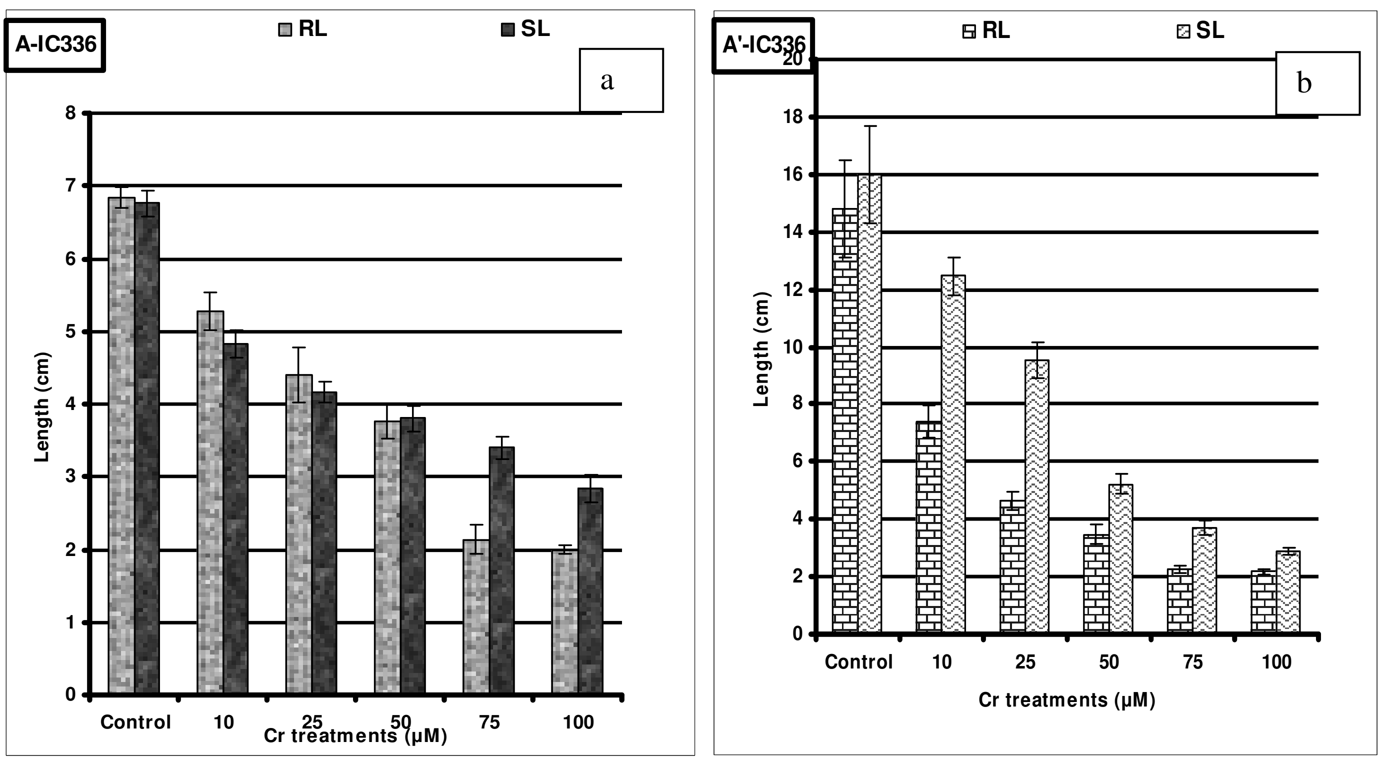

Figure-3a and b

Effect of different concentrations of Cr^{6+} on shoot and root length of 7 and 14 days old IC-336684 variety of *Oryza nivara* seedlings. [NB: A-7 days grown; A'- 14 days grown]

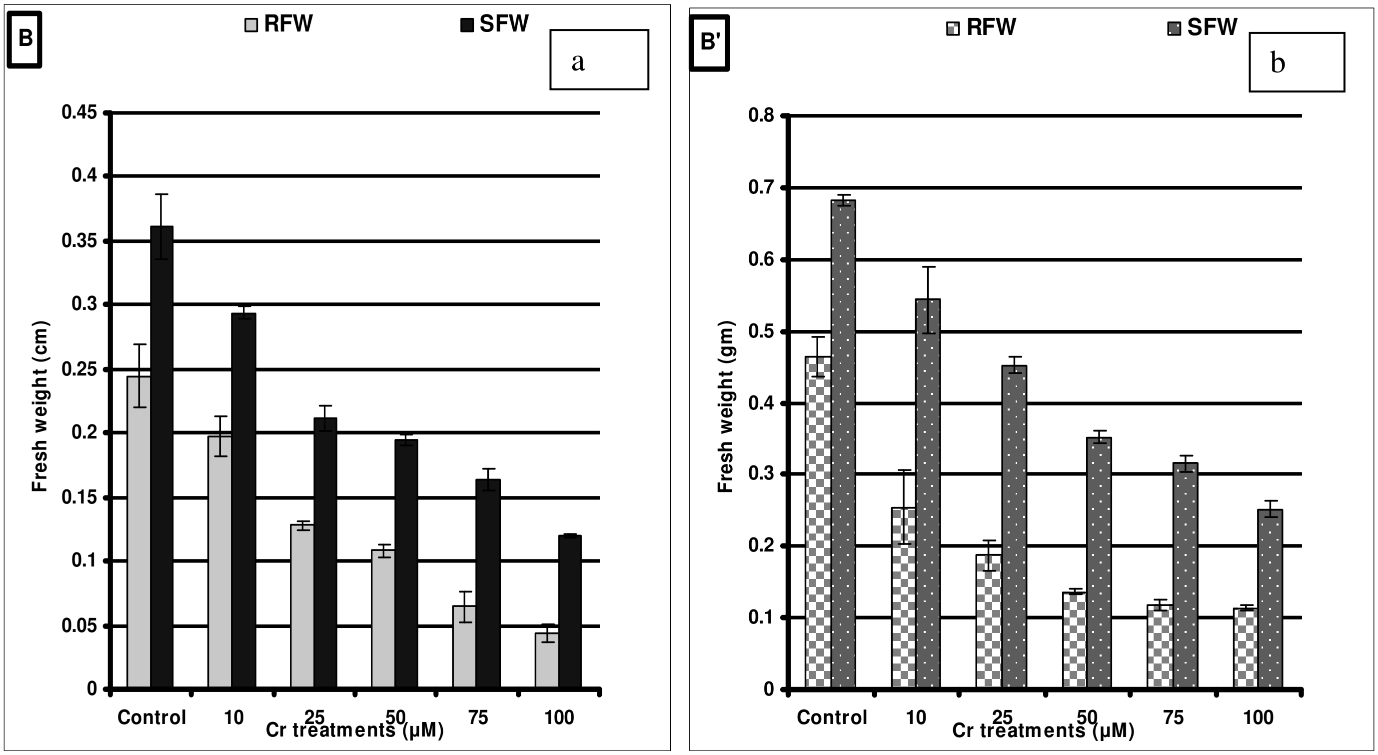

Figure-4a and b

Effect of different concentrations of Cr^{6+} on root fresh weight and shoot fresh weight of 7 and 14 days old IC-283169 variety of *Oryza nivara* plant. [NB: B-7 days grown; B'- 14 days grown]

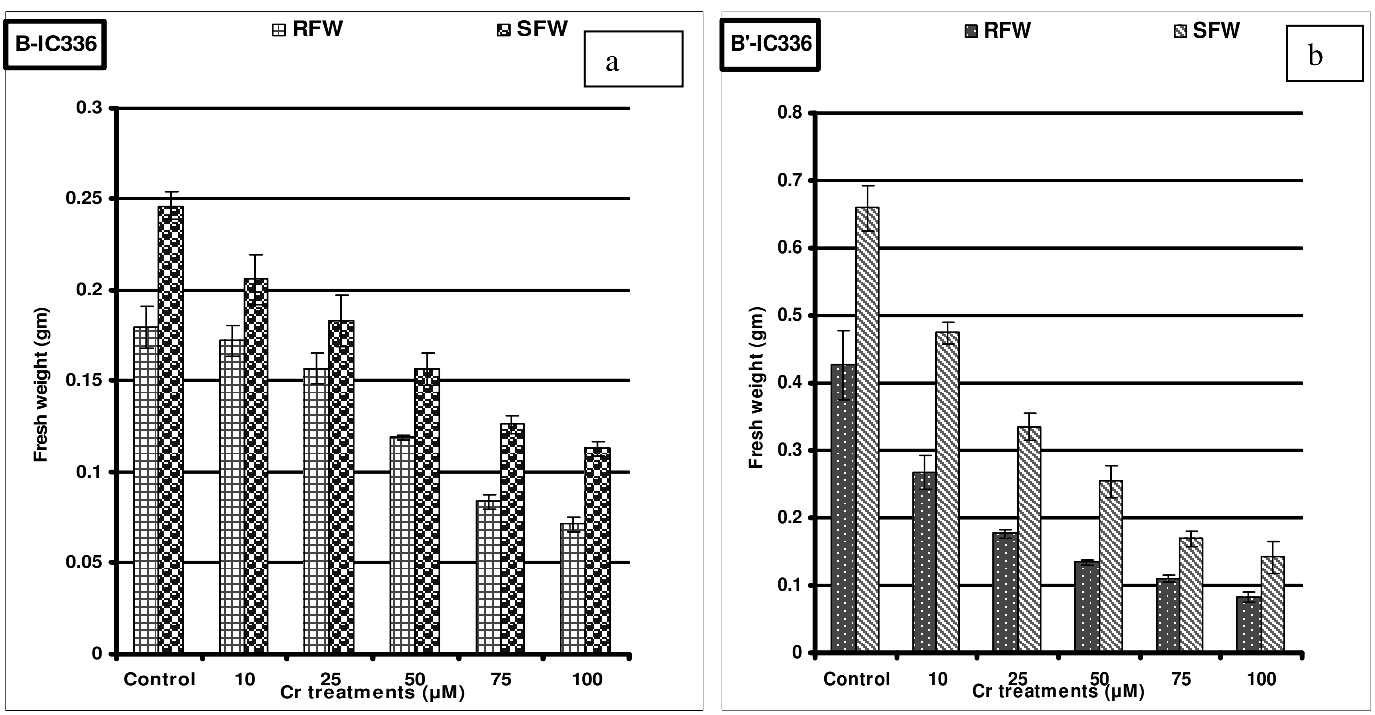

Figure-5a and b

Effect of different concentrations of Cr^{6+} on root fresh weight and shoot fresh weight of 7 and 14 days old IC-336684 variety of *Oryza nivara* plant. [NB: B-7 days grown; B'- 14 days grown]

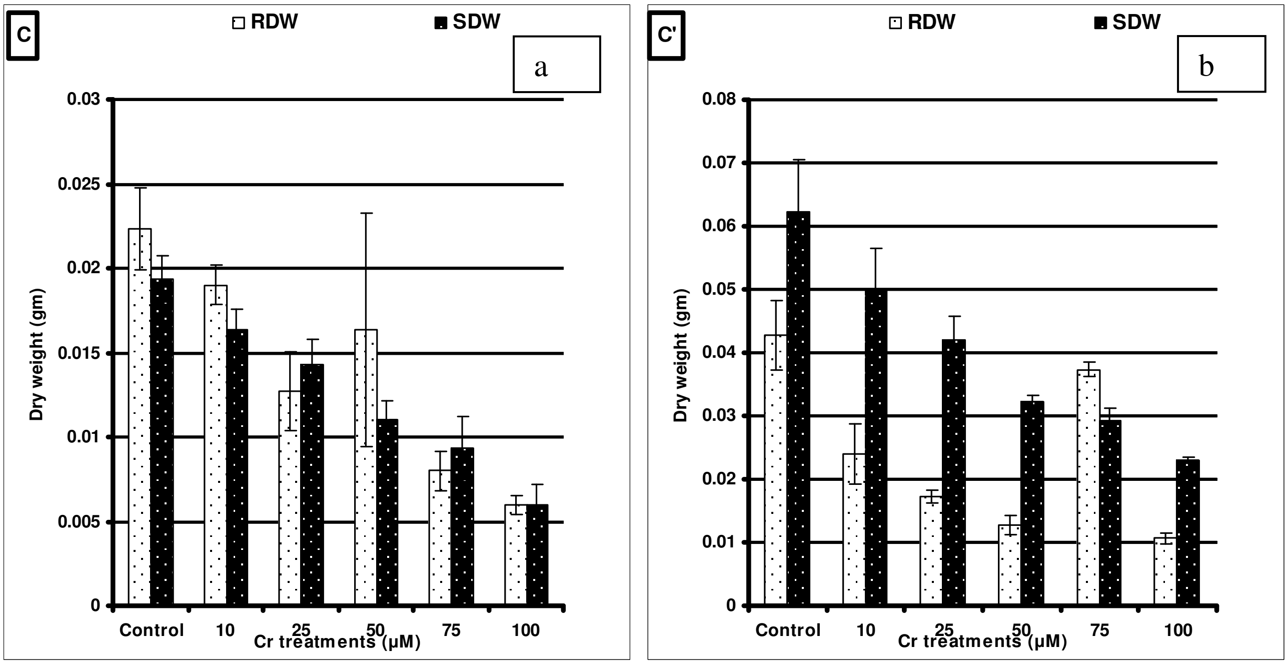

Figure-6a and b

Effect of different concentrations of Cr^{6+} on root dry weight and shoot dry weight of 7 and 14 days old IC-283169 variety of *Oryza nivara* plant. [NB: C-7 days grown; C'- 14 days grown]

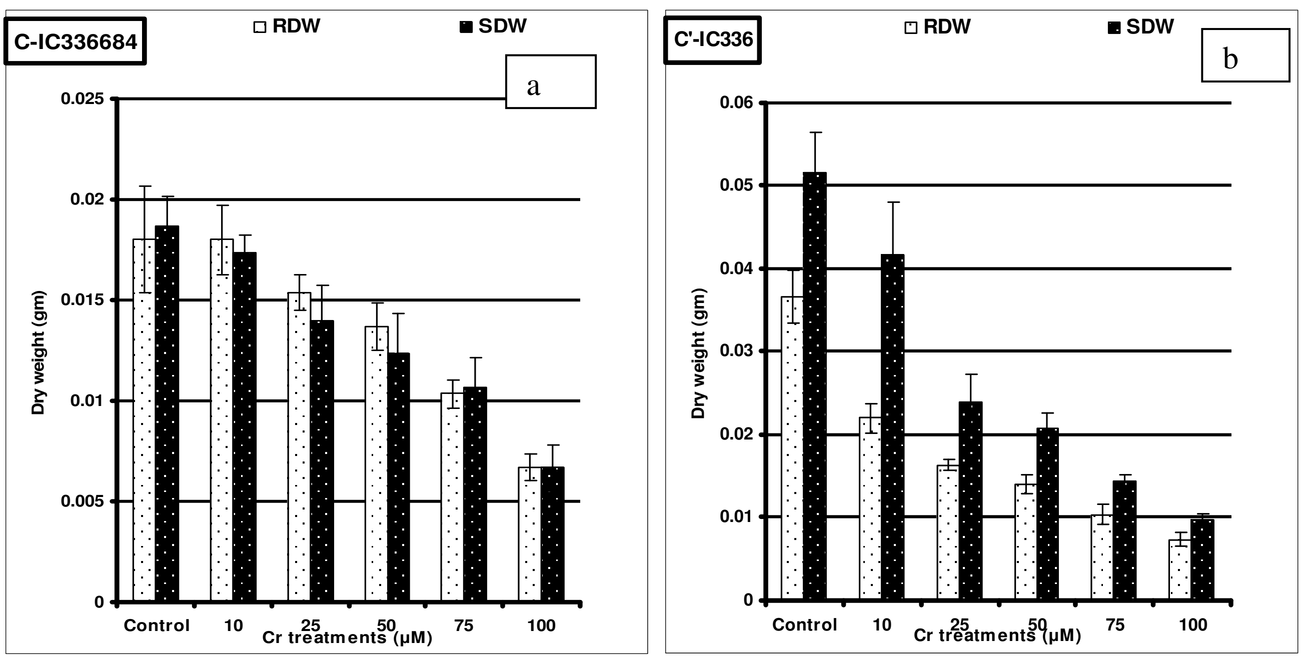

Figure-7a and b

Effect of different concentrations of Cr^{6+} on root dry weight and shoot dry weight of 7 and 14days old IC-336684 variety of *Oryza nivara* plant. [NB: C-7 days grown; C'- 14 days grown]

Effects on Chlorophyll and Carotenoid Content: By comparing the total chlorophyll content of two cultivates of *O. nivara*, it was noted that IC-336684 showed high Chlorophyll content in comparison to IC-283169 cultivar. Gradual decrease in chlorophyll content was found with increased treatment concentrations of Cr^{6+} except for treatment of 10 µM Cr^{6+}. Maximum chlorophyll content was recorded in 10µM-Cr^{+6} and minimum in 100µM-Cr^{+6} treated seedlings. Analogous trend of increase/decrease in carotenoid content was observed (figure-8a and 8b).

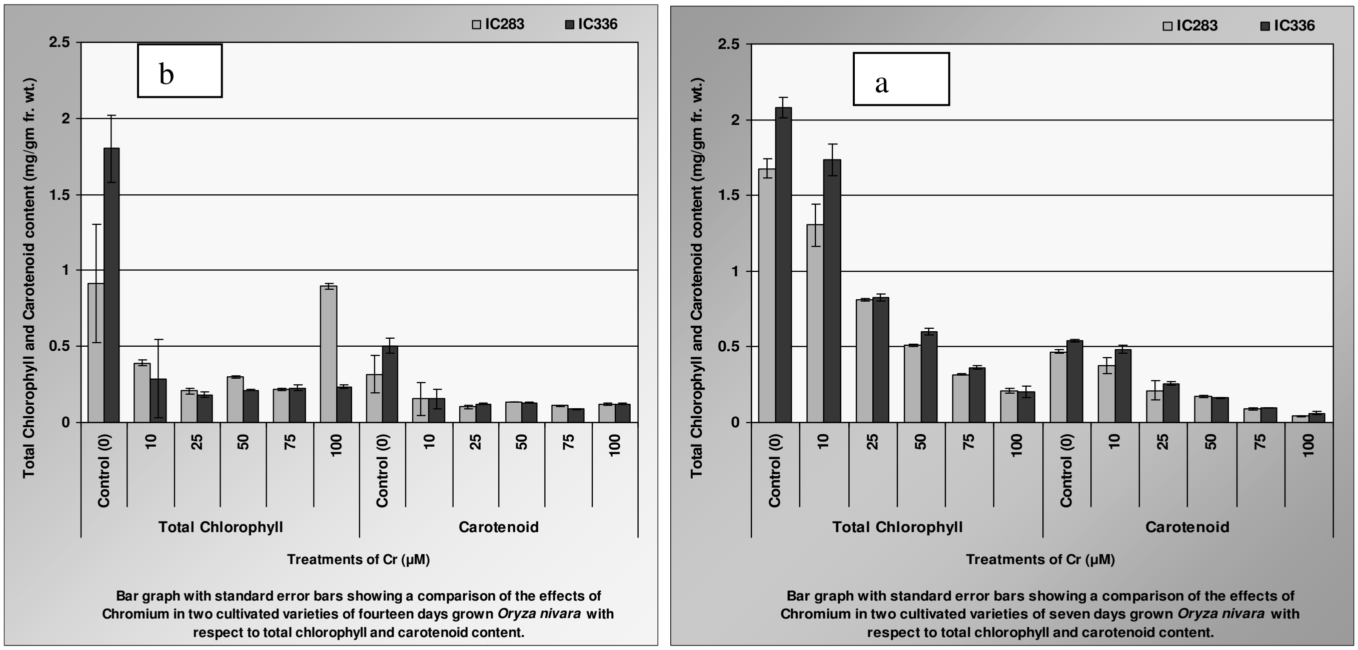

Figure-8a and b
Effect of different concentrations of chromium on chlorophyll and carotenoid content (mg / gm fresh wt.) of two cultivars of *Oryza nivara* seedlings after 7 and 14 days exposure

Accumulation of proline: Higher proline content was found with increased concentration of Cr^{6+}. Elevated proline accumulation was noted in seedlings treated with 100µM-Cr^{+6} treatments for IC-283169 cultivar and 75µM-Cr^{+6} treatments for IC-336684 cultivar for both 7 and 14 days old *O. nivara* seedling. A comparative proline accumulation in two cultivars of Indian wild rice shows that IC-283169 cultivar has higher proline accumulation (figure-9a and 9b).

Alterations in antioxidative enzyme activity: Variable responses were exhibited by antioxidants like catalase, peroxidase activities and carotenoids to different chromium treatments (figure-10a, 10b, figure-11a, 11b, and figure-8a, 8b). The activity of peroxidase (POX) increased at high chromium stress of 100µM. Enhanced peroxidase activity was observed in

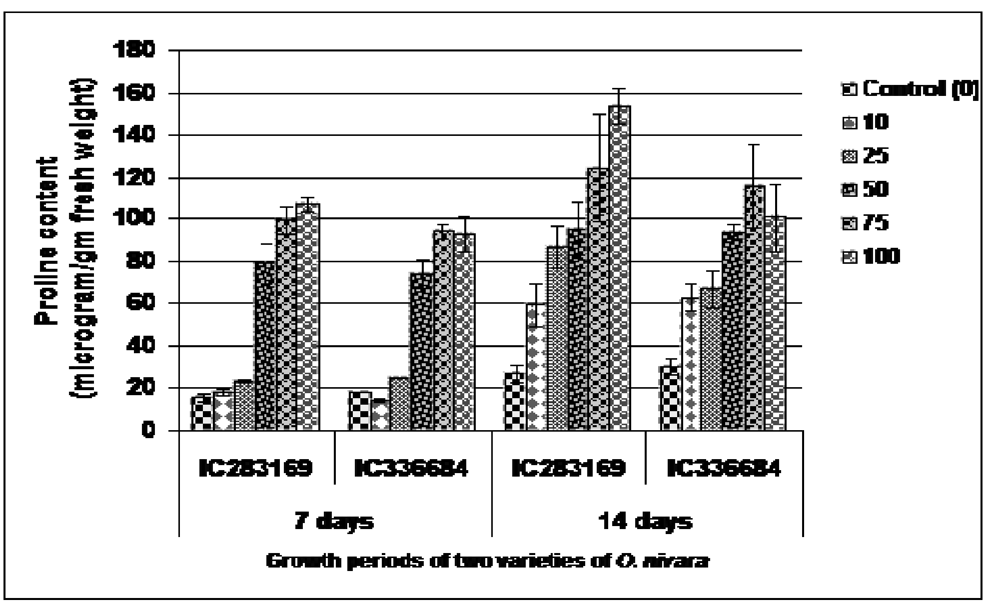

Figure-9
Comparative proline accumulation in two cultivars of *Oryza nivara* seedlings after 7 and 14 days exposure

IC-336684 cultivar than other cultivar. Catalase (CAT) activity showed decreasing trend with increased chromium concentrations for IC-283169 cultivar whereas in IC-336684 cultivar the activity was significantly enhanced at 50 µM treatment of Cr^{6+} than other treatments after 14 days exposure. An imbalance arises between generation and removal of ROS in plant tissues subjected to excess chromium or any other heavy metal exposure[18].

Discussion: Though chromium induced phytotoxic effects (reduced rate of growth, damage to cell wall, cell membranes and changes in the metabolic status of plants) was reported long time back, still there is a huge dearth of information regarding

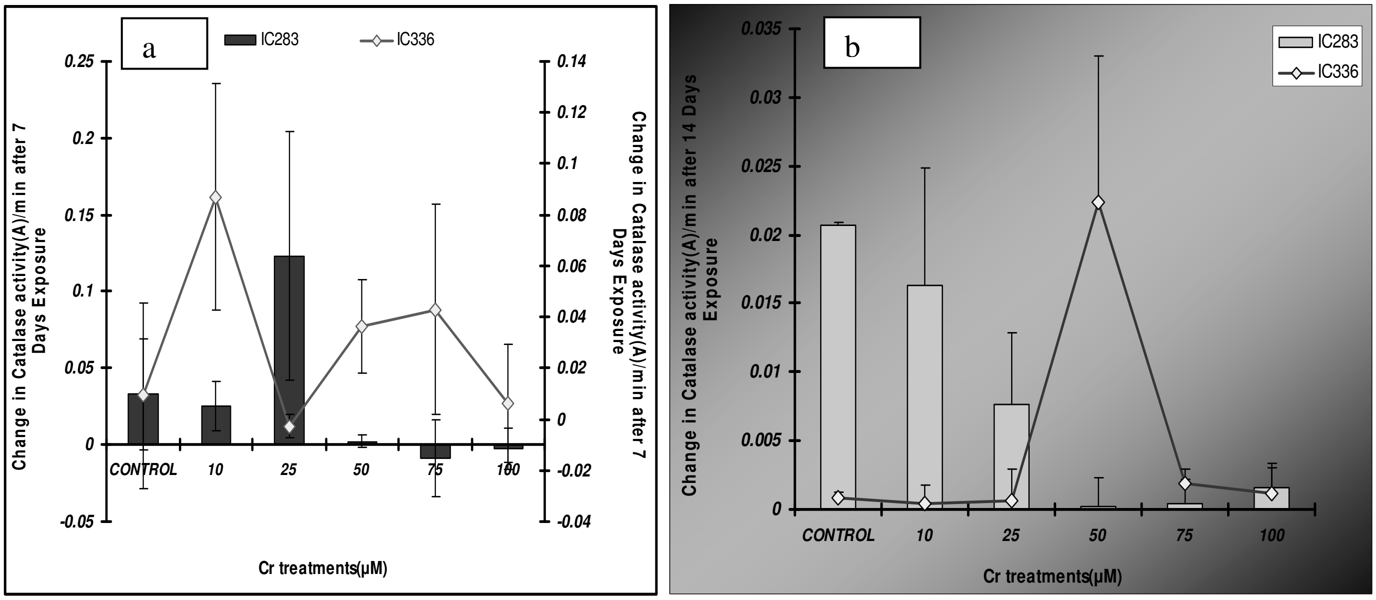

Figure-10a and b
Effect of different concentrations of Cr^{6+} on catalase activity in two cultivars of *Oryza nivara* after 7 and 14 days exposure

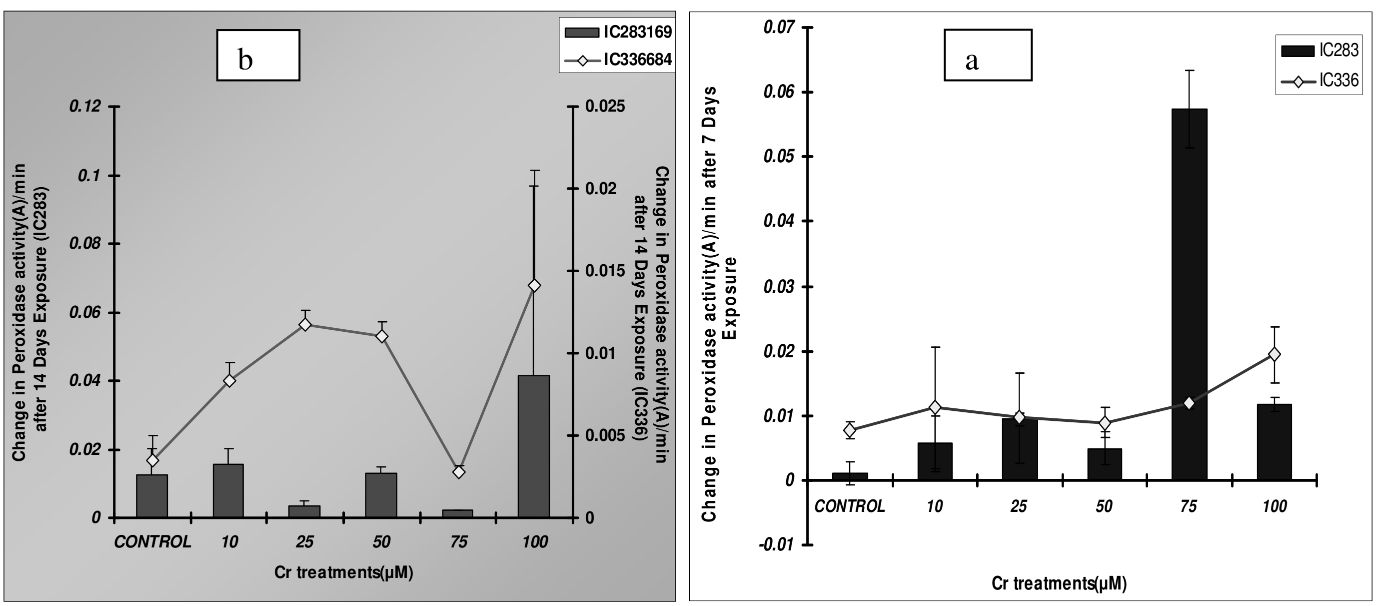

Figure-11a and b
Effect of different concentrations of Cr^{6+} on peroxidase activity in two cultivars of *Oryza nivara* after 7 and 14 days exposure

its impact in wild cultivars of Indian wild rice. In view of the seriousness if Cr pollution, the present study has been undertaken with an effort to assess the phytotoxic impacts with special reference to biochemical lesions in 7days and 14 days grown *Oryza nivara* seedlings. The part of the present hydroponics study provides a promising start for revising and comparing the level of chromium toxicity in two varieties of *Oryza nivara* with a potentiality of their tolerance after exposure to varying concentrations of Cr. The work signifies the potential of *Oryza nivara* plants towards Cr phytotoxicity and tolerance.

Study of proline accumulation in response to heavy metal stress is one of the important indicators in plants under stress. Increased proline provide osmoprotection, regulates the redox potential, scavenges hydroxyl radicals which will denature of various macromolecules and thus protects the macromolecules from damage. Non-enzymic free radical detoxifications are often associated with proline accumulation[16]. The increased proline biosynthesis in the Cr^{6+} treated seedlings signifies a stress regulating factor that gives protection in plants under heavy metal toxicity environment[17].

Essential components of plant antioxidant defence system are associated with activities of POX and CAT. Earlier studies on elevated POX activity in nickel treated *O. sativa*, suggests its role in the detoxification of H_2O_2[19]. Plants with enhanced POX activity in response to excess chromium and cadmium supply might suffer from peroxidative damage of the thylakoid membrane or lower auxin and protein contents in tissues[20]. Though CAT and POX activities protect the plant metabolic system[21] still it has negative impacts with its excess. Considering the above phytotoxic effects, Cr removal from pollutes sites becomes very much essential for which different biosorbents are effectively used[22-24].

Conclusion

Plant tolerance to heavy metals is the ability to survive in contaminated soil which is manifested by an interaction between a genotype and its environment. Above studies reveal some interrelationship between different metabolic effects induced by chromium in a wild plant like *Oryza nivara.* Intensive future research on the effects of accumulation of Cr on plant metabolism is essential. Further the ability of different cultivars of this plant for increasing phytoaccumulation potential needs to be tried alongwith various biosorption models. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated toxic chromium from mining environment. The overall view of the impact of varying doses of hexavalent chromium on tolerance and sensitivity ability of two cultivars of Indian wild rice suggest removal of these toxic contaminants from soil by potent application of weeds like Indian wild rice.

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