RESEARCH

27(97), January 1, 2015

ISSN 2278–5469 EISSN 2278–5450

Discovery

Altered Germination Index and Chlorophyll Biosynthesis in Seedlings of Wild Rice Cultivars in Response to Hexavalent Chromium Stress

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Publication History

Received: 29 October 2014

Accepted: 11 December 2014

Published: 1 January 2015

Citation

Samantaray S, Das S, Mohanty RC, Mohanty M, Pradhan C. Altered Germination Index and Chlorophyll Biosynthesis in Seedlings of Wild Rice Cultivars in Response to Hexavalent Chromium Stress. *Discovery*, 2015, 27(97), 27-35

ABSTRACT

Chromium is a potent environmental phytotoxicant. The impact of varying doses of hexavalent chromium on the seed germination and photosynthetic responses in a weed plant like Indian wild rice (*Oryza nivara*) has been assessed in the present study. After exposure to different concentrations of toxic hexavalent chromium, the two cultivars of *Oryza nivara* i.e. IC-283169 and IC-336684 showed significant changes in its seed germination and physiological parameters. The present hydroponic study exhibited toxic effects of chromium on germination and plant photosynthetic response after one and two weeks exposure period. IC-336684 cultivar of wild rice showed better germination of seeds than IC-283169 cultivar. Photosynthetic pigment content like chlorophyll and carotenoid content showed 50% reduction beyond a treatment dose of 25 µM Cr^{6+}. Present preliminary study screens the tolerance and sensitivity of wild rice cultivars to toxic doses of Cr^{+6}.

Keywords: Chromium, Chlorophyll, Antioxidative enzymes, Protein, Hydroponically

1. INTRODUCTION

A number of biotic and abiotic stresses pose serious threats to environment. Plants are exposed to various types of plants in our surrounding. Heavy metal stress is one of them. An assortment of natural and manmade activities is the causative factors for increased heavy metal content in the environment. Heavy metals discharge from various anthropogenic activities leads to soil and water pollution and pose threat to mankind. 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 (Mohanty and Patra, 2011a).

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 (Mohanty and Patra, 2011a). Cr 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 (Cervantes et al., 2001; Mc Grath, 1982). Presence of excess amount of chromium in soil and irrigated water beyond the tolerance limit will cause harm to crop growth and yield. Delayed seed germination, reduced seedling growth, less pigment content, nutrient content and enzyme activities of various are the common phytotoxic effects induced by Cr(VI) in plants are (Pattnaik et al., 2013). Toxicity of Cr also affects human being and cattles when it entered through food chain, causing bronchitis and cancer (Mohanty and Patra, 2013).

Oryza nivara Sharma et Shastry, is a wild progenitor of the cultivated rice *Oryza sativa* L. It is found growing in swampy areas, at edge of pond and tanks, beside streams, in ditches, in or around rice fields. It is an annual short (usually <2 m) seasonal grass found growing in swampy areas, at edge of pond and tanks, beside streams, in ditches. The present preliminary study assesses the phytotoxic effects and photosynthetic alterations induced by Cr^{6+} in different plant parts. 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 by phytoremediation technology.

2. MATERIALS AND METHODS

2.1. Plant Material

Dry graded seeds 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.

2.2. Germination study

Disinfected [with 0.1% mercuric chloride ($HgCl_2$)] uniform sized seeds were placed in sterilized petriplates over saturated tissue paper for germination under varying concentrations (0 µM as Control, 5µM, 25 µM, 50µM, 75µM and 100µM) of Cr [source: $K_2Cr_2O_7$] in different petriplates at 25° C in dark for two days. Germinated seeds with emergence of 2 mm radicle in different treatment petriplate were analysed for calculating Germination percentage using following formula.

% of Germination = No. of seeds germinated/ Total no. of seeds taken X 100

2.3. Seedling growth

Germinated seeds were grown in hydroponics in controlled laboratory conditions in growth chambers. Well aerated hydroponic culture vessels containing Hoagland's nutrient solution (half strength) was treated as control and Hoagland's solution supplemented with different concentrations of Cr for seedling growth. The seedlings were grown under 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.

2.4. Analysis of Chlorophyll Content

One week and two week old seedling leaves were grinded in 10 ml of 80% cold acetone was used for spectrophotometric analysis of chlorophyll (Porra, 2002). 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.

3. RESULTS

Germination study

Seed germination reduced to 40% to 45% with highest dose of Cr. i.e. 100 µM Cr^{6+} treatments in both the varieties of *Oryza nivara* (IC-283169 and IC-336684). The seed germination was recorded as 38 % and 42% in IC-283169 and IC-336684 cultivars respectively (Fig. 1). Changes were visible as observed from the plates (Plate 1 and Pate 2).

Effects on Chlorophyll Content

Total chlorophyll content of two cultivars of *O. nivara*, showed 50% decline at 25µM-Cr^{+6}. Cr treated seedlings of IC-336684 cultivar showed high chlorophyll content in comparison to IC-283169 cultivar. Gradual decrease in chlorophyll content was found with increased treatment concentrations of Cr^{6+} (Fig. 2 and Fig. 3).

Treatment of different hexavalent chromium concentrations (5µM,25µM,50 µM,75µM,100µM) along with control showed marked changes in the chlorophyll content of 7 days and 14 days old *Oryza nivara* seedlings grown under Cr^{6+} stress. A marked increase in total chlorophyll content was observed in the seedlings treated with half strength Hoagland nutrient solutions as control. The total chlorophyll content decreased with increase in Cr^{6+} concentrations. In IC-283169 variety the total chlorophyll content was maximum under control condition after 7 days as well as 14 days seedlings. In IC-336684 variety, the maximum chlorophyll content was found in control condition after 7 days seedlings but in 14 days seedlings, the least chlorophyll content was found in Cr^{6+}(25 µM) condition. The order of total chlorophyll content treated with different chromium concentration was as follows-

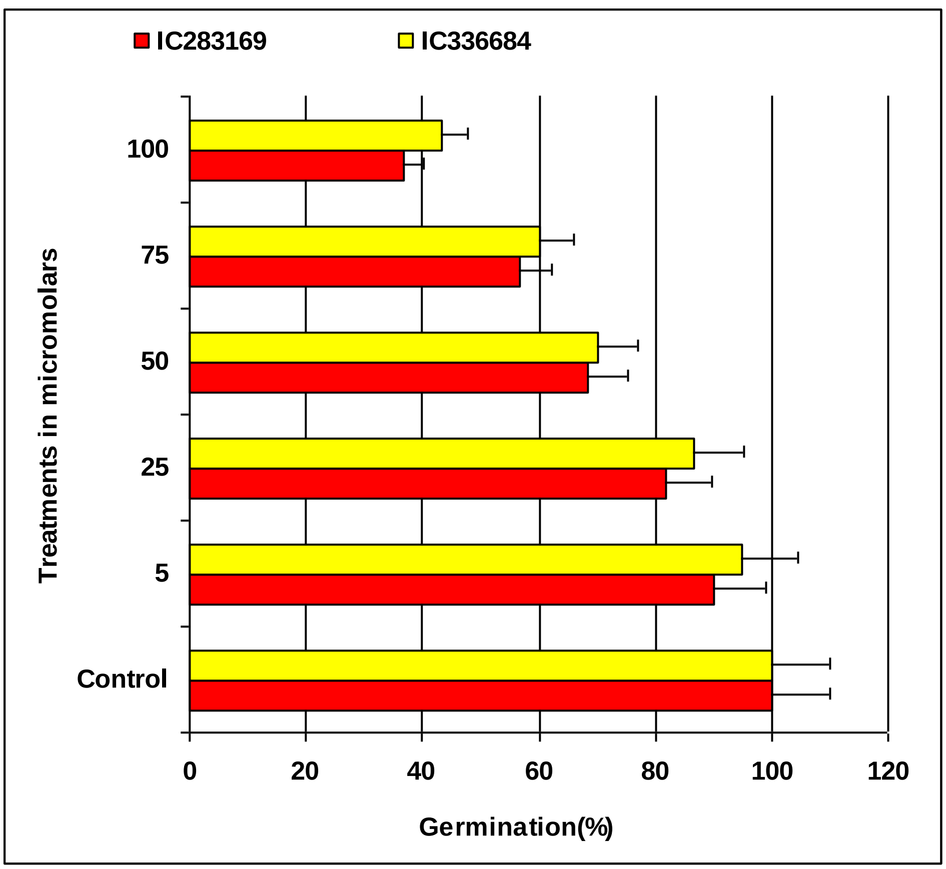


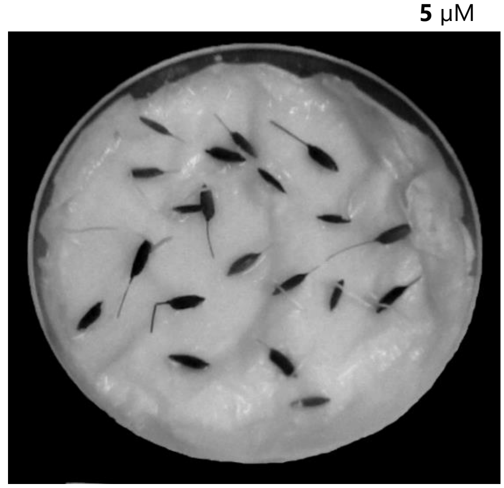
Figure 1

Bar graph showing the effects of Hexavalent Chromium on seed germination of two varieties of *Oryza nivara* Seeds

Control (**0**µM)

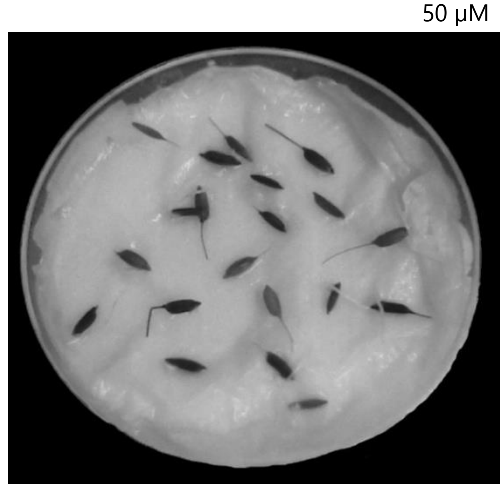
5 µM

25 µM



50 µM

75 µM



100 µM

Plate 1

Effects of Hexavalent Chromium concentrations (µM) on seed germination of IC-283169 in different concentrations

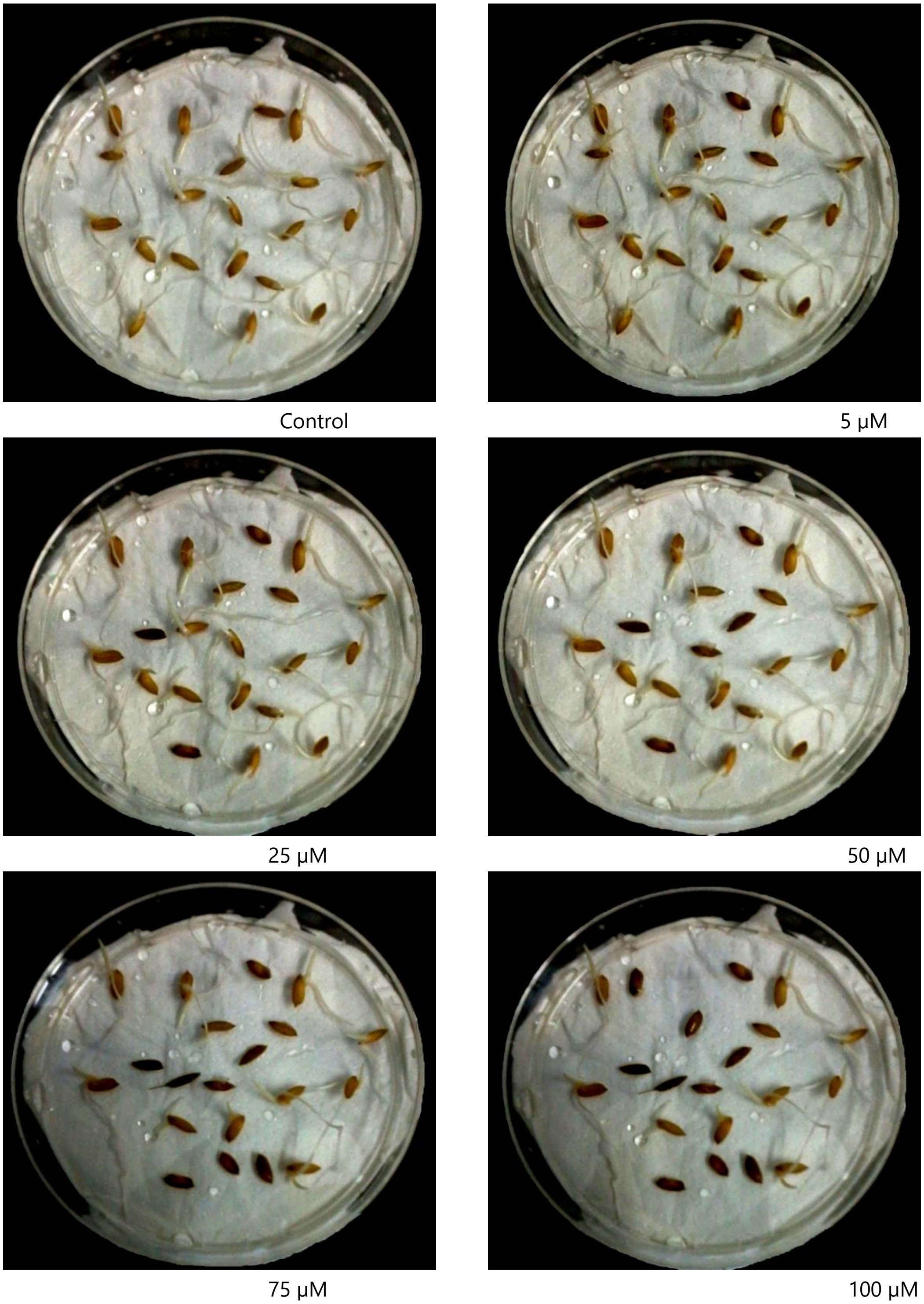


Plate 2

Effect of Hexavalent Chromium on seed germination of IC-336684 in different concentrations

After 7 days,

In IC-283169 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)> Cr^{6+}(25μM)>Cr^{6+}(50μM)>Cr^{6+}(75μM)>Cr^{6+}(100μM).

In IC-336684 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)> Cr^{6+}(25μM)>Cr^{6+}(50μM)>Cr^{6+}(75μM)>Cr^{6+}(100μM).

After 14 Days,

In IC-283169 variety of *Oryza nivara* :

Control > Cr^{6+}(100μM)>Cr^{6+}(5μM)>Cr^{6+}(50μM)>Cr^{6+}(25μM)>Cr^{6+}(75μM).

In IC-336684 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)>Cr^{6+}(100μM)>Cr^{6+}(75μM)>Cr^{6+}(50μM)>Cr^{6+}(25μM).

Effects on Carotenoid content

A marked increase was found in carotenoid content of the seedling grown in controlled condition with increase in growth period in IC 283169 seedlings of *O. nivara* But with increased Cr^{6+} concentrations the carotenoid content was decreased. (Fig 2 and Fig.3)

After 7 days treatment the decreasing trend of carotenoid at different concentrations of chromium is as follows,

In IC-283169 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)> Cr^{6+}(25μM)>Cr^{6+}(50μM)>Cr^{6+}(75μM)>Cr^{6+}(100μM).

In IC-336684 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)> Cr^{6+}(25μM)>Cr^{6+}(50μM)>Cr^{6+}(75μM)>Cr^{6+}(100μM).

After 14 days, treatment the decreasing trend of carotenoid at different concentrations of chromium is as follows,

In IC-283169 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)>Cr^{6+}(50μM)>Cr^{6+}(75μM)>Cr^{6+}(100μM)>Cr^{6+}(25μM).

In IC-336684 variety of *Oryza nivara* :

Control > Cr^{6+}(5μM)>Cr^{6+}(50μM)>Cr^{6+}(25μM)>Cr^{6+}(100μM)>Cr^{6+}(75μM).

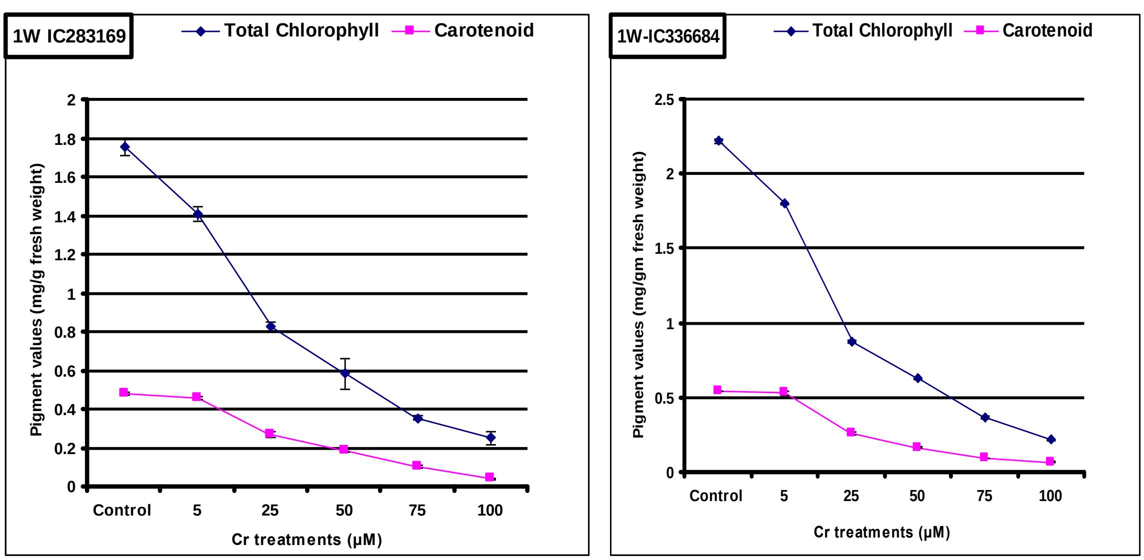


Figure 2(a)

Effect of different concentrations of chromium on chlorophyll and carotenoid content (mg / gm fresh wt.) of 7 days treated *Oryza nivara* seedlings

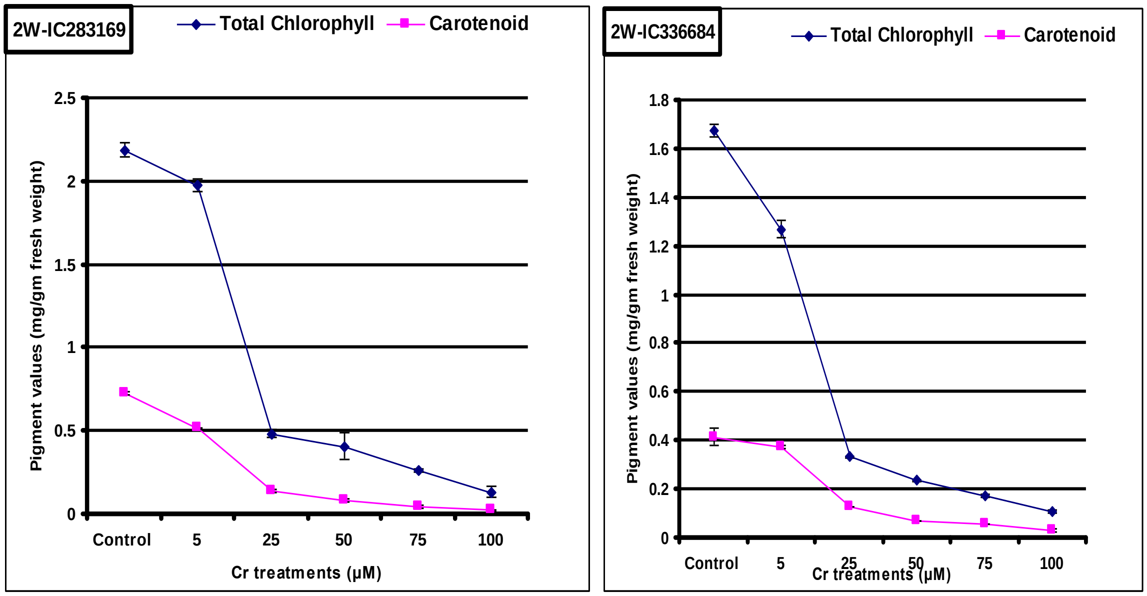


Figure 2(b)

Effect of different concentrations of chromium on chlorophyll and carotenoid content (mg / gm fresh wt.) of 14 days treated *Oryza nivara* seedlings

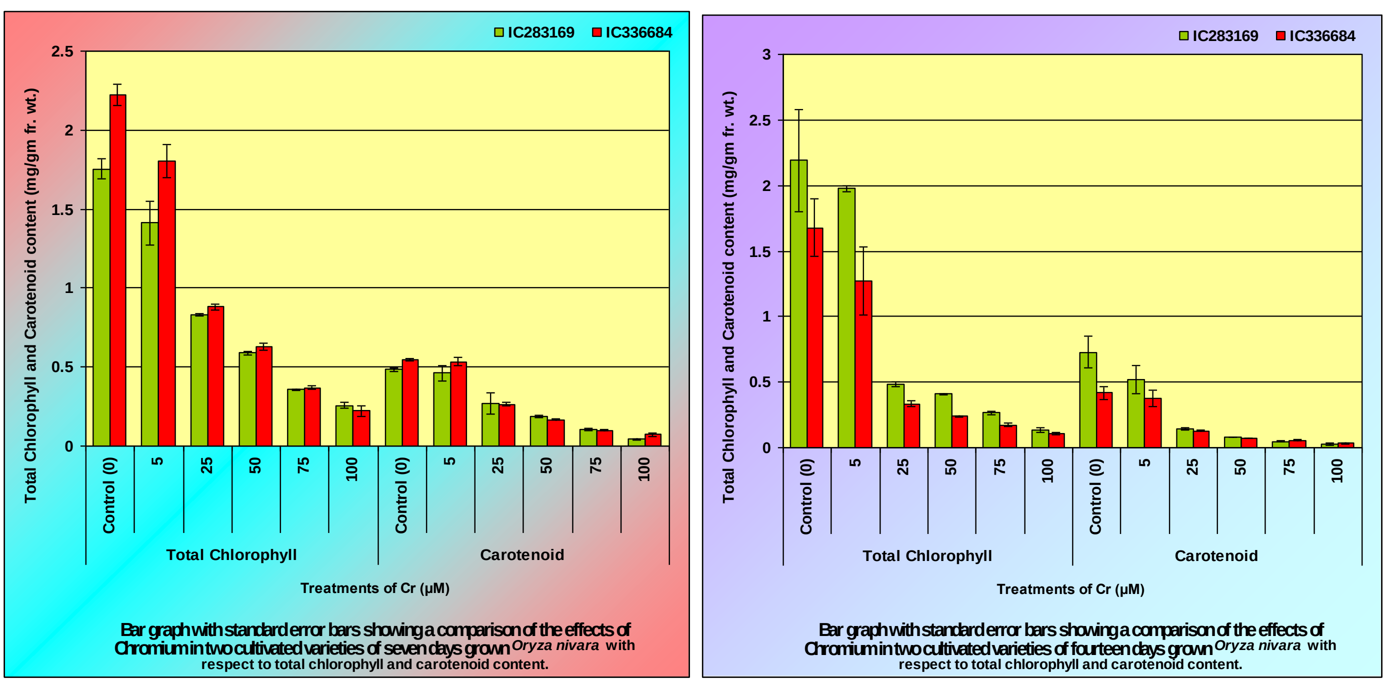


Figure 3

Comparative effect of different concentrations of chromium on chlorophyll and carotenoid content (mg / gm fresh wt.) of *Oryza nivara* seedlings

4. 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 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. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis. An excessive supply of chromium seems to prevent the incorporation of iron into the protoporphyrin molecule, resulting in the reduction of chlorophyll pigment [Datta et al. 2011]. Similar events of reduced pigment biosynthesis have been reported under salt stress [Mohanty and Patra, 2011 b]. Chromium degrades δ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting δ-aminolevulinic acid (ALA) utilization; this results in the buildup of ALA and reduction of the level of chlorophyll [Vajpayee et al., 2000]. Chromium, mostly in its hexavalent form, can replace Mg ions from the active sites of many enzymes. Cr(VI) also causes Fe deficiency in stressed plants, disrupting chlorophyll biosynthesis [Liu et al., 2008].

5. CONCLUSION

The above studies reveal some interrelationship between the different metabolic effects induced by chromium in a wild plant like *Oryza nivara* Intensive future research on the effects of accumulation of heavy metal on plant metabolism is essential. Further the ability of different plants for increasing phytoaccumulation potential needs to be tried. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated toxic chromium from the mining environment. Plant tolerance to heavy metals is manifested by an interaction between a genotype and its environment. Above studies reveal interrelationship between different metabolic effects induced by chromium in a wild plant like *Oryza nivara*.

FUTURE ISSUES

Precise study on impact of Cr accumulation in plant metabolism is essential. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated toxic chromium from mining environment.

SUMMARY OF RESEARCH

1. Seed germination reduced to 40% to 45% with highest dose of Cr. i.e. 100 µM
2. Gradual decrease in chlorophyll content was found with increased treatment concentrations of Cr^{6+}.
3. A marked increase was found in carotenoid content of the seedling grown in controlled condition with increase in growth period in IC 283169 seedlings of *O. nivara* But with increased Cr^{6+} concentrations the carotenoid content was decreased.
4. Total chlorophyll and carotenoid content in IC36684 variety of O. nivara seedlings decreased with increased exposure period of plants to Cr.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by UGC-DRS-SAP scheme to Department of Botany, Utkal University Bhubaneswar, India.

REFERENCE

1. Cervantes C, Garcia JC, Devars S, Corona FG, Tavera HL. Carlos Torres-guzman J, Sanchez RM. Interactions of chromium with micro-organisms and plants, *FEMS Microb. Rev.*, 2001, 25, 335–347.
2. McGrath SP. The uptake and translocation of tri- and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytologist*, 1982, 92, 381–390
3. Pattnaik N, Mohanty M, Patra HK. Effect of chelating agents and metal ions on nickel bioavailability and chlorophyll fluorescence response in wheat- An approach for attenuation of Ni stress, *J. Stress Physiol. Biochem.*, 2012, 8(3), 99-112
4. Mohanty M, Patra HK. Effect of Chelate assisted Hexavalent Chromium on Physiological changes, Biochemical alterations and Cr Bioavailability in Crop Plants - An *in vitro* Phytoremediation Approach, *Bioremediat. J.* 2012, 16(3), 147–155
5. Mohanty M, Patra HK. Effect of ionic and chelate assisted hexavalent chromium on mung bean seedlings (*Vigna radiata* L. wilczek. var k-851) during seedling growth, *J. Stress Physiol. Biochem*,. 2013, 9(2), 232-241
6. Liu DH, Zou JH, Wang M, Jiang WS. Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L.", *Bioresource Technology*, 2008, 99 , 2628–2636,.

7. Vajpayee P, Tripathi RD, Rai UN, Ali MB, Singh SN. Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L., *Chemosphere,* 2000, 41, 1075– 82,.
8. J.K. Datta, A. Bandhyopadhyay, A. Banerjee, N.K. Mondal, "Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) Cultivars under laboratory condition", *Journal of Agricultural Technology*, 2011, 7 (2), 95-402.
9. Mohanty M, and Patra HK. Effect of Cr^{+6} and chelating agents on growth, pigment status, proline content and chromium bioavailability in rice seedlings, *International Journal of Biotechnology Applications*, 2011b, 3, (3), pp. 91-96,.
10. Mohanty M, Patra HK. Attenuation of chromium toxicity by bioremediation technology, *Review of Environmental Contamination and Toxicology,* 2011a, 210, 1-34.