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Antioxidative Enzyme Response and Proline Biosynthesis in Two Cultivars of Indian Wild Rice under Hexavalent Chromium Stress

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Abstract: ***Chromium (Cr) is one of the most toxic heavy metal and is discharged into the environment through various anthropogenic and mining activities. Current investigation have made a comparative assessment on toxic impacts of varying doses of hexavalent chromium with respect to its antioxidative enzyme (Catalase and Guaiacol Peroxidase) response and proline accumulation in two cultivars (IC-283169 and IC-336684) of Indian wild rice (Oryza nivara) grown in hydroponics. Catalase activity showed significant gradual reduction with increasing treatment dose of Cr^{6+} in IC-283169 cultivar of O. nivara after 14 days of exposure period. A constant insignificant impact of Cr^{6+} up to the treatment concentration of 25 µM was observed in IC-336684 cultivar of O. nivara. Catalase activity was highly stimulated showing a value of 20 µkat g^{-1} fresh weights with the treatment of 50 µM Cr^{6+} for 14 days of seedling growth. At an early stage of seedling growth i.e. for 7 days treatment, seedlings of IC-283169 cultivar of O. nivara showed high peroxidase activity (maximum at 75 µM Cr^{6+} treatment) as compared to that of IC-336684 cultivar. After 14 days treatment, IC-336684 cultivar showed high peroxidase activity (maximum at 100µM Cr^{6+} treatment). Proline content was maximum in IC-283169 cultivar of O. nivara after 14 days growth period at 100µM Cr^{6+} treatment. Above results revealed the toxic impacts of Cr and plant response in respect to its free radical scavenging potentiality as an antioxidative protective system.***

Keywords: Chromium, Catalase, Peroxidase, Proline, Tolerance index

1. Introduction

Chromium (Cr) is one of the most toxic heavy metal and is discharged into the environment through mining activities and various human activities like extensive use of chromium in electroplating, tanning and textile dyeing and as a biocide in powder plant cooling water [1]. The pace of release of chromium into the environment is growing exponentially and poses potentially serious risks to human health. It has been estimated that about 1, 12, 000 tons of chromium was discharged annually into the world's aquatic ecosystems and worldwide annual mining of the chromate ($FeCr_2O_4$) exceeded a level of 10 million tons [1]. Chromium is not destroyed by degradation and therefore accumulates in the environment. Cr, in contrast to other toxic trace metals like cadmium, lead, mercury and aluminum, has received little attention from plant scientists. Its complex electronic chemistry has been a major hurdle in unraveling its toxicity mechanism in plants. Phytotoxic impacts of Cr on plant physiology depend on its oxidation state, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system. Cr exist in two most common and stable forms i.e. the trivalent Cr(III) or Cr^{3+} and the hexavalent Cr(VI) or Cr^{6+} form. Various phytotoxic effect induced by Cr^{6+} in different plants has been reported [2] [3] time to time. 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 [4] [5]. Toxicity of Cr magnifies after it entered through food chain, causing various ailments in humans and animals [3]. Cr toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. Taking into view of serious phytotoxic impacts of Cr it becomes the need of the hour to remediate such heavy metals from the cultivated land in the contaminated areas. Present investigation is a phytoremediation approach through testing the potentiality of a weed species i.e. Indian wild rice.

Oryza nivara Sharma et Shastry, is a wild progenitor of the cultivated rice Oryza sativa L 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. Current investigation measures phytotoxic effects induced by Cr^{6+} and the corresponding defense mechanism in terms of their antioxidative enzyme system and proline biosynthesis in Indian wild rice. Present investigation will be helpful for screening the potentiality of different cultivars of Indian Wild rice the tolerance and sensitivity of wild rice cultivars to toxic doses of Cr^{6+}.

2. Materials and Methods

2.1 Plant Material and seed germination

Dry graded Indian wild rice (Oryza nivara Sharma et Shastry; Accesion No. IC-283169 and IC-336684) seeds were collected from Central Rice Research Institute (CRRI), Cuttack. Uniform sized seeds were selected and disinfected with 0.1% mercuric chloride ($HgCl_2$). They were placed in sterilized petriplates over saturated tissue paper for germination at 25º C in dark for two days. Germinated seeds with emergence of 2 mm radicle were used for hydroponic culture experiments

2.3 Seedling growth

Germinated seeds were grown in hydroponics in controlled laboratory conditions in growth chambers under varying concentrations (0 µM as Control, 5µM, 25 µM, 50µM, 75µM and 100µM) of Cr^{6+} [source: $K_2Cr_2O_7$] in different labeled culture vessels. 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. Tolerance index was calculated as per the method of Jali et al. [6]

2.4. Analysis of Proline Content

For the analysis of proline, the primary leaves of growing crop seedlings from different treatments were taken. For estimation of proline content, the primary leaves of different crop seedlings were homogenized with 3% aqueous sulfo-salicylic acid and centrifuged at 3000 rpm for 10 min. Proline was estimated as per method of Mohanty and Patra [3].

2.5. Extraction and assay of enzymes

The activity of catalase (1:11:1:6) and guaiacol peroxidase (1:11:1:7) was assayed according to the method of Patra and Mishra, [7] with slight modifications. One unit of peroxidase activity is expressed in terms of purpurogallin formed, which increased the absorbancy at 420 nm, by 0.1 per minute under the assay condition described above. The process was repeated for the two cultivars in Indian wild rice seedlings grown in different treatments.

2.6. Statistical Analysis

All of the treatments were conducted in triplicates and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates..

3. Results and Discussion

3.1 Proline accumulation in response to Cr^{6+} stress

Proline content in 7 days and 14 days old plants of Oryza nivara varieties(i.e. IC-283169 and IC-336684) increased with increasing the dose of Cr^{6+} in nutrient solution. In IC-283169 variety,the maximum proline biosynthesis was observed under Cr^{6+}(100µM) after 7 days and 14 days. In Ic-336684 variety, proline content under Cr^{6+}(10µM) was less than others after 7 days seedlings and after 14 days seedlings ,proline content under Cr^{6+}(75µM) was maximum (Fig. 1). The order of decrease in proline content at different concentrations of chromium is as follows:

After 7 Days,

In IC-283169 variety of Oryza nivara : Cr^{6+}(100µM)>Cr^{6+}(75µM)>Cr^{6+}(50µM)>Cr^{6+}(25µM)>Cr^{6+}(10µM)>Control.

In IC-336684 variety of Oryza nivara : Cr^{6+}(75µM)>Cr^{6+}(100µM)>Cr^{6+}(50µM)>Cr^{6+}(25µM)>Control>Cr^{6+}(10µM).

After 14 Days,

In IC-283169 variety of Oryza nivara : Cr^{6+}(100µM)>Cr^{6+}(75µM)>Cr^{6+}(50µM)>Cr^{6+}(25µM)>Cr^{6+}(10µM)>Control.

In IC-336684 variety of Oryza nivara : Cr^{6+}(75µM)>Cr^{6+}(100µM)>Cr^{6+}(50µM)>Cr^{6+}(25µM)>Cr^{6+}(10µM)>Control.

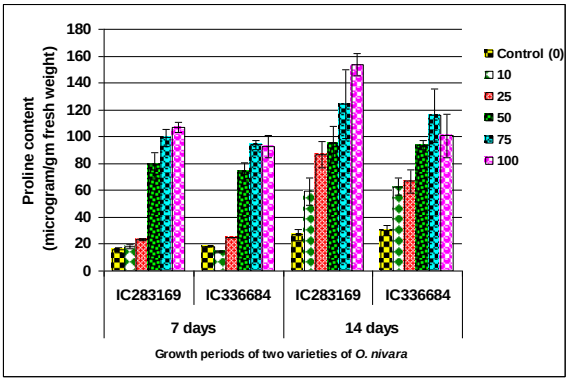

Figure 1: Clustered Bar graph showing the comparative values of proline content in in two varieties of seven days and fourteen days grown Oryza nivara.

A marked increase in proline content was found in the seedlings grown under Cr^{+6} (100µM) treatments **and** 75µM (Fig. 1) in IC283169 and IC336684 cultivars of Indian wild rice respectively**.** Proline accumulation is an important parameter to recognize the stress impact on plants [8] [9]. For example, stress induced proline biosynthesis was observed in many plants in response to salt stress [4] [9] [10]. Proline accumulation may also help in non-enzymic free radical detoxifications [10]. An increasing proline level is considered to help the cells in osmoprotection as well as regulating the redox potential, scavenging hydroxyl radicals in the protection against denaturation of various macromolecules [10]. In the present context, a uniform increase in the proline level was noticed in 7 and 14-day-old IC283169 variety seedlings subjected to Cr^{+6} 100 µM treatment. An increase in

the proline content in seedlings may provide nontoxic sinks for carbon and nitrogen preservation as reported for other plants under salt stress [10] [9]. Maximum proline bioaccumulation in IC336684 cultivar of Indian wild rice was recorded at 75 µM Cr^{6+} treatment which has lost its tolerance beyond it.

3.2 Changes in Catalase and Peroxidase activities

Treatment of different hexavalent chromium concentrations (10µM, 25µM, 50µM, 75µM, 100µM) along with control showed marked changes in the catalase activity of 7 days and 14 days old Oryza nivara seedlings grown under Cr^{6+} stress. In IC-283169 variety, Cr^{6+} (25 µM) shows the highest catalase activity after 7 days treatment and after 14 days treatment Cr^{6+}(50 µM) shows the least catalase activity. In IC-336684 variety,Cr^{6+}(10 µM) and Cr^{6+}(50 µM) shows the highest catalase activity after 7 days treatment and after 14 days treatment respectively (Fig. 2 and Fig. 3).

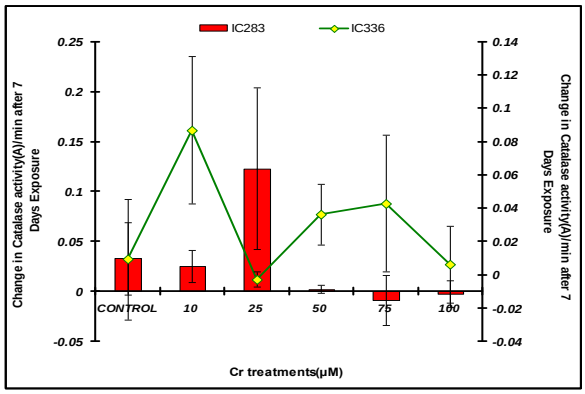

Figure 2: Effect of different concentrations of Cr^{6+} on catalase activity in two varieties of 7 days grown Oryza nivara .

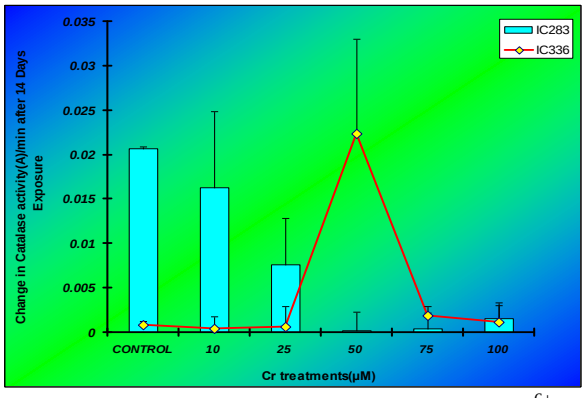

Figure 3: Effect of different concentrations of Cr^{6+} on catalase activity in two varieties of 14 days grown Oryza nivara

The order of catalase activity treated with different chromium concentration was as follows:

After 7 Days,

In IC-283169 variety of Oryza nivara:
Cr^{6+}(25µM)>Control>Cr^{6+}(10µM)>Cr^{6+}(50µM)>Cr^{6+}(100µM)>Cr^{6+}(75µM).

In IC-336684 variety of Oryza nivara:
Cr^{6+}(10µM)>Cr^{6+}(75µM)>Cr^{6+}(50µM)>Cr^{6+}(100µM)>Control>Cr^{6+}(25µM).

After 14 Days,

In IC-283169 variety of Oryza nivara:
Control>Cr^{6+}(10µM)>Cr^{6+}(25µM)>Cr^{6+}(100µM)>Cr^{6+}(75µM)>Cr^{6+}(50µM).

In IC-336684 variety of Oryza nivara:
Cr^{6+}(50µM)>Cr^{6+}(75µM)>Cr^{6+}(100µM)>Control>Cr^{6+}(25µM)>Cr^{6+}(10µM).

Peroxidase activity increased significantly with increase in chromium treatment in all the tested cultivars. The activity was increased with the increasing dose of chromium treatment. In IC-283169 variety, the maximum peroxidase activity was observed in Cr^{6+}(75µM) treatment after 7 days seedlings and after 14 days seedlings,Cr^{6+}(100µM) shows the maximum peroxidase activity(Fig. 4 and Fig. 5).

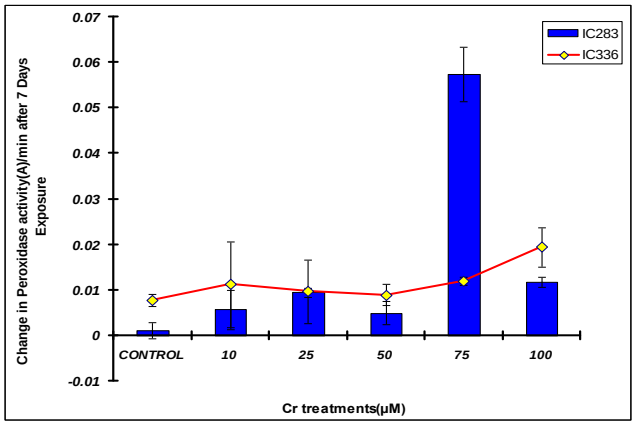

Figure 4: Effect of different concentrations of Cr^{6+} on peroxidase activity in two varieties of 7 days grown Oryza nivara

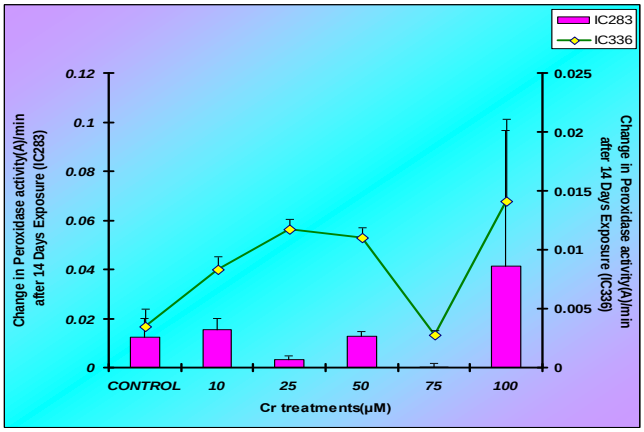

Figure 5: Effect of different concentrations of Cr^{6+} on peroxidase activity in two varieties of 14 days grown Oryza nivara

In IC-336684 variety, the maximum peroxidase activity was observed in Cr^{6+}(100µM) treatment after 7 days as well as 14 days seedlings. The order of peroxidase activity treated with different chromium concentration was as follows:

After 7 Days,

In IC-283169 variety of Oryza nivara :

Cr^{6+}(75µM) >Cr^{6+}(100µM) >Cr^{6+} (25µM) >Cr^{6+} (10µM) > Cr^{6+} (50µM)>Control .

In IC-336684 variety of Oryza nivara : Cr^{6+}(100µM)>Cr^{6+}(75µM)>Cr^{6+}(10µM)>Cr^{6+}(25µM)>Cr^{6+}(50µM)>Control.

After 14 Days,

In IC-283169 variety of Oryza nivara : Cr^{6+}(100µM)>Cr^{6+}(10µM)>Control>Cr^{6+}(50µM)>Cr^{6+}(25µM)>Cr^{6+}(75µM).

In IC-336684 variety of Oryza nivara : Cr^{6+}(100µM)>Cr^{6+}(25µM)>Cr^{6+}(50µM)>Cr^{6+}(10µM)>Control >Cr^{6+}(75µM).

These enzymes are mostly associated with the scavenging of toxic free radicals produced as a result of Cr stress [11] [12]. These enzymes plays important role to detoxify and sequester Cr, and therefore, the roots appear to be the major site of enzyme synthesis. This finding is consistent with the results obtained from previous studies with Cr tolerance in green gram conducted by Mohanty and Patra, [9]

3.3 Toxicological effects of Cr on plant growth

Tolerance index (TI) represents the relative growth rate of the plants and is equal to the growth of seedlings under Cr+6 treatment divided by the growth in control. TI of root length and fresh weight are commonly used to quantify plant metal tolerance [13].

The higher the TI, the better is the tolerance. Results from the seedling tolerance studies showed that Fresh weight TI was better than root tolerance index (Table1). Root lengths are less substantially impaired by Cr stress than fresh weights (Table 1). The data for IG% and tolerance index indicates that Indian wild rice is less sensitive to chromium and develops tolerance beyond 50µM Cr^{6+} treatment.

Table 1: Toxicological interpretations in 14 days grown O. nivara seedlings under Cr+6 stress

IC283169			
Treatments of Cr^{6+} (µM)	Germination Index (IG%)	Root Tolerance index (RTI)	Shoot Tolerance index (STI)
Control (0)	100	1	1
5	67.2045	0.75	0.78
25	42.28893	0.52	0.70
50	25.25641	0.37	0.45
75	12.43902	0.22	0.38
100	5.916198	0.74	0.62
IC336684			
Treatments of Cr^{6+} (µM)	Germination Index (IG%)	Root Tolerance index	Shoot Tolerance index
Control (0)	100	0.46	1
5	43.97626	0.30	0.75
25	25.58853	0.27	0.59
50	18.90208	0.23	0.41
75	13.97626	0.20	0.33
100	8.55094	0.46	0.29

4. Conclusion

Above studies assess the comparative antioxidative enzyme protective response against Cr stress in two cultivars of Indian wild rice i.e. Oryza nivara. Proline accumulation as a symbol of stress response showed the variation in plant tolerance in two cultivars of Indian wild rice. Further research on screening the cultivars for Cr tolerance potentiality through assessing the other physiological and biochemical parameters is needed. The ability of these plants as potent tools for phytoremediation could be enhanced through supplemental nutrition for increasing phytoaccumulation potential needs to be tried.

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