Evaluation of Phenotypic Responses of Selected Rice (*Oryza sativa* L.) Cultivars to Hexavalent Chromium Stress in Soil

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**ABSTRACT**

The current work is designed to search for suitable rice (*Oryza sativa* L.) cultivars capable of growing on *Cr(VI)*, hexavalent chromium contaminated soils. The study of tolerance and phenotypical changes of three selected rice cultivars like Bina Dhan 11, Kalachampa, and Pratikshya, at the seedling stages, was done under soil *Cr(VI)* concentrations up to 300 mg kg⁻¹ of soil. The 7-day seedlings of these rice cultivars growing on *Cr(VI)* treated soils were found to exhibit a significant reduction in shoot and root growth at *p* ≤ 0.05. The experimental results support that 7-day seedlings of Bina Dhan 11 were found to be the best among the three cultivars under soil *Cr(VI)* stress conditions. The present work may help in selecting suitable rice cultivar for paddy cultivation on *Cr(VI)* contaminated crop lands present in mining and industrial belts. Further work on this aspect may be useful in increasing rice productivity, catering to the increase in demand for food.

**INTRODUCTION**

Chromium (Cr), commonly used as a metal corrosion inhibitor is an important heavy metal with multiple oxidation states and has wide applicability in industrial sectors (Das et al. 2021a). It is the 17th most hazardous substance for the living biota (Hou et al. 2019). It is widely introduced to the environmental components with the release of industrial effluents, irrigation of contaminated water, and disposal of sludge and solid wastes. (Majhi & Samantaray 2020). The *Cr(VI)* is a hazardous soil chemical constituent and is treated as a highly toxic heavy metal by the USEPA (Das et al. 2017).

The natural sources of hexavalent chromium contamination of croplands can takes place through volcanoes, eroded rocks, and soils (Wang et al. 2017). The human involved activities like tanning of leathers, electroplating, burning of fossil fuels, dyeing of textiles, welding, polishing, and mining are the major sources of release of chromium into the environment (Das et al. 2018). The high chromium concentration of 2000-5000 mg L⁻¹ in the released effluents contributes to the pollution of major rivers supporting irrigation systems in India (Alka 2017). The release of 2000-3000 tons of chromium per annum from tanneries in India to surroundings shows the magnitude of chromium pollution (Ahmed et al. 2021). It is not only polluting the terrestrial ecosystem but also the aquatic ecosystems due to leaching and percolations (Das et al. 2021b).

The grains of cereals like rice (*Oryza sativa* L.) are a nutritious food to remove hidden hunger from the growing world population (Khush & Virk 2000). These grains contain enough polysaccharides, proteins, vitamins, fibers, minerals, and antioxidants (Swain et al. 2017). It is a food of demand across the globe, especially in Asia, as billions of people are consuming rice and its products in multiple forms. Despite its demand, the productivity of rice per unit hectare in India is low as it is only 2.23 tons (Jain 2019). The high solubility of *Cr(VI)* renders to be more toxic to the growing rice crops on cultivable lands in the vicinity of industrial and mining areas. It may show a loss in the yield of the crop during exposure to *Cr(VI)* (Dheeba et al. 2014).

Many rice varieties are released so far but are not free from constraints to grow on contaminated soils. The present study was designed to find out an improved rice cultivar tolerant to *Cr(VI)* toxicity, at early seedling stages. It may help in increasing the yield of grains per unit area of land under rice cultivation.

**MATERIALS AND METHODS**

**Experimental Environment**

The experimental study was done in two successive Kharif seasons from July to December in the years 2019 and 2020. It was done under greenhouse conditions at the Centre for Biotechnology, Ghatikia, Bhubaneswar. It was located at...
85°46’31” E and 21°17’02” N in the subtropical belt of India. The mean temperature and humidity maintained inside the greenhouse during the experimental growth and monitoring were 27 ± 1°C and 82 ± 2%, respectively. The mild acidic clayey soil was used for seedling growth during the experiment with pH ranged in between 5.9 to 6.2.

Selection of Cultivars

The certified seeds of ten *Oryza sativa* L. cultivars were collected randomly and used for preliminary screening. Three rice cultivars Pratiksha (ORS 201-5), Bina Dhan 11 (Cihera Sub-1), and Kalachampa (FV-152), meeting the requisite criteria were selected for the present experimental study (Table 1).

Experimental Design

The healthy and intact seeds of three selected rice cultivars were used in this experimental study. The selected seeds were surface sterilized with 0.1% mercuric chloride. 200 pre-germinated, sterilized seeds of each selected cultivar were sown in rows of trays containing soils moistened with distilled water. These trays were maintained at a temperature of 27 ± 1°C in dark. After germination, the experiment was designed with a selection of 7-day-old seedlings of three cultivars based on a randomized block design with triplicate seedlings in each group. The selected cultivars were allowed to grow on pots having the capacity to carry 4 kg of homogenous soils. One triplicate group of seedlings was allowed to grow as a control on 3 kg of homogenous soil without having any Cr(VI) contamination. The 7-day old seedlings of each replication of all the treatment conditions and control were sampled and analyzed for various phenotypic parameters like thousand grains weight, seed germination efficiency, seedlings survival percentage under Cr(VI) contaminated soil conditions, and variation in growth of shoots and roots with the change in soil Cr(VI) concentrations under treatment conditions.

Phenotypic Study of Selected Cultivars

The phenotypic parameters of the selected cultivars were determined following the standard methodologies (Yoshida et al. 1976).

**Thousand Seeds Weight**

Three rice cultivars having three replicates of each were constituted by taking 1000 randomly selected intact seeds. These randomly selected seed samples were used for the determination of 1000 grain weights.

**Seed Germination Efficiency**

The three replicates of each of these three rice cultivars having 50 healthy seeds each were selected randomly for the determination of this parameter. The seed germination efficiency was determined based on the percent germination of seeds at 27 ± 1°C.

Germination per cent = \( \frac{Gs}{Ts} \times 100 \)

Where,

\( Gs = \) Number of seeds germinated to produce normal seedlings

\( Ts = \) Total number of seeds taken for germination

**Seedlings Survival Percentage**

The three replicates of each of these three rice cultivars like Bina Dhan 11, Kalachampa, and Pratiksha having 30 healthy seedlings each were selected randomly for the determination of this parameter.

Seedling survival percentage = \( \frac{Ssn}{Stn} \times 100 \)

Where,

\( Ssn = \) Number of normal seedlings survived after 7 days under Cr(VI) stress soil conditions.

\( Stn = \) Total number of normal seedlings taken for the study.

**Seedlings’ Growth and Variations**

The seedlings’ growth in terms of shoot, root, and leaf length of three replicates of each of the selected rice cultivars like Bina Dhan-11, Kalachampa, and Pratiksha was recorded.

**Statistical Analysis**

The statistical analysis was done using a statistical package for the social sciences (IBM SPSS statistics 21) analytical

<table>
<thead>
<tr>
<th>Selected cultivars</th>
<th>Range of maturity (in days)</th>
<th>Cultivar type</th>
<th>Grains type</th>
<th>Range of cultivar productivity [t.ha⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bina Dhan 11</td>
<td>95-105</td>
<td>Early group</td>
<td>Medium Slender</td>
<td>5.83-6.12</td>
</tr>
<tr>
<td>Kalachampa</td>
<td>143 - 158</td>
<td>Late group</td>
<td>Medium bold</td>
<td>6.86-7.14</td>
</tr>
<tr>
<td>Pratikshya</td>
<td>138 - 152</td>
<td>Medium late group</td>
<td>Long slender</td>
<td>7.06-7.38</td>
</tr>
</tbody>
</table>
software. The analysis of variance (ANOVA) in respect of the mean response of cultivars to variation in Cr(VI) treatment concentrations was done under a split-plot design. The differences between treatment means were estimated using Tukey’s HSD test at $p \leq 0.05$.

**RESULTS AND DISCUSSION**

Rice is a major food crop in the Indian sub-continent and India is the second-largest rice-producing nation in the world after China. A person in India consumes approximately 68.2 kg of milled rice per year which confirms the importance of rice as a major staple food for the Indian population (Das et al. 2020). The yield of rice is highly affected due to contamination of agricultural soil with toxic industrial effluents rich in heavy metals like Cr(VI). The current work, therefore, strived to select the improved cultivars that could resist and grow well under high concentrations of soil Cr(VI) content.

**Seed Characteristics of Selected Rice Cultivars**

The rice cultivar, Bina Dhan 11 records 94 per cent germination of seeds and it is maximum as compared to other cultivars like Kalachampa and Pratikshya (Fig. 1). The per cent germination of seeds in Kalachampa and Pratikshya are 88 and 86 per cent, respectively. In the present experiment, the per cent germination of seeds shows an inverse relationship with the weight of thousand seeds of the selected rice cultivars. It is supported by the findings of another study on *Pisum sativum* L. (Peksen et al. 2004). Based on a comparison of three selected cultivars, Bina Dhan 11 shows maximum seed germination (94 per cent) and simultaneously minimum thousand seeds weight (59.09 g). A thousand seeds weight is maximum in Pratikshya (63.34 g) and recorded minimal seeds germination as 86 per cent (Table 2).

**Seedlings Survival Percentage**

The survival of seedlings on croplands is an important parameter to getting a better yield. It is required for farmers’ acceptance and for meeting their socio-economic needs. The Bina Dhan- 11 is found to be a suitable rice cultivar as compared to Kalachampa and Pratikshya based on its survival percentage at the early seedling age of 7 days. It is observed that the survival of rice cultivar Pratikshya is intermediate and is ranked in between the other two cultivars studied together (Fig. 1).

Cr at a very low concentration (0.06–1.0 mg.L$^{-1}$) is found to enhance growth and yield in *Oryza sativa* L. However,
a higher concentration of toxic heavy metals hinders the survival of plants. It affects the germination process and negatively impacts the growth of rice crops (Javaid et al. 2020). This may be the possible reason behind the reduced survivability among the cultivars with an increase in soil Cr(VI) concentrations.

**Seedlings Growth and Variations**

An increase in Cr(VI) load in the soil led to a gradual reduction in the shoot and root length of three rice cultivars during the 7-day treatment. The mean shoot growth of Bina Dhan 11 is 2.10 ± 0.01 cms on soil contaminated with 270 mg.kg⁻¹ soil is low as compared to its growth on 0 mg.kg⁻¹ soil, treated as a control in the experiment. Similarly, the mean root growth of Bina Dhan 11 is 13.47 ± 0.20 cms on soil contaminated with 270 mg.kg⁻¹ soil is low as compared to its growth as a control. The mean shoot and root growth of rice cultivar Pratikshya are more as compared to the other two cultivars Bina Dhan 11 and Kalachampa on soil contaminated with 210 mg.kg⁻¹ soil but this cultivar is unable to grow on soils contaminated with a higher concentration of Cr(VI) (Table 3). Plants treated with 270 mg.kg⁻¹ soil Cr(VI) showed maximum reduction in the shoot (35.97% for Bina Dhan 11) and roots (16.07% for Bina Dhan 11) about controls (Table 4).

The Cr to be responsible for bringing about a reduction in the root and shoot of crop plants is supported by the findings of previous studies (Purohit et al. 2003, Sundaramoorthy et al. 2010). The reduction in morphological parameters of *Oryza sativa* L. due to Cr stress has received strong support from the earlier studies (Gill et al. 2015, Shahid et al. 2017). The reduction in length of seedlings may be due to Cr stress-led constraint in nutrient uptake as well as alterations in the ultra-structural framework of mesophyll cells present in the rice leaves (Hussain et al. 2018). The decrease in chlorophyll content of leaves due to the loss of both chlorophyll a and b is observed under stress conditions (Das et al. 2008). The parameter like total sugar and starch content of seedlings are determining the tolerance of rice cultivars to stress conditions (Das et al. 2009).

The Cr has been reported to form insoluble complexes with soil nutrients, thus hindering their uptake in plants (Chigonum et al. 2019). The Cr has been playing a major role in disturbing the nutrient balance in plants. Excess of Cr has been reported to bring about a corresponding decrease in micro and macronutrients in *Oryza sativa* L. which may be attributed to the displacement of the nutrients from physiologically important binding sites and thus their reduced translocation into the crop (Shahzad et al. 2018).

**Correlation Studies**

An increase in soil Cr(VI) concentrations resulted in a corresponding decrease in both the shoot and root length of the selected rice cultivars implying the existence of a negative correlation between the studied parameters (Fig. 2). Among the three selected rice cultivars the correlation between soil Cr(VI) and shoot length of Bina Dhan 11 (r= -0.795) was much less as compared to Kalachampa (r= -0.926), and Pratikshya (r= -0.913). A similar trend was obtained between

<table>
<thead>
<tr>
<th>Soil treatment code / Cr(VI) concentration in mg.kg⁻¹ soil</th>
<th>Shoot and root growth of 7-day seedlings of selected rice cultivars under soil Cr(VI) treatment conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean shoot growth ± S.E.M</td>
</tr>
<tr>
<td></td>
<td>(cm)</td>
</tr>
<tr>
<td>Bina Dhan 11</td>
<td>Kalachampa</td>
</tr>
<tr>
<td>T0 (control) / 0</td>
<td>3.28±0.03</td>
</tr>
<tr>
<td>T1 / 30</td>
<td>3.18±0.01</td>
</tr>
<tr>
<td>T2 / 60</td>
<td>3.12±0.03</td>
</tr>
<tr>
<td>T3 / 90</td>
<td>3.12±0.01</td>
</tr>
<tr>
<td>T4 / 120</td>
<td>2.88±0.02</td>
</tr>
<tr>
<td>T5 / 150</td>
<td>2.68±0.02</td>
</tr>
<tr>
<td>T6 / 180</td>
<td>2.67±0.03</td>
</tr>
<tr>
<td>T7 / 210</td>
<td>2.48±0.02</td>
</tr>
<tr>
<td>T8 / 240</td>
<td>2.39±0.04</td>
</tr>
<tr>
<td>T9 / 270</td>
<td>2.10±0.01</td>
</tr>
<tr>
<td>T10 / 300</td>
<td>-</td>
</tr>
</tbody>
</table>

Significant at p ≤ 0.05; ‘-’ indicates the non-existence of cultivars at the specified concentration of soil Cr(VI).
Table 4: Mean percentage reduction in length of rice seedlings under variable soil Cr(VI) concentrations for 7 days.

<table>
<thead>
<tr>
<th>Soil treatment code / Cr(VI) concentration in mg.kg(^{-1}) soil</th>
<th>Mean Percentage reduction</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot growth</td>
<td>Root growth</td>
</tr>
<tr>
<td></td>
<td>Bina Dhan-11</td>
<td>Kalachampa</td>
</tr>
<tr>
<td>T1 / 30</td>
<td>3.04</td>
<td>4.41</td>
</tr>
<tr>
<td>T2 / 60</td>
<td>6.09</td>
<td>33.82</td>
</tr>
<tr>
<td>T3 / 90</td>
<td>6.09</td>
<td>38.72</td>
</tr>
<tr>
<td>T4 / 120</td>
<td>12.10</td>
<td>39.95</td>
</tr>
<tr>
<td>T5 / 150</td>
<td>18.29</td>
<td>46.56</td>
</tr>
<tr>
<td>T6 / 180</td>
<td>18.59</td>
<td>49.01</td>
</tr>
<tr>
<td>T7 / 210</td>
<td>24.39</td>
<td>-</td>
</tr>
<tr>
<td>T8 / 240</td>
<td>27.13</td>
<td>-</td>
</tr>
<tr>
<td>T9 / 270</td>
<td>35.97</td>
<td>-</td>
</tr>
<tr>
<td>T10 / 300</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 2: Graph showing the correlation between varied soil Cr(VI) concentration and (a) shoot length of Bina Dhan 11 (b) shoot length of Kalachampa (c) shoot length of Pratikshya (d) root length of Bina Dhan 11 (e) root length of Kalachampa, and (f) root length of Pratikshya.
the reduction in mean root length and an increase in soil Cr(VI) concentrations. This confirms the better suitability of Bina Dhan 11 to Cr(VI) soil stress as compared to the other two cultivars.

**Cr(VI) Mediated Variations in Leaf Length**

In the present study, the impact of Cr(VI) on the three selected rice cultivars was studied. It is observed that the size of the leaves varied negatively with a proportionate increase in Cr(VI) content in the soil. The leaf length gradually decreased with an increase in soil Cr(VI) content from 30 mg.kg\(^{-1}\) soil to 270 mg.kg\(^{-1}\) soil (Fig. 3). A leaf is a vital organ in plants and helps mostly in the process of photosynthesis. The total area of a leaf in a plant determines the efficiency of photosynthesis (Srivastava et al. 2021). It is observed that Cr(VI) shows a negative impact on leaf morphology in *O. sativa*. It is supported by the findings of another study on paddy (Sundaramoorthy et al. 2010). The Cr(VI) has also been found to impart toxic effects on other plants. The *Prosopis laevigata* when exposed to nutrient media enriched with a 3.4mM Cr(VI) concentration resulted in a reduced number of leaves (Buendía-González et al. 2010). In a similar study, *Lolium perenne* L. grown in nutrient media spiked with 0.5 mM of Cr(VI) resulted in the wilting of the leaves (Vernay et al. 2007). Chlorosis in the leaves of *Saccharum officinarum* was observed when exposed to a soil Cr(VI) concentration of 40 mg.kg\(^{-1}\). On increasing the toxic metal concentration to 80 mg.kg\(^{-1}\), the leaves of the plant exhibited severe necrosis (Radha et al. 2000). Cr(VI) has been found to inhibit the biosynthesis of chlorophyll pigments in plants (Sharma et al. 2019), a possible reason behind reduced leaf area in the current study. An increase in Cr(VI) concentrations may lead to a reduction in the chlorophyll content which may be due to the inactivation of chlorophyll biosynthesis enzymes (Zlobin et al. 2015, Sharma et al. 2020).

Another probable reason for reduced leaf length can be attributed to either reduction in the number of leaf cells or a probable reduction in the size of the cells under Cr(VI) stress. Reduced cell size may also negatively affect the intracellular spaces thus leading to stunted growth of the leaves (Joshi et al. 1999).

**CONCLUSION**

The current experimental approach is to find out a suitable rice cultivar capable of growing on Cr(VI) stress soil conditions. All the three rice cultivars Bina Dhan-11, Kalachampa, and Pratikshya have been able to survive on Cr(VI) stress soil conditions up to 180 mg.kg\(^{-1}\). The evaluation of selected phenotypic parameters reveals that Bina Dhan 11 is a suitable rice cultivar at the early seedling stage to grow on Cr(VI) stress soil conditions up to 270 mg.kg\(^{-1}\) soil. Further work
on this aspect is useful in getting better paddy yield from Cr(VI) contaminated soil conditions.

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