Nature Environment and Pollution Technology
An International Quarterly Scientific Journal
p-ISSN: 0972-6268 (Print copies up to 2016)
e-ISSN: 2395-3454
Vol. 21 | No. 3 | pp. 1341-1347 | 2022
Original Research Paper
https://doi.org/10.46488/NEPT.2022.v21i03.041
Open Access Journal

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

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 29-09-2021
Revised: 12-12-2021
Accepted: 22-12-2021

Key Words:
Rice cultivars
Hexavalent chromium stress
Phenotypic responses
Seedlings

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 $p \leq 0.05$. 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 17^{th} 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 Pratikshya (ORS 201-5), Bina Dhan 11(Ciherang 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 3kg of homogenous soil without having any Cr(VI) contamination. The seven-day seedlings of each cultivar were allowed to grow on 3 kg of Cr(VI) contaminated homogenous clayey soil of variable concentrations ranging between 30 $mg.kg^{-1}$ soil to 300 $mg.kg^{-1}$ soil with a regular gap of 30 $mg.kg^{-1}$ of soil. 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 = (Gs / Ts) x 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 Pratikshya having 30 healthy seedlings each were selected randomly for the determination of this parameter.

Seedling survival percentage = (Ssn / Stn) x 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 Pratikshya was recorded.

Statistical Analysis

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

Table 1: Characteristics of rice cultivars selected for the experiment.

Selected cultivars	Range of maturity (in days)	Cultivar type	Grains type	Range of cultivar productivity [$t.ha^{-1}$]
Bina Dhan 11	95-105	Early group	Medium Slender	5. 83-6.12
Kalachampa	143 - 158	Late group	Medium bold	6.86-7.14
Pratikshya	138 - 152	Medium late group	Long slender	7.06-7.38

Table 2: Seed characteristics of selected rice cultivars.

Sl. No.	Cultivars	Thousand seeds weight [g]	Germination of seeds [%]
1	Bina Dhan 11	59.09	94
2	Kalachampa	61.45	88
3	Pratikshya	63.34	86

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,

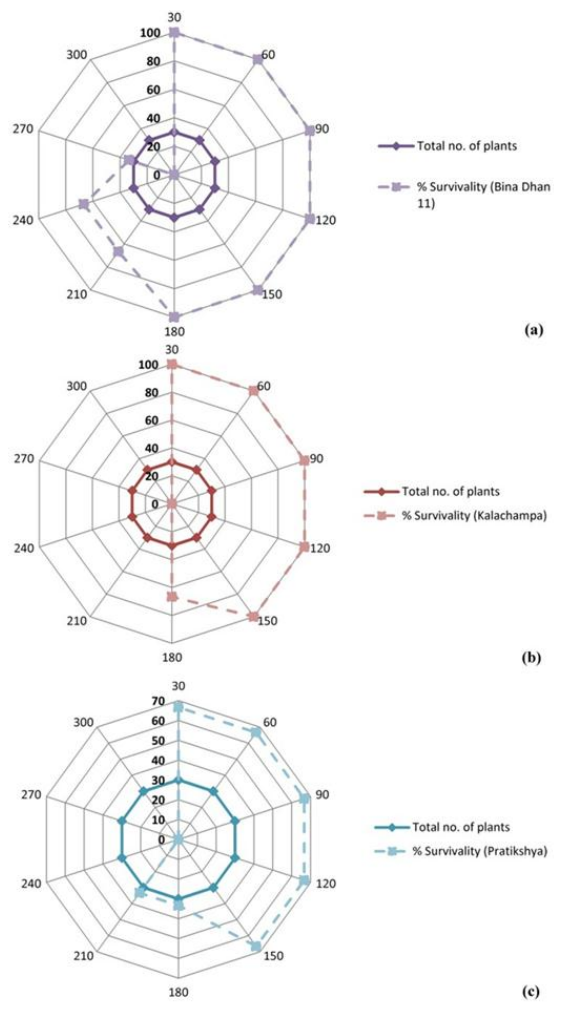

Fig. 1: Survival of seedlings of selected rice cultivars (a) Bina Dhan-11, (b) Kalachampa, and (c) Pratikshya under different soil Cr(VI) concentrations. (The values around the periphery of the graph show the different levels of soil Cr(VI) in mg.kg^{-1} and the values marked in bold denote the survival percentage of seedlings. The solid lines mark the number of 7-day-old seedlings subjected to treatment, and the dotted lines express the survival percentage at each concentration of soil Cr(VI)).

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 3: Effect of 7-day treatment of variation in soil Cr(VI) concentration on seedlings growth of selected rice cultivars.

Soil treatment code / Cr(VI) concentration in $mg.kg^{-1}$ soil	Shoot and root growth of 7-day seedlings of selected rice cultivars under soil Cr(VI) treatment conditions					
	Mean shoot growth ± S.E.M (cm)			Mean root growth ± S.E.M (cm)		
	Bina Dhan 11	Kalachampa	Pratikshya	Bina Dhan 11	Kalachampa	Pratikshya
T0 (control) / 0	3.28±0.03	4.08±0.05	5.66±0.08	16.05±0.18	21.16±0.16	22.29±0.33
T1 / 30	3.18±0.01	3.90±0.02	5.50±0.06	15.52±0.18	18.82±0.16	22.24±0.23
T2 / 60	3.12±0.03	2.70±0.01	4.60±0.04	15.43±0.19	15.49±0.13	21.27±0.25
T3 / 90	3.12±0.01	2.50±0.01	4.23±0.11	15.03±0.02	14.74±0.16	19.38±0.22
T4 / 120	2.88±0.02	2.45±0.02	4.14±0.10	15.00±0.26	14.56±0.12	19.08±0.21
T5 / 150	2.68±0.02	2.18±0.06	3.81±0.03	14.64±0.23	14.19±0.09	18.70±0.17
T6 / 180	2.67±0.03	2.08±0.03	3.69±0.04	14.15±0.31	13.81±0.13	18.36±0.21
T7 / 210	2.48±0.02	-	3.53±0.04	14.09±0.15	-	18.07±0.20
T8 / 240	2.39±0.04	-	-	13.77±0.20	-	-
T9 / 270	2.10±0.01	-	-	13.47±0.20	-	-
T10 / 300	-	-	-	-	-	-

Significant at $p \leq 0.05$; '-' 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.

Soil treatment code / Cr(VI) concentration in mg.kg^{-1} soil	Mean Percentage reduction					
	Shoot growth			Root growth		
	Bina Dhan-11	Kalachampa	Pratikshya	Bina Dhan-11	Kalachampa	Pratikshya
T1 / 30	3.04	4.41	2.82	3.30	11.05	0.22
T2 / 60	6.09	33.82	18.72	3.86	26.79	4.57
T3 / 90	6.09	38.72	25.26	6.35	30.34	13.05
T4 / 120	12.10	39.95	26.85	6.54	31.19	14.40
T5 / 150	18.29	46.56	32.68	8.78	32.93	16.10
T6 / 180	18.59	49.01	34.80	11.83	34.73	17.63
T7 / 210	24.39	-	37.63	12.21	-	18.93
T8 / 240	27.13	-	-	14.20	-	-
T9 / 270	35.97	-	-	16.07	-	-
T10 / 300	-	-	-	-	-	-

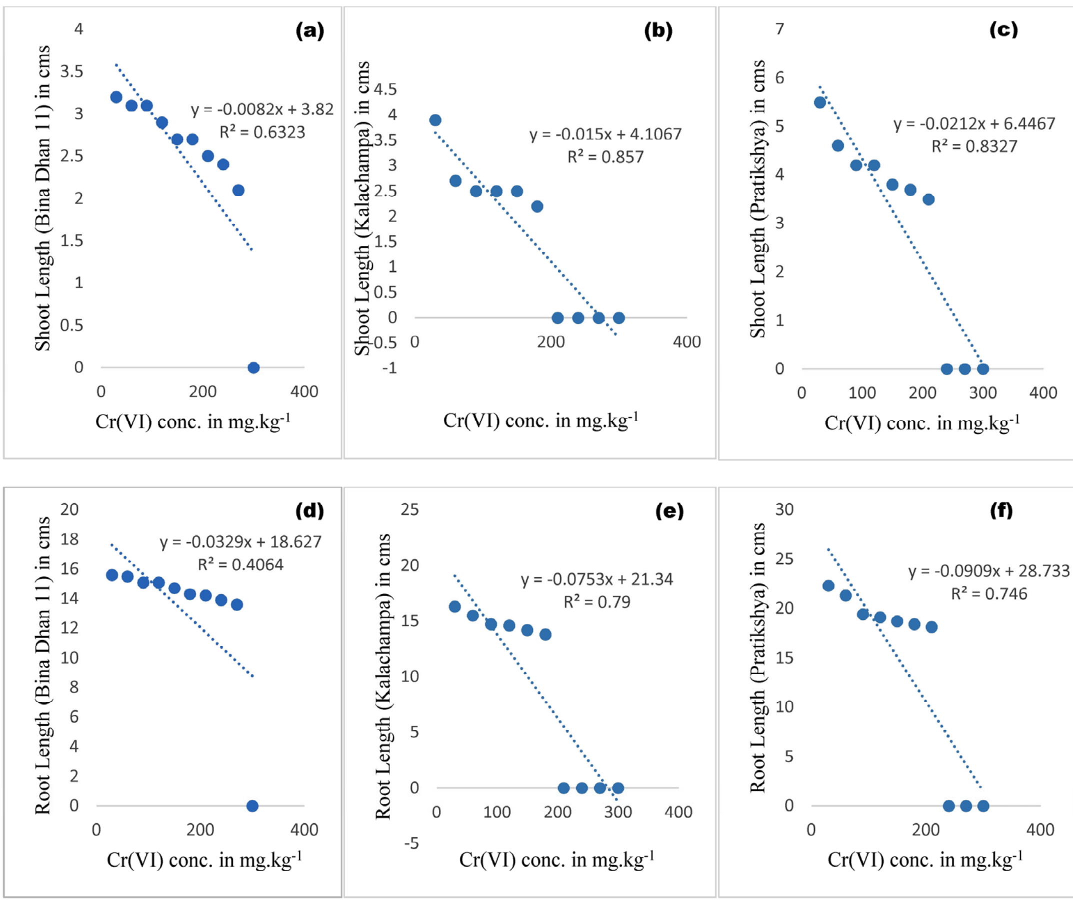

Fig. 2: Graph showing the correlation between varied soil Cr(VI) concentration and **(a)** shoot length of Bina Dhan 11 **(b)** shoot length of Kalachampa **(c)** shoot length of Pratikshya **(d)** root length of Bina Dhan 11 **(e)** root length of Kalachampa, and **(f)** root length of Pratikshya.

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 upto 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

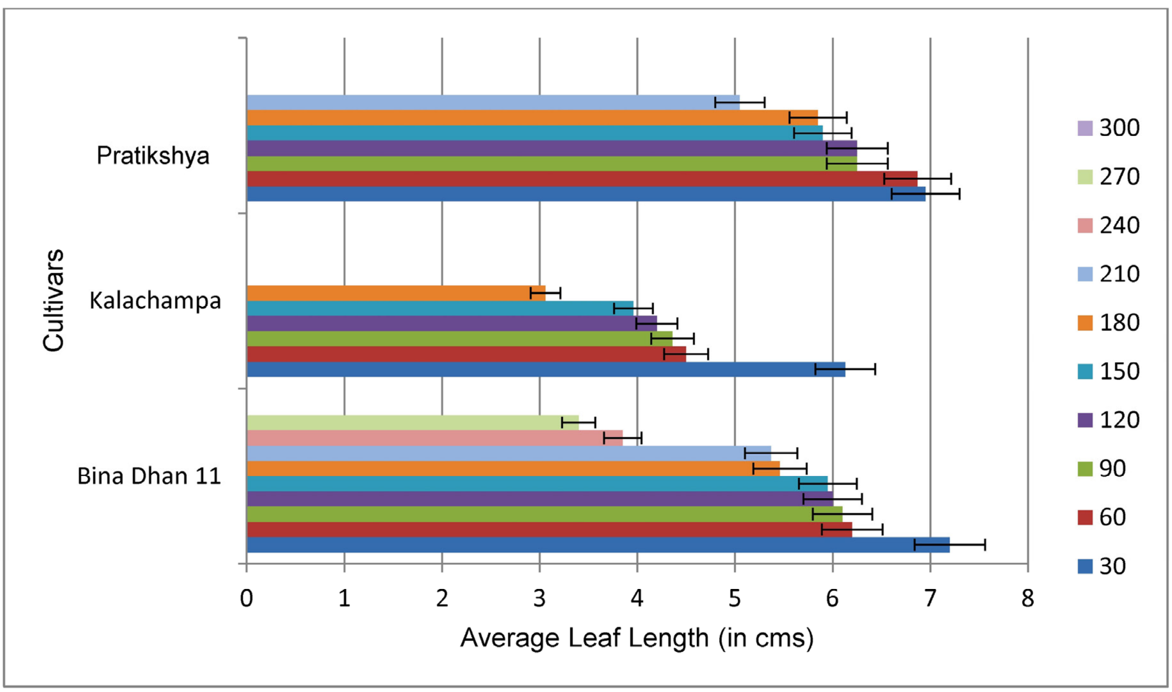

Fig. 3: Leaf length of rice cultivars under different concentrations (30-270 $mg.kg^{-1}$ soil) of Cr(VI).

on this aspect is useful in getting better paddy yield from Cr(VI) contaminated soil conditions**.**

ACKNOWLEDGEMENT

The authors acknowledge the support provided by Siksha 'O' Anusandhan (Deemed to be University) during the course of this research.

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