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Effect of chromium induced stress on seed germination and physiological parameters of Barley (*Hordeum vulgare* L.)

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Abstract

Crop plants are negatively affected by heavy metals. Chromium is also a heavy metal that harms crops. This study aims to examine the effects of several chromium treatments on the germination and physiological processes of barley seedlings. A Petri culture experiment was performed to accomplish this purpose. In this experiment, sterilized barley seeds were sown in Petri dishes lined with Whatman No. 1 filter paper in triplicate. Filter papers were wetted with four concentrations of chromium (25, 50, 75, and 100 µM), and distilled water was used as a control (0 µM) treatment. The seedlings were measured on the fourteenth day. Resulted, various Cr treatments insignificantly decreased the germination percentage (GP) and speed of germination index (SGI). The number of lateral roots was reduced significantly by all Cr treatments. Treatments with Cr decreased the growth of the seedling and biomass (fresh and dry) of seedlings. Higher treatments (75 and 100 µM) resulted in significant decreases in root length, and the treatments with 50, 75, and 100 µM of Cr caused significant reductions in shoot length and total seedling length. All chromium treatments significantly decreased the fresh weight of root, shoot, and total seedlings. Higher Cr treatments (50, 75, and 100 µM) caused significant reductions in root dry weight. Several chromium treatments caused significant decreases in shoot and total seedling dry weight. Different Cr treatments diminished the seedling vigor index (SVI) and tolerance index (TI). By increasing the chromium treatments, the percentage difference from the control (% DFC) for germination and the percentage of phytotoxicity (PP) were found to increase.

Keywords: *Hordeum vulgare* L., chromium, speed of germination index, tolerance index, biomass

1. Introduction

Various heavy metals are present in the atmosphere and contaminate the environment. Oversaturation of soil and water with heavy metals adversely affects crop plants and their yield (Sangwan *et al.*, 2014) [1]. In these heavy metals, chromium is also contaminated the environment and secures second rank to contaminate soil and water (Kumar *et al.*, 2017; Kumar *et al.*, 2021) [2, 3]. Chromium is an extremely poisonous heavy metal for living things, with several negative consequences seen in people, animals, plants, and microorganisms (Cervantes *et al.*, 2001; Lushchak 2011) [4, 5]. A negative impact of chromium on the physiological and biochemical processes of crop plants had been documented (Ali *et al.*, 2011a) [6]. There are various physiological functions of plants, including photosynthesis, plant water relation, and mineral nutrient absorption in plants that can be negatively impacted by excessive Cr (Ali *et al.*, 2011b; Ali *et al.*, 2012) [7, 8]. In recent decades, Cr has been widely used in a wide range of industrial activities, like leather tanning, extracting, and electroplating (Ertani *et al.*, 2017) [9]. Exposure to Cr is expected to cause seed germination toxicity, and it is the initial physiological impact of Cr on plants. Plants are impacted by chromium through a number of metabolic activities, including germination of seeds and early seedling development, root and shoot growth, biomass, and chlorosis deficits in photosynthetic capacity, as well as plant death on the whole (Scoccianti *et al.*, 2006) [10]. A decrement was found in the length and weight (fresh and dry) of seedlings by chromium toxicity (Anuradha and Tejasvi 2022) [11].

Animal feed, malt, and human meals all use barley grain in some capacity. In a number of developing nations, notably India, barley straw gets used as food for livestock (Cavallero *et al.*, 2002) [12].

The possible health benefits of barley are now the principal benefit of using grain in diets. It has been widely observed that barley lowers blood cholesterol when combined with b-glucans (Behall *et al.*, 2004) [13] and the glycemic index (Cavallero *et al.*, 2002) [12]. The purpose of the present study was to investigate the effects of various concentrations of chromium on seed germination and seedling growth of barley.

2. Materials and Methods

A Petri culture experiment was carried out in the Department of Botany, Agra College, Agra, Uttar Pradesh, India. In this experiment, distilled water was used as the control (0 µM), and four chromium concentrations (25, 50, 75, and 100 µM) were applied. $CrCl_3.6H_2O$ was used to provide chromium treatments. A completely randomized design was used in the experiment with three replicates.

Barley seeds were disinfected with 0.1% $HgCl_2$ (mercuric chloride) for one minute, then washed several times with distilled water. After this, the seeds were dried between two filter sheets. Sterilized petri plates were lined with Whatman number 1. Filter paper. Twenty seeds of barley (*Hordeum vulgare* var. RD 2552) were sown in each petri dish and three replicates were used for each treatment. The filter papers were wetted with four different concentrations of chromium solution; consisting of 25, 50, 75, and 100 µM. Distilled water was used as a control treatment (0 µM). The seeds were allowed to sprout for 5 to 7 days in a dark growth chamber. Seeds were considered to have germinated with a radical length of 2 mm (Mohammadi, 2009) [14]. Growth parameters were measured on the fourteenth day of seeding.

2.1 Growth parameters

The germination percentage was calculated according to the given formula which was described in the earlier study (Anuradha and Tejasvi 2023) [15]. Number of lateral roots was counted manually. The root length and shoot length were measured by using a scale. Fresh weight of the root and shoot was taken immediately after removing the plant from the filter paper, and dry weight was measured after drying the plants when weight was until stabled.

Germination percentage was computed by using this formula

Germination % = [Number of germinated seeds / Total number of seeds] x 100

Speed of germination index (SGI) The speed of germination index was calculated by the following formula given by (Carley and Watson 1968) [16].

SGI = 5 (5G + 4G + 3G + 2G + G)

Where G is the number of germinated seeds after 24 hours (1 day), the sum of germinated seeds was multiplied with 5 to calculate the germination percentage.

% DFC for germination: % DFC was computed by using (Mhatre and Chaphekar's 1982) [17] formula.

% DFC = [Germination % of control - Germination % of test solution / Germination % of control] x 100

Seedling vigour index I was calculated by using formula proposed by (Abdul-Baki and Anderson, 1973) [18].

Seedling Vigour index I (SVI I) = Germination percentage × root length + shoot length (cm).

Seedling vigour index II was calculated by using formula given by (Hossein and Kasra, 2011) [19].

SVI II = Germination Percentage × Dry Weight of Seedling (mg).

Percentage of phytotoxicity: PP for shoot and root was calculated according to the formula of (Chou *et al.*, 1978) [20].

% Phytotoxicity = [root or shoot length of control – root or shoot length of test solution / root or shoot length of control] x 100.

Tolerance index (TI) of root and shoot was determined according to the formula of (Turner and Marshal 1972) [21] as follows.

TI = [mean length of longest root or shoot in test solution / mean length of longest root or shoot in control] x 100.

2.2 Statistical analysis

The experiment was a completely randomized design with three replicates. The obtained data from germination and growth characteristics of barley by statistically analyzed to determine the level of individual variation by the mean and standard error (N = 3) and then one-way ANOVA was performed using SPSS software and the Least significance difference (LSD) at 0.05 probability.

3. Result and discussion

The effects of chromium were examined on barley var. RD 2552 and its results have been assessed for seed germination and physiological characteristics such as germination percentage, number of lateral roots, length, and biomass of seedlings.

3.1 Effect of chromium on seed germination

The results of the present study indicated in Table 1 and found that Cr showed deleterious effects on seed germination at all levels. The maximum value of germination percentage (100%) was at the control, and the minimum GP value (86.67%) was found at 100 µM concentrations of chromium. The speed of seed germination was also affected by the chromium treatments. It was the maximum in the control-treated plant and minimum under 50 µM chromium level (Table 1). But the effect of chromium on germination percentage and speed of germination index was not significant.

Figure 1 shows that the % DFC for germination increased with chromium levels increased. It was 5%, 8.33%, 11.66%, and 13.33% under 25, 50, 75, and 100 µM levels of Cr treatment, respectively. Various studies show that the sprouting of plants is inhibited by chromium stress as, Hou *et al.* (2014) [22] studied on 5 different crop plants; Sharma *et al.* (2016) [23] studied on rice; Lei *et al.* (2021) [24] studied on wheat.

3.2 Number of lateral roots / plant

Lateral roots increase the amount of soil reached by roots, provide the anchor, and participate in the absorption and transport of water and nutrients. Data shown in Table 1; the numbers of lateral roots were decreased by various chromium levels significantly. At all Cr levels 25, 50, 75, and 100 µM, it declined 8.53%, 17.76%, 26.74%, and 32.55% respectively, compared with the control. Many studies indicates that the number of lateral roots were decreased by chromium such as, Zeid *et al.* (2001) [25] in *Pharsalus vulgaris*, Mallick *et al.* (2010) [26] reported in *Zea mays*, and Lopez-Bucio *et al.* (2015) [27] seen in *Arabidopsis thaliana*.

3.3 Length of seedlings

As shown in Table 2, root length was reduced by all chromium treatments. It was significantly elevated at 75 and 100 µM and negligible at chromium levels of 25 and 50 µM. The root length was reduced by 4.65%, 10.9%, 17.72%, and 22.25% at 25, 50, 75, and 100 µM treatments respectively, relative to the control. It is known as the percentage of phytotoxicity in the root (Figure 3a).

The shoot length was diminished by all doses of chromium (Table 2). A significant reduction was seen at the 50, 75, and 100 µM levels, and the % reduction was 10.05%, 15.48%, and 22.12% respectively. But at low treatment (25 µM), the reduction was 1.18%, which was not significant in comparison with the control. It is called the percentage of phytotoxicity in the shoot (Figure 3a).

Chromium was toxic for the total seedling length (Table 2). The total seeding length shows an insignificant reduction to less treatment (25 µM) with a % reduction of 2.45%. It was significant at 50, 75, and 100 µM treatments with 10.34%, 16.27%, and 22.21% respectively, compared to the control. Similar results were recorded in previous studies by various researchers, Akinci & Akinci (2010) [28] measured in melon, Handa *et al.* (2018) [29] examined *Brassica juncea*, Anuradha and Tejasvi (2022) [11] investigated on barley, and reported that the seedling length reduced by chromium.

3.4 Weight of seedling (fresh and Dry)

According to the findings listed in Table 3a, all amounts of chromium significantly reduced the fresh weight of the seedlings' root, shoot, and total weight. The root fresh weight decreased by 8.2% at 25 µM, 18.3% at 50 µM, 27.3% at 75 µM, and 35.2% at 100 µM treatments versus control. At 25, 50, 75, and 100 µM Cr treatments, the reduction in shoot fresh weight was 3.17%, 6.52%, 10.22%, and 12.75% lower than the control, respectively. Under 25, 50, 75, and 100 µM Cr concentrations, respectively the fresh weight of total seedlings decreased by 3.97%, 8.39%, 12.94%, and 16.28% above the control.

According to the results shown in Table 3b, all chromium concentrations caused negative impacts on the seedling's dry weight and significantly decreased the dry weight of the shoot and total seedlings. The root dry weight declined significantly, at 50, 75, and 100 µM treatments, the reduction was 9.07%, 16.08%, and 21.6% respectively, and insignificantly at 25 µM, reduction was 3.37%, compared to the control. At 25, 50, 75, and 100 µM treatments, the decrease in shoot dry weight was 7.2%, 11.94%, 16.71%, and 19.41%, respectively as compared to control. At treatments of 25, 50, 75, and 100 µM chromium, the dry weight of total seedlings decreased by 6.3%, 11.26%, 16.57%, and 19.95% in comparison to control-treated plants. Our results show similarities with the given studies; Ashfaque *et al.* (2017) [30] worked on mustard, Joshi *et al.* (2019) [31] investigated some Indian crops, Anuradha and Tejasvi (2022) [11] researched on barley and recorded the plants' biomass was reduced by various Cr levels.

3.5 Seedling vigor index (SVI)

The chromium treatments harmed the seedling vigor index. The seedling vigor index (cm) decreased significantly at higher treatments (50, 75, and 100 µM) but not at the lowest treatment (25 µM). The greatest SVI (cm) Value (1951) was recorded under control treatment, and the lowest SVI (cm) value (1317.93) with 100 µM Cr dosage, as shown in Figure 2a.

The seedling vigor index (mg) dramatically decreased at all chromium treatments. The maximum SVI (mg) was 4451, discovered under control, while the lowest value was 3087.77, recorded at a 100 µM Cr treatment (Figure 2b). Our findings are consistent with Amin *et al.* (2013) [32], Amin *et al.* (2014) [33], and Murtaza *et al.* (2017) [34], they discovered that chromium lowered the seedling vigor index.

3.6 Tolerance index (TI)

The root and shoot TI values decreased considerably under 75 and 100 µM Cr treatments, but the reduction was not significant at lower treatments (25 and 50 µM). The highest root TI value was 92.41 for the 25 µM and 50 µM treatments. Under a 100 µM Cr level, the lowest value was 78.65 (Figure 3b).

According to Figure 3b, at a 25 µM treatment, the highest shoot TI value was 96.99, whereas the 100 µM treatment recorded the lowest value (79.39). Several researchers, including Amin *et al.* (2013) [32], Amin *et al.* 2014 [33], Murtaza *et al.* (2017) [34], and Ashfaque *et al.* (2017) [30] found that higher chromium treatments considerably decreased the tolerance index compared to lower treatments that dropped insignificantly.

Table 1: Effects of various Cr treatments on the germination percentage, speed of germination index (SGI) and number of lateral roots of barley

Chromium treatments (µM)	Germination percentage	Speed of germination index (SGI)	Number of lateral roots / plant
0	100.00±0	1386.66	7.44±0.16
25	95.00±2.88^{ns}	1283.33^{ns}	6.77±0.16^{a}
50	91.66±6.00^{ns}	963.33^{ns}	6.11±0.06^{ab}
75	88.33±6.00^{ns}	1116.66^{ns}	5.44±0.06^{abc}
100	86.66±3.33^{ns}	1106.66^{ns}	5.0±0.11^{abc}

Data are mean of 3 replicates, mean± SE (standard error); "ns" for non-significant at $p<0.05$; "a" to be significant at $p<0.05$ and compare with the control; "b" for significantly at $p<0.05$ and compare to 25 µM; "c" for significant at $p<0.05$ and compare with 50 µm of Cr treatments.

Table 2: Effects of various Cr treatments on root length, shoot length, and total length of seedlings (cm) of barley.

Chromium treatments (µM)	Root Length (cm)	Shoot Length (cm)	Total length of seedlings (cm)
0	7.42±0.17	12.08±0.09	19.50±0.26
25	7.08±0.26^{ns}	11.94±0.05^{ns}	19.02±0.29^{ns}
50	6.61±0.16^{ns}	10.86±0.07^{ab}	17.47±0.10^{ab}
75	6.11±0.22^{a}	10.21±0.12^{abc}	16.32±0.09^{ab}
100	5.75±0.17^{ab}	9.41±0.16^{abc}	15.16 0.32^{abc}

Data are mean of 3 replicates, mean± SE (standard error); "ns" for non-significant at $p<0.05$; "a" to be significant at $p<0.05$ and compare with the control; "b" for significantly at $p<0.05$ and compare to 25 µM; "c" for significant at $p<0.05$ and compare with 50 µm of Cr treatments.

Table 3a: Effects of various Cr treatments on root fresh weight, shoot fresh weight, and total fresh weight (mg) of seedlings of barley

Chromium treatments (µM)	Root fresh weight (mg)	Shoot fresh weight (mg)	Fresh weight of total seedlings (mg)
0	48.44±1.03	258.66±0.19	307.11±0.97
25	44.44±0.73^{a}	250.44±1.45^{a}	294.88±1.03^{a}
50	39.55±1.03^{ab}	241.77±0.80^{ab}	281.33±1.82^{ab}
75	35.11±0.57^{abc}	232.22±0.86^{abc}	267.33±0.29^{abc}
100	31.44±1.23^{abc}	225.66±0.90^{abc}	257.11±1.61^{abc}

Data are mean of 3 replicates, mean± SE (standard error); "ns" for non-significant at $p<0.05$; "a" to be significant at $p<0.05$ and compare with the control; "b" for significantly at $p<0.05$ and compare to 25 µM; "c" for significant at $p<0.05$ and compare with 50 µm of Cr treatments.

Table 3b: Effects of various Cr treatments on root dry weight, shoot dry weight, and total dry weight (mg) of seedlings of barley.

Chromium treatments (µM)	Root dry weight (mg)	Shoot dry weight (mg)	Dry weight of total seedlings (mg)
0	10.67±0.13	33.83±0.16	44.51±0.04
25	10.31±0.05^{ns}	31.4±0.44^{a}	41.71±0.39^{a}
50	9.7±0.06^{ab}	29.8±0.45^{ab}	39.5±0.40^{ab}
75	8.95±0.12^{abc}	28.17±0.19^{abc}	37.13±0.16^{abc}
100	8.36±0.06^{abc}	27.26±0.34^{abc}	35.63±0.29^{abc}

Data are mean of 3 replicates, mean± SE (standard error); "ns" for non-significant at $p<0.05$; "a" to be significant at $p<0.05$ and compare with the control; "b" for significantly at $p<0.05$ and compare to 25 µM; "c" for significant at $p<0.05$ and compare with 50 µm of Cr treatments.

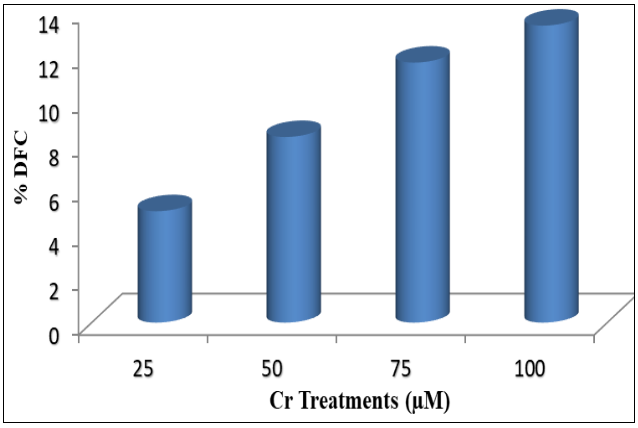

Fig 1: Impacts of different Chromium treatments on % difference from control for germination of *Hordeum vulgare* L. var. RD 2552

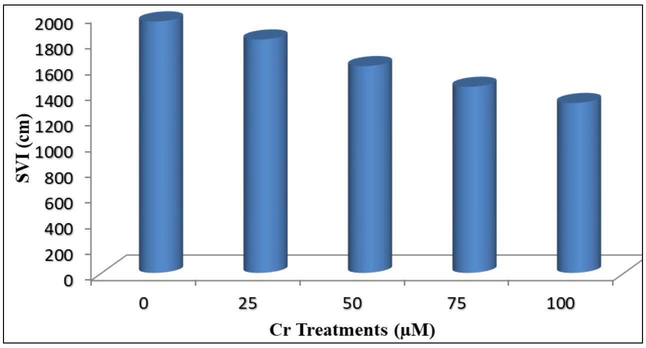

Fig 2a: Impacts of different Chromium treatments on seedling vigor index (cm) of *Hordeum vulgare* L. var. RD 2552.

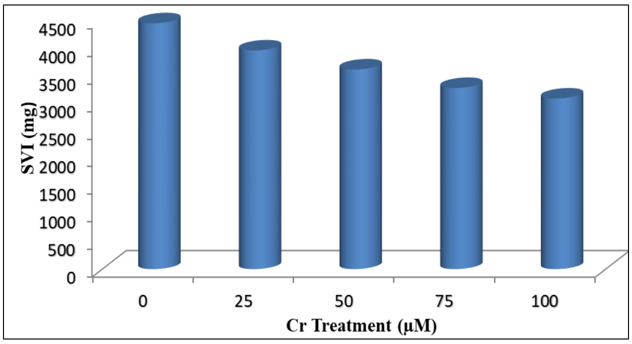

Fig 2b: Impacts of different Chromium treatments on seedling vigor index (mg) of *Hordeum vulgare* L. var. RD 2552

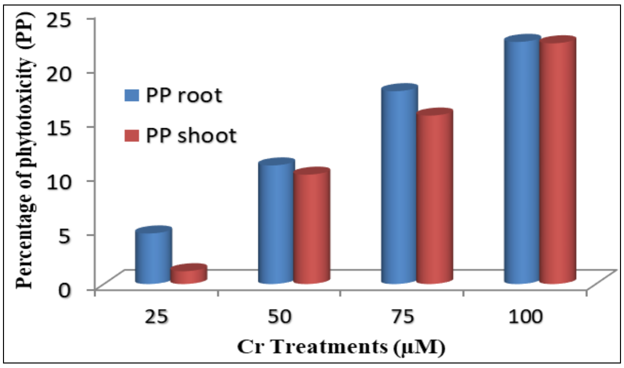

Fig 3a: Impacts of different Chromium treatments on percentage of phytotoxicity (PP) of *Hordeum vulgare* L. var. RD 2552

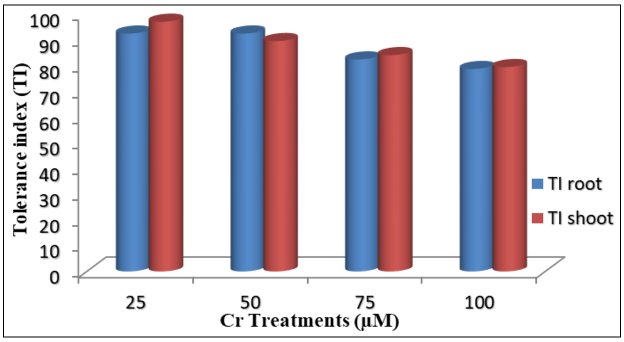

Fig 3b: Impacts of different Chromium treatments on tolerance index (TI) of Hordeum vulgare L. var. RD 2552

Conclusion

The study's findings demonstrated that chromium hurt the germination percentage and seedling growth of barley (*Hordeum vulgare*). The germination percentage, speed of germination index (SGI), seedling vigor index (SVI), and tolerance index (TI) were negatively affected by all measured concentrations of this heavy metal (Cr; 25, 50, 75, and 100 µM). When Cr was used to treat the roots and shoots, their growth, biomass, and number of lateral roots were severely hampered. This study may provide more protection guidelines for crop plants for Cr in soils and water and also helps to prepare Cr-tolerant varieties of barley.

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