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Comparative physiological and agronomic evaluation of barley genotypes under different levels of chromium stress

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ABSTRACT

Chromium (Cr) is one of the most toxic metals, posing serious threats to crop productivity, environmental quality, and human health. Cultivars differ in their ability to tolerate heavy metals. Therefore, this study was performed to evaluate the response of different barley cultivars against Cr stress. The study contained different barley cultivars: Sultan-17, Haider-93, Jau-17, Pearl-21, and Jau-21 and Cr stress levels 20 and 40 mg kg^{-1} soil. The findings demonstrate a significant decline in barley growth, physiological, biochemical, yield and ionic responses under chromium stress. Among varieties, Haider-93 showed maximum decrease in plant height (33.1%), shoot fresh and dry weight (62.2%, 53.4%), root fresh and dry weight (53.6%, 57.7%) under Cr stress, while Sultan-17 exhibited comparatively lower reduction in these parameters: plant height (19.8%), shoot fresh and dry weight (26.1%, 22.4%), root fresh and dry weight (26.4%, 21.9%) under Cr stress. Chromium stress also negatively affected physiological traits, with Haider-93 showing a significant decline in relative water content (47.9%) and chlorophyll a (55.3%), while Sultan-17 had lower reductions (21.8% and 18.5%, respectively) in under 40 mg kg^{-1} Cr stress. Sultan-17 exhibited a stronger antioxidant response, with a 23.1% increase in ascorbic acid content, compared to 17.7% in Haider-93. Moreover, Haider-93 showed higher levels of H_2O_2 and MDA production as well as yield reduction compared to Sultan-17. The results suggest that Sultan-17 showed the highest tolerance to Cr toxicity. These findings provide insights to develop tolerant crops for Cr contaminated soils.

Keywords: antioxidants, barley chromium, ionic homeostasis, stress tolerance, yield.

INTRODUCTION

Environmental pollution has become one of the most significant issues posing serious challenges to plants and human health threats (Maqsood et al., 2022). Heavy metals, e.g., chromium, mercury, lead, and copper have become a serious challenge owing to their persistence and toxicity (Balali-Mood et al., 2021). The main sources of heavy metal pollutants include industrial emissions, farming practices, mining, and city construction. In the environment, they can exist in complex compounds, since heavy metals are thermodynamically unstable (Chaplygin et al., 2024). With time, they become more challenging to clean up, they contaminate soil, water, and the human food chain, as well as threaten both human and ecological wellbeing (Terron-Camero et al., 2019; Mohammadi et al., 2022). Chromium is a toxic pollutant (Gill et al., 2016) that enters soil and water bodies related to tannery and electroplating processes (Srivastava et al., 2021). The agricultural land has a bearable amount of Cr in the soil. In addition to this level, it causes toxicity to both plants and animals (Ullah et al., 2023). Due to its solubility, Cr (VI) is believed to be a hazardous ion capable of marching through the food chain (Zia-ul-Haq et al., 2024). The chromium from soils enters into human through contaminated foods, thereby cause carcinogenic hazards (Li et al., 2020). It negatively affects plant growth and functioning (Ali et al., 2011; Saleem et al., 2022; Hassan et al., 2024; Hassan and Su, 2026). The excessive accumulation of Cr causes oxidative stress, membrane damage, and reduces nutrient uptake as well as enzyme activity involved in photosynthesis and nitrogen assimilation (Kumar and Seth, 2022).

$HCrO_4$ and $CrO_4^{2-}$are the most prevalent Cr compounds found in the soil, are readily taken up by plants, and pollute the soil (Sharma et al., 2022). Plant take up Cr from their roots, and some of it is moved to the above-ground portions, which negatively affect growth and development (Ao et al., 2022). Excessive Cr concentrations cause root cell wilt and plasmid wall detachment as well as trigger an increased rate of chromosomal abnormality in root tip cells, resulting in suppressed root cell division and differentiation, reduced volume, and the number of root cells (Saud et al., 2022). Moreover, the toxicity of Cr was also demonstrated in stems and leaves of plants, which may cause toxic effects by disrupting plant normal functioning, including nutrient uptake as well as photosynthesis, leading to ROS, membrane damage, alteration of antioxidant synthesis, the decreasing photosynthetic pigments, plant biomass, and protein concentrations (Hamzah et al., 2025). The stress caused by chromium decreased the leaf area that further lowers the photosynthesis and production of assimilates (Dey et al., 2023). The Cr toxicity also triggers leaf rolling, leaf wilting, and chlorosis that subsequently decrease the crop growth and productivity (Nikolaou et al., 2022).

Barley (*Hordeum vulgare* L.) is the fourth most produced cereal globally. Barley is one of the early domesticated crops, historically speaking (Geng et al., 2021). Today, it is consumed in food, animal feed, brewing, distillation, forage, and most other industrial applications (Bouhraoua et al., 2024). As a nutritionally rich, stress-resilient cereal crop, barley has the potential to grow under stressed soil conditions, but its response to Cr stress has not been explored. The cultivars have variable responses to heavy metal toxicity. Thus, it was hypothesized that the tolerant cultivars will display superior performances through physiological, biochemical, and ionic regulation, as compared to sensitive cultivars. Therefore, this study was performed with the following objective: to examine the morphological, physiological, antioxidant, yield, and ionic responses of different barley genotypes to Cr stress.

MATERIALS AND METHODS

Experimental site

This experiment was run at the wire house of the Agronomy Farm, University of Agriculture, Faisalabad, during November-April 2023–2024. The experimental site has a maximum temperature of up to 28 °C and a mean minimum 25 °C. The plastic pots (length: 46 cm, diameter: 25 cm) were filled with a mixture of soil and silt (3: 1). A total of 45 pots were used to carry out this experiment, and each pot contained 9 kg of soil. The plastic pots were filled with soil, which was taken from the agronomy field. A sub-sample of soil was taken for its physicochemical analysis. Soil was loamy with alkaline pH (7.35), organic matter (0.88%), nitrogen (N: 0.041%), phosphorus (P) 12.68, and potassium (K) 164 mg kg^{-1}, and chromium 0.002 mg kg^{-1}. The experimental design included two Cr levels; (20 and 40 mg kg^{-1}) and five barley varieties (Sultan-17, Haider-93, Jau-17, Pearl-21, and Jau-21). Barley seeds were obtained from Ayub Agriculture Research Institute and sterilized by seventy percent ethanol to prevent impurity and subsequently rinsed using distilled water to eliminate any residues. The study was done in a completely randomized design (CRD) with three replicates in a factorial layout.

The source of chromium was potassium dichromate ($K_2Cr_2O_7$) to achieve various levels of Cr stress. The potassium dichromate was poured into the soil and homogenized. Then it was allowed to stand for one day, during which the chromium salt was completely absorbed in the soil. Barley seeds were planted on the first week of November 2023 and 12 seeds were planted in every pot with a depth of 2 cm. The pots were irrigated on basis of the crop requirement. Recommended doses of nitrogen, phosphorus and potash (25 kg $acre^{-1}$, 12 kg $acre^{-1}$, 6 kg $acre^{-1}$) were used. In the case of these nutrients; Single super phosphate (SSP) (0.74 g pot^{-1}), Urea (0.54 g pot^{-1}) and Muriate of potash (MOP) (10 g pot^{-1}) fertilizers were applied. The experiment contained different varieties of barley: Sultan-17, Haider-93, Jau-17, Pearl-21 and Jau-21 and Cr stress levels: control, 20 and 40 mg kg^{-1} Cr stress.

Morphological traits

Three plants from each pot were picked out in order to measure morphological features. Plant height and leaf area were taken, and the number of leaves per plant was counted to obtain the mean values. These three plants were picked very carefully in order to detach roots and shoots. The roots were then rinsed completely, and the fresh weight of roots and shoots was noted. Such samples were then dried in an oven at 105 °C to ascertain the dry weight.

Physiological traits

The collection of fresh leaves was followed by their fresh weight (FW) in terms of grams using an electronic balance. The leaves were subsequently placed in pure water (distilled water) and left to attain full turgidity after 24 h. They were weighed again to obtain turgid weight (TW). Lastly, to determine the dry weight (DW) the samples were dried in an oven at 70 °C. Relative water content (RWC) was determined based on the works of Karrou and Maranville (1995) by following the formula: RWC (%)=FW−DR/TW−DR×100. Electrolyte leakage was assessed to measure the stability of cell membranes according to the technique provided by Blum and Ebercon (1981). Use fresh leaf (0.5 0g) and place it in distilled water, and allow to stand at room temperature for 30 minutes. The initial conductivity (EC1) should be measured using an EC meter. This was followed by heating of samples in a water bath at 90 °C for 30 min to ascertain the final conductivity (EC2). The formula is as follows to calculate electrolyte leakage (%); Electrolyte leakage (%) = $EC_1/ EC_2 \times 100$.

Photosynthetic pigments

The calculation of chlorophyll a, chlorophyll b and carotenoids was performed as recommended by Lichtenthaler (1987). Leaves (0.5 g) have been cut into fine pieces and immersed in 5 mL of 80 percent methanol solution for 24 hours. The solution was there after centrifuging at 10.000 rpm for 10 minutes, after which the absorbance of the supernatant was read at 663, 645 and 480 nm respectively.

Hydrogen peroxide and malondialdehyde content

Homogenization of 0.5 gram of leaf tissues in 5 ml of 5% trichloroacetic acid (TCA) was done and centrifuged at 10.000 rpm for fifteen minutes at 4 °C. One mL of supernatant was added to one mL of potassium iodide and 100 microliters of phosphate buffer, and absorbance was taken at 390 nm and incubated at room temperature for 30 minutes (Velikova et al. 2000). The content of MDA was calculated based on the Buege and Aust (1978) method. A 0.5 gram of leaf sample was crushed in one percent TCA, centrifuged at 15,000 revolutions per minute at a temperature of 4 °C for 15 minutes. One milliliter of supernatant was added to 1 mL of 0.5% trichloroacetic acid (TCA) and one milliliter of twenty percent thiobarbituric acid (TBA), and the mixture was incubated at 95 °C for 50 min, followed by rapid cooling. The measurements of absorbance were carried out at 532 nm and non-specific turbidity at 600 nm was corrected. The inaction coefficient of 155 mM^{-1} cm^{-1} was used to find MDA content and it was given in nmol g^{-1} FW.

Free amino acids and total soluble proteins

The amount of soluble proteins was quantitatively measured according to Bradford (1976). Five milliliters of fifty mM potassium phosphate buffer were added to the leaf tissues (0.5 g) that were ground and centrifuged at 12,000 rpm for fifteen minutes. The supernatant (100 µL) was combined with 3 ml Bradford reagent, and for 15 minutes incubate it at 25 °C, and reading was recorded at 590 nanometer. The method of estimation of free amino acids was taken according to the Hamilton and Van Slyke (1943). A quantity of 1 milliliter of extract was combined with 1 milliliter of 2 percent ninhydrin and 1 milliliter of 10 percent pyridine, then it was incubated at 90 degrees Celsius in a duration of 30 minutes and cooled at room temperature and then the absorbance was measured at 570 nano meter.

Activities of antioxidant enzymes

In the analyses of antioxidant responses, leaf materials (1 gram) were placed in 10 milliliters of 50 millimolar potassium phosphate buffer and homogenized. The resulting product was centrifuged at 14,000 rpm and at 40 °C for 30 minutes. Analysis of the enzyme was carried out on the supernatant. Catalase (CAT) readings was measured according to Chance and Maehly (1955) as follows: 0.1 milliliter of extract was combined with 0.1 milliliter H_2O_2 and 2.5 milliliter of buffer; readings was recorded at 240 nanometers. The action of peroxidase (POD) was determined by mixing 100 microliter of extract with 100 µL of 180 millimolar H_2O_2 and 100 µL of 180 millimolar guaiacol, which was combined with 700 µL of 50 mM phosphate buffer, and absorbance was measured at 470 nanometer. Asada and Takahashi's (1987) process was followed in ascorbate peroxidase (APX) activity by combining 100 mL enzyme extract with 100 mL ascorbic acid, 6.1 millimolar H_2O_2, and seven hundred milliliter buffer as well as measuring absorbance at 290 nanometer. Measurement of ascorbic acid was done as presented by Mukherjee and Choudhuri (1983). The samples of leaves (0.5 gram) were homogenized in 5 milliliters of 10 percent TCA and centrifuged at 15,000 rpm in 15 minutes. Then, 0.5 milliliter of DTC reagent was added to 2 milliliters of the performing of extraction and was incubated at a temperature of 37 °C for 3 hours. It was then cooled on ice, and 2.5mL of chilled H_2SO_4 was added drop wise and shaken for 30 min. The detection was measured at 520 nanometers.

Yield attributes

The parameters relating to yield, including the number of tillers/plant, productive spikes, and grains per spike, were obtained manually and averaged. After the harvest, in order to determine the biological yield, harvested plants were weighted then the spike of each plant was threshed out to obtain the grain yield per pot, and the weight of 1000 grains was calculated by weighing a subsample of 1000 seeds.

The accumulation of chromium in plant parts

Oven-dried samples of the plant (roots, leaves and grains) were then reduced to a fine powder and digested using 2:1 ratio of HNO_3 and $HClO_4$ as explained by Jones and Case (1990). The level of chromium obtained after digestion in various parts of the plant was discovered using atomic absorption spectrophotometry (AAS, PerkinElmer Analyst^{tm} 800). The following formula was used to determine the content of chromium in different plant parts (ug g^{-1} of dry matter): Cr concentration = (AAS reading) (dilution factor)/ sample dry weight.

Statistical analysis

The analysis of variance technique presented in STATISTIX 8.1 was used to analyze the recorded data. The significance of the treatment means was examined at 5% probability level using the least significant difference (LSD) test (Steel and Torrie, 1997).

RESULTS

Growth traits

Chromium stress (20 and 40 mg kg^{-1}) exhibited varying effects on the growth of different barley genotypes (Table 1). In the Sultan-17 plant, height was reduced by 13.6% and 19.8% in Cr1 and Cr2. The leaf area was decreased by 7.0% (Cr1) to 15.5% (Cr2) and leaves were reduced to 13.0% (Cr1) and 26.1% (Cr2). Shoot fresh weight reduced by 16.1% (Cr1), 26.1% (Cr2), and shoot dry weight by 9.5% (Cr1), 22.4% (Cr2), root fresh weight by 12.4% (Cr1), 26.4% (Cr2) and root dry weight by 7.2% (Cr1) and 21.9% (Cr2). In Haider-93, PH, LA and leaves were found to decrease by 24.1, 45.1 and 36.4 % in Cr1, respectively, whereas Cr2 resulted in further loss of 33.1, 66.1 and 54.5% in PH, LA and leaves, respectively. The fresh weight of shoots reduced by 47.9% (Cr1) and 62.2% (Cr2), and dry weight 44.9% and 53.4%, respectively. The root fresh and dry weight declined by 42.9 and 40.5% (Cr1), 53.6, and 57.7% (Cr2). SFW decreased by 24.2% (Cr1) and 38.3% (Cr2) and SDW 26.3% and 31.6% and a reduction in root FW by 14.9%, 31.0% and root DW 17.3%, 32.6% under Cr1 and Cr2, respectively (Table 1). In Jau-21, PH had decreased by 21.7% (Cr1), 30.3 % (Cr2) and LA by 34.6% and 45.8% and leaves/plant by 27.3% and 36.4%. The decrease in SFW and SDW was by 26.9% (Cr1), 45.2% (Cr2), and 27.1% (Cr1), 38.9% (Cr2). Root fresh weight was reduced by 22.3% (Cr1) and 35.8% (Cr2) and simultaneous RDW also reduced by 18.3% (Cr1, 36.8% (Cr2) (Table 1). These findings demonstrate that chromium stress adversely affects barley growth, with the magnitude of impact varying across different varieties, with Haider-93 being the most sensitive and Sultan-17 the least affected when compared to other varieties.

Table 1 contains mean value of three replications ± SE and various letters indicate difference of significance at ($P \leq 0.05$) according to HSD test. PH: Plant height, LA: Leaf area, No L/P: No of leaves per plant, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight. Cr1; 20 mg kg^{-1}, Cr2; mg kg^{-1}.

Physiological traits

Dose dependent effects of chromium stress resulted in the reduction of photosynthetic

Table 1. Effect of different chromium levels on growth of different barley varieties

Varieties	Treatments	PH (cm)	LA (cm^2)	No L/P	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Sultan-17	Control	97.66±0.72a	47.33±0.27a	7.66±0.27a	8.03±0.44a	2.1±0.081a	3.03±0.027a	1.83±0.027a
	Cr_1	84.33±0.27b	44±0.47b	6.66±0.47abc	6.73±0.21abcd	1.9±0.047ab	2.66±0.054bcd	1.7±0.047ab
	Cr_2	78.33±0.27d	40±0.47cd	5.66±0.27bcd	5.93±0.19bcde	1.63±0.054bc	2.23±0.027ef	1.43±0.027cd
Haider-93	Control	96.66±0.25a	41.33±0.54bc	7.33±0.27ab	7.03±0.47abc	1.93±0.053ab	2.8±0.047abc	1.56±0.026bc
	Cr_1	73.33±0.24f	22.66±0.72i	4.66±0.27de	3.66±0.27fg	1.06±0.098de	1.6±0.047h	0.93±0.024h
	Cr_2	64.66±0.27i	14±0.47j	3.33±0.27e	2.66±.27g	0.9±0.047e	1.3±0.047i	0.66±0.027i
Jau-17	Control	97.33±0.24a	43±0.47bc	7.33±0.72a	7.4±0.24ab	2±0.047ab	3±0.047a	1.63±0.027b
	Cr_1	81.66±0.27c	38±0.47d	6.33±0.27abcd	5.83±0.26bcde	1.7±0.081abc	2.6±0.047cd	1.36±0.037de
	Cr_2	72±0.24f	32.33±0.72e	5.33±0.27cd	5±0.23def	1.4±0.047cd	2.23±0.027ef	1.23±0.024ef
Pearl-21	Control	96.66±0.22a	42.66±0.27bc	7.21±0.27ab	7.83±0.13a	1.9±0.081ab	2.9±0.047ab	1.73±0.037ab
	Cr_1	78±0.22d	31.66±0.27ef	6±0.47abcd	5.93±0.28bcde	1.4±0.047cd	2.46±0.027de	1.43±0.027cd
	Cr_2	69.66±0.27g	27±0.47gh	5±0.47cde	4.83±0.13ef	1.3±0.047cde	2±0.047fg	1.16±0.024fg
Jau-21	Control	96.33±0.24a	44.33±0.72ab	7.30±0.27ab	7.3±0.28ab	1.96±0.072ab	2.96±0.027a	1.63±0.024b
	Cr_1	75.66±0.27e	29±0.47fg	5.33±0.42cd	5.33±0.27cdef	1.43±0.072cd	2.3±0.047e	1.33±0.024def
	Cr_2	67.33±0.27h	24±0.47hi	4.66±0.27de	4±0.47ef	1.2±0.081de	1.9±0.047g	1.03±0.027gh

Note: Mean value of three replications ± SE and various letters indicate difference of significance at ($P \leq 0.05$) according to HSD test. PH – plant height, LA – leaf area, No L/P – no of leaves per plant, SFW – shoot fresh weight, SDW – shoot dry weight, RFW – root fresh weight, RDW – root dry weight. Cr_1 – 20 mg kg^{-1}, Cr_2 – mg kg^{-1}.

pigments, relative water content (RWC), and an increment of membrane damage markers (Figure 1). Chlorophyll a decreased by 12.1 (Cr1) and 18.5 (Cr2)%, chlorophyll b by 6.8 and 13.6% and carotenoids by 5.4 and 9.2% in Sultan-17. There was a drop in RWC 10.9% (Cr1) and 21.8% (Cr2) and an increase in EL 9.0% and 17.0%. In Haider-93, the magnitude of pigment losses was worse: Chl a, 34.3% (Cr1) and 55.3% (Cr2), carotenoids, 47.9% (Cr1) and 61.9%, and RWC, 33.6% (Cr1) and 47.7%. EL rose by 32.9% (Cr1) and 43.3% (Cr2). Moderate decreases were also recorded in Jau-17; Chl a: 23.3 % (Cr1) and 29.9 % (Cr2), Chl b: 10.0% and 23.2%, carotenoids: 19.8% and 27.8, RWC: 19.2 and 23.1, EL increased by: 10.9 and 18.7%. In Pearl-21, chlorophyll a reduced by 24.5% (Cr1) and 34.9% (Cr2), carotenoids by 29.9% and 34.7%, RWC by 24.1% and 34.8% and the EL rose by12.2% and 20.1%. Jau-21 exhibited Chl a reduction of 29.7% (Cr1) and 40.0% (Cr2), Chl b 21.6% and 30.8%, and carotenoids 31.4% and 35.7% (Figure 1). The reductions in RWC were of 25.2% and 35.4%, an increase of 19.7% and 30.9% in EL. These results suggest that chromium stress significantly impacts pigment content, RWC, and membrane stability, with Haider-93 showing the most pronounced reductions and Sultan-17 the least, as compared to other varieties (Figure 1).

Antioxidant activity

In tolerant varieties, there was a high increase in antioxidant enzymes. In Sultan-17, with Cr1, there is a 32.8% rise in POD, 39.5% rise in APX, 26.3% rise in CAT and 64.7% anthocyanin (Figure 2). It also increased Cr2 by 43.7% (POD), 47.4% (APX), 33.3% (CAT) and 71.5 in anthocyanin. TSP went up with (Cr1) 87% (Cr2) 99%, free amino acids increased with (Cr1) 31.8%, (Cr2) 44.8%, and ascorbic acid 15.4% and 23.1%, respectively. The H_2O_2 levels, increased by 4.1% (Cr1) and 21.6% (Cr2), whereas the level of MDA was elevated by 28.6% and 30.3%. In Haider-93, POD rose by 8.0% (Cr1) and 24.3% (Cr2), CAT by 4.7% and 7.1%, APX by 6.1% and 7.8% and (25%, 36.4%) in anthocyanin at Cr1 and Cr2. TSP went up by 8% (Cr1) and 33% (Cr2), whereas free amino acids went up by 8.3% (Cr1) and 22.3% (Cr2). The ascorbate increased by 11.1 % and 17.7%. H_2O_2 increased by 45.0% (Cr1) and 65.5% (Cr2), and MDA increased by 65.7 and 79.9%, which revealed oxidative overload. Jau-17 was found to have excellent antioxidant responses: POD, 20.5% (Cr1) and 39.2% (Cr2) higher; APX, 35.1% and 43.4%; CAT 16.1% and 24.7% and anthocyanin (41.8%, 54.5%) at Cr1 and Cr2 (Figure 2). These findings demonstrate the variability in antioxidant responses under chromium stress across different barley varieties. The results

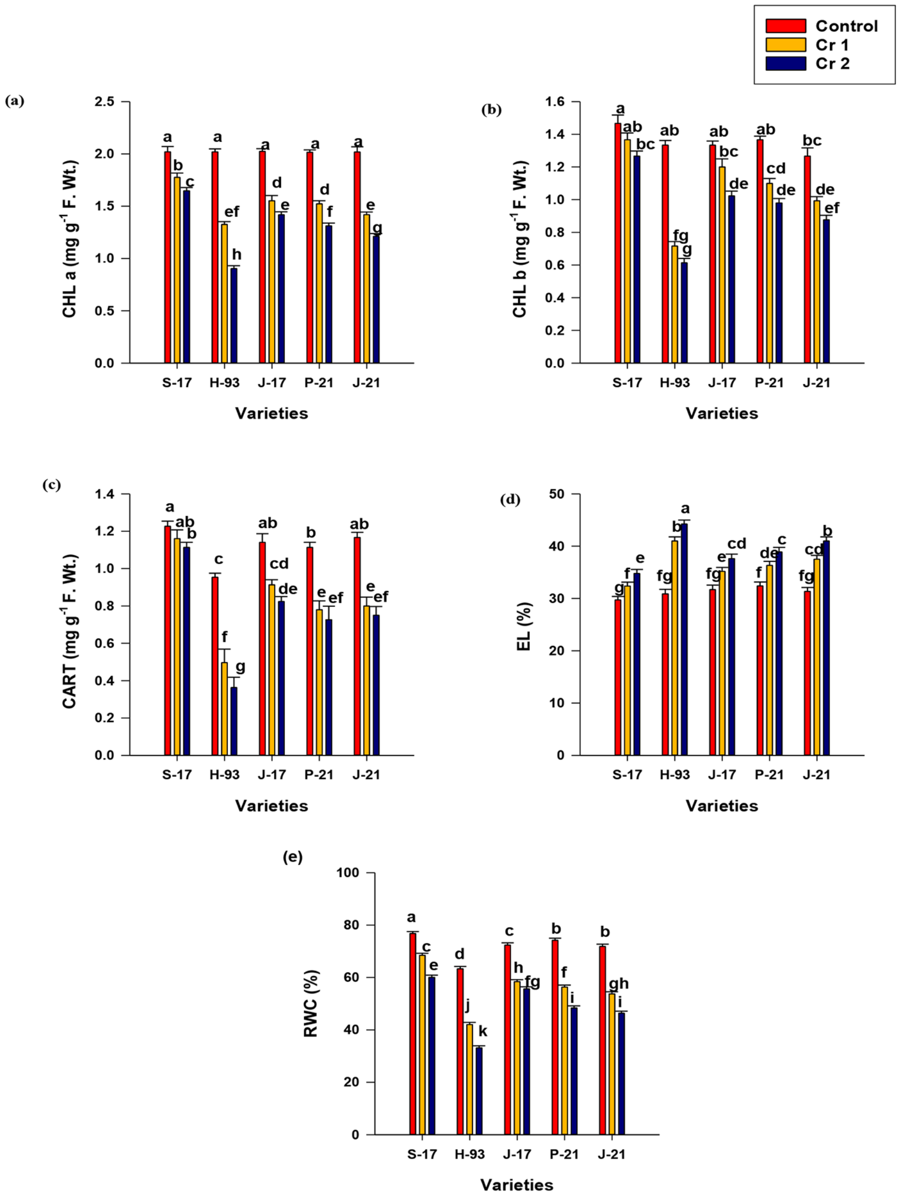

Figure 1. Effect of chromium stress on chlorophyll a (a), Chlorophyll b (b), Carotenoids (c), Electrolyte leakage (d) and relative water content (e) of different barley varieties. Where S-17: Sultan-17, H-93: Haider-93, J-17: Jau-17, P-21: Pearl-21, J-21: Jau-21, CHL: Cr_1; 20 mg kg^{-1}, Cr_2; 40 mg kg^{-1}. Same letters on bars do not indicate significant difference among means. The means of three replicates accompanied by the bars indicating the average of standard deviation of replications (3) and the various letters on the same treatment indicate the significant differences at $p < 0.05$

also showed that Cr stress decreased the TSP, FAA and ascorbic acid contents with cultivar Haider-93 showed maximum reduction (Figure 3).

Yield traits

Chromium stress significantly affected the yield of different barley varieties, including Sultan-17, Haider-93, Jau-17, Pearl-21, and Jau-21. Sultan-17 performed better than other varieties (Table 2). In cultivar Sultan-17, tillers/plant were decreased by 14.3% (Cr1), 21.4% (Cr2), grain per spike by 12.5%, 21.9%, productive tillers by 8.33%, 16.66%, 1000-grain weight by 10.3, 17.8%, grain yield by 19.2, 28.4, and biological yield by 5.8 and 9.9%. Moreover, tillers (36.4 and 54.5%), grains/spike (27.8 and 39.3 %), productive tillers (54%, 63.6%), 1000-GW (50 and 54.3%), grain yield (36.9 and 51.6%), and biological yield (43.8 and 63.4%) were significantly decreased under lower and higher levels of Cr stress (Table 2). The moderate reductions observed in Pearl-21 were faced with more severe

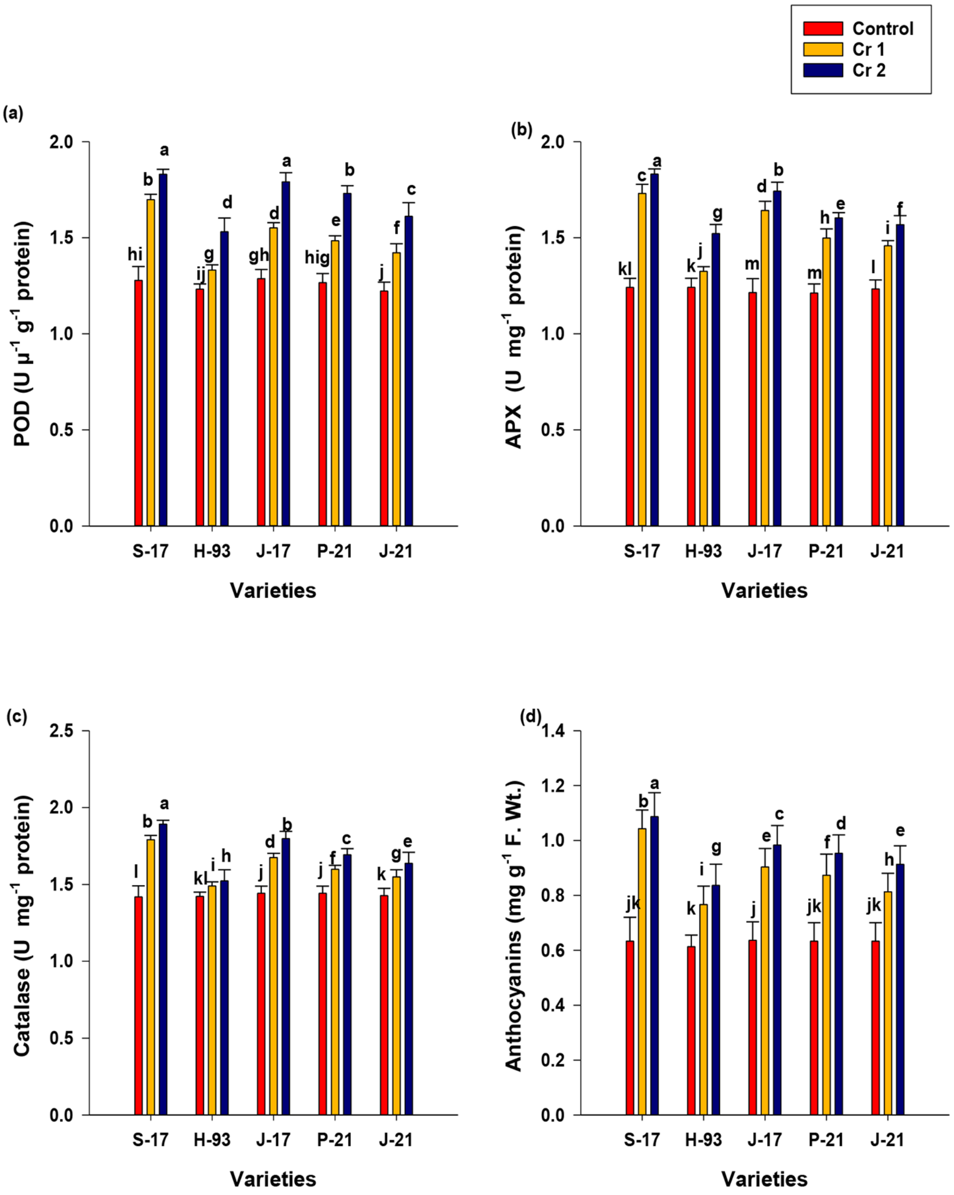

Figure 2. Effect of chromium stress on peroxidase (a), ascorbate per oxidase (b), catalase (c), and anthocyanins (d), of different barley varieties. Where S-17: Sultan-17, H-93: Haider-93, J-17: Jau-17, P-21: Pearl-21, J-21: Jau-21, CHL: Cr_1; 20 mg kg^{-1}, Cr_2; 40 mg kg^{-1}. Same letters on bars do not indicate significant difference among means. The means of three replicates accompanied by the bars indicating the average of standard deviation of replications (3) and the various letters on the same treatment above the bars indicate the significant differences at $p < 0.05$

reductions (Table 2). These findings underscore the negative effects of chromium stress on barley yield, with Haider-93 and Jau-21 showing the most significant reductions in yield-related parameters, while Sultan-17 exhibited the least when results are compared with other varieties (Table 2).

Ionic traits

The severity of stress was accompanied by the accumulation of chromium in tissues. In Sultan-17, maximum Cr in leaf rose by 4 times, while in grain and roots it increase by 5.2 times, and 5 times in Cr2 compared to their controls (Figure 4). In Haider-93, maximum Cr in leaves increased by 7.9 times, in grain by 8 times, and by 7.5 times in the root in Cr2, as compared to their respective controls (Figure 4). These results highlight that Haider-93 showed the highest chromium accumulation across all plant parts, while Sultan-17 exhibited the lowest levels of chromium (Figure 4).

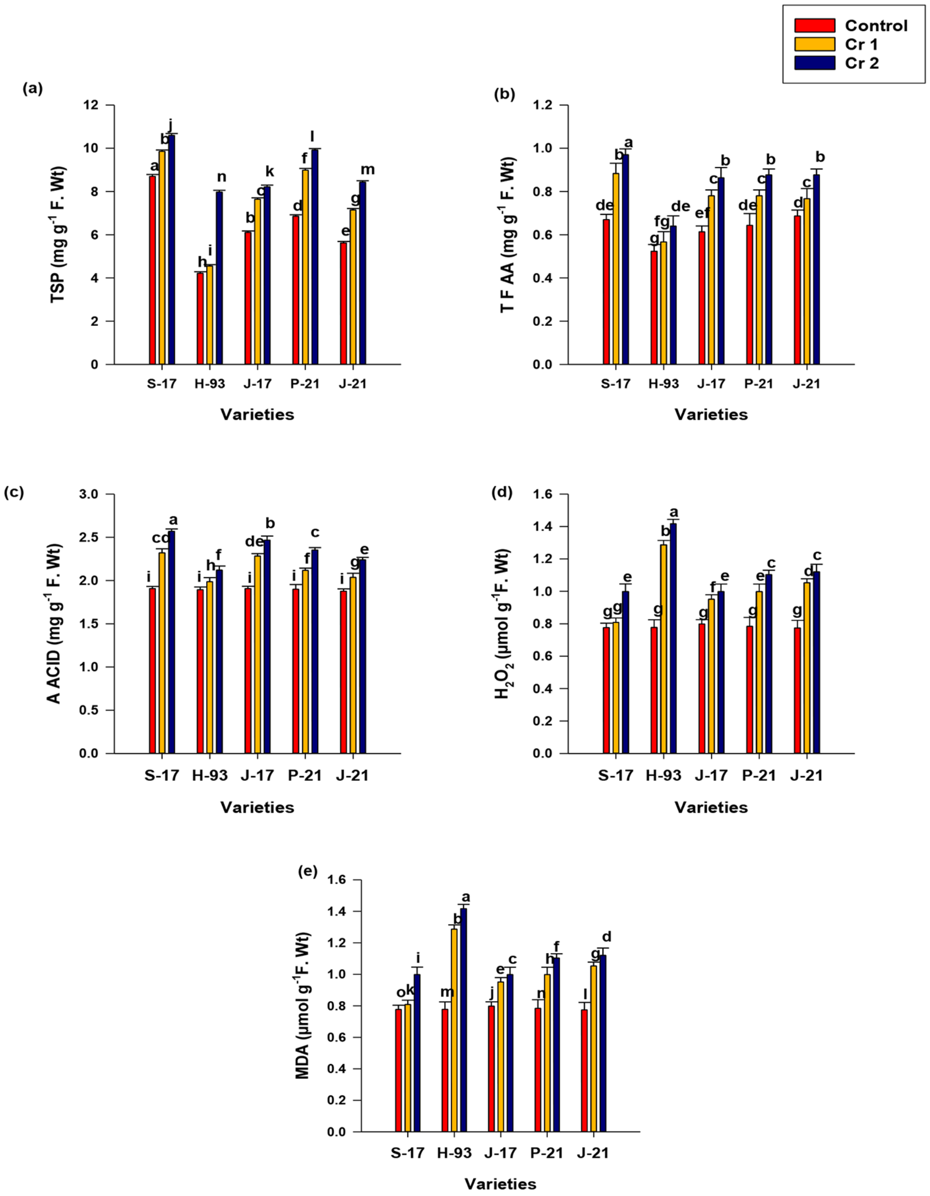

Figure 3. Effect of chromium stress on total soluble proteins (a), Total free amino acids (b), Ascorbic acid (c), Hydrogen peroxide (d) and Malondialdehyde (e) of different barley varieties. Where S-17: Sultan-17, H-93: Haider-93, J-17: Jau-17, P-21: Pearl-21, J-21: Jau-21, CHL: Cr1; 20 mg kg^{-1}, Cr2; 40 mg kg^{-1}. Same letters on bars do not indicate significant difference among means. The means of three replicates accompanied by the bars indicating the average of standard deviation of replications (3) and the various letters on the same treatment above the bars indicate the significant differences at $p < 0.05$

Heatmap with dendrogram

The scaling of the colors to the right of the heatmap depicts the strength of the relationship between the traits and crop varieties, which goes between -2 (dark blue) and 2 (dark red), with 0 as a neutral or something closer to the baseline (Figure 5). The visualization of the data in this color scale is easy, and the darker the shade of red, the greater the positive correlation, while dark blue shads indicate the negative correlations (Figure 5). Notably, Cr1V2 and Cr2V2 show a positive correlation with a number of characteristics, such as GY, PH, and grains per spike. On the other hand, Cr0V2 and Cr0V1 have a negative relation to most of the traits, such as SDW, CHL.a, and CARO, among others, which is indicated by the blue point. This is an indication that such treatments do not have a negative impact in these parameters (Figure 5).

Table 2 contains the mean value of three replications ± SE and various letters indicate

Table 2. Effect of different chromium levels on yield of different barley varieties

Varieties	Treatments	No T/P	No G/S	PT/P	B Yield (g)	G Y/P (g)	1000-G W (g)
Sultan-17	Control	4.66±0.27a	21.33±0.24a	4±0.47a	44.06±0.05a	23.07±0.49a	39.48±0.30a
	Cr_1	4±0.27ab	18.66±0.27bc	3.66±0.27a	39.17±0.07cd	18.65±0.42b	35.41±0.23b
	Cr_2	3.66±0.26abc	16.66±0.27d	3.33±0.27ab	34.13±0.06f	16.53±0.27cd	32.44±0.27c
Haider-93	Control	3.66±0.47abc	20.33±0.23ab	3.66±0.26a	37.16±0.07e	23.74±0.25a	37.43±0.24ab
	Cr_1	2.33±0.27bc	14.66±0.47efg	1.66±0.24bc	20.54±0.17j	14.98±0.27def	18.67±0.25e
	Cr_2	1.66±0.24c	12.33±0.47h	1.33±0.27c	18.47±0.30k	11.48±0.21g	17.08±0.03e
Jau-17	Control	4.33±0.2 ab	20.66±0.26a	3.66±0.27a	40.14±0.07bc	23.37±0.30a	38.81±0.62a
	Cr_1	3.66±0.23abc	17.33±0.27cd	3.33±0.26ab	33.43±0.23f	17.43±0.32bc	32.73±0.30c
	Cr_2	3.33±0.27abc	15.66±0.27def	2.66±0.24abc	29.07±0.07h	15.8±0.29cde	28.37±0.34d
Pearl-21	Control	4.66±0.25a	21±0.24a	3.66±0.26a	41.06±0.09b	23.43±0.23a	38.11±0.55a
	Cr_1	3.33±0.27abc	16.33±0.23de	3±0.27abc	33.77±0.01f	16.4±0.24cd	31.71±0.29c
	Cr_2	3±0.26abc	14.33±0.54fg	2.33±0.26abc	29.06±0.07h	14.73±0.25def	27.37±0.30d
Jau-21	Control	4.33±0.27ab	20.66±0.0.27a	3.33±0.27ab	39.06±0.03d	22.66±0.27a	38.39±0.52a
	Cr_1	3±0.54abc	15.66±0.23def	2.66±0.24abc	31.77±0.36g	14.33±0.27ef	31.43±0.33c
	Cr_2	2.33±0.47bc	13.66±0.24gh	1.66±0.26g	27.10±0.05i	13.33±0.26fg	27.10±0.05d

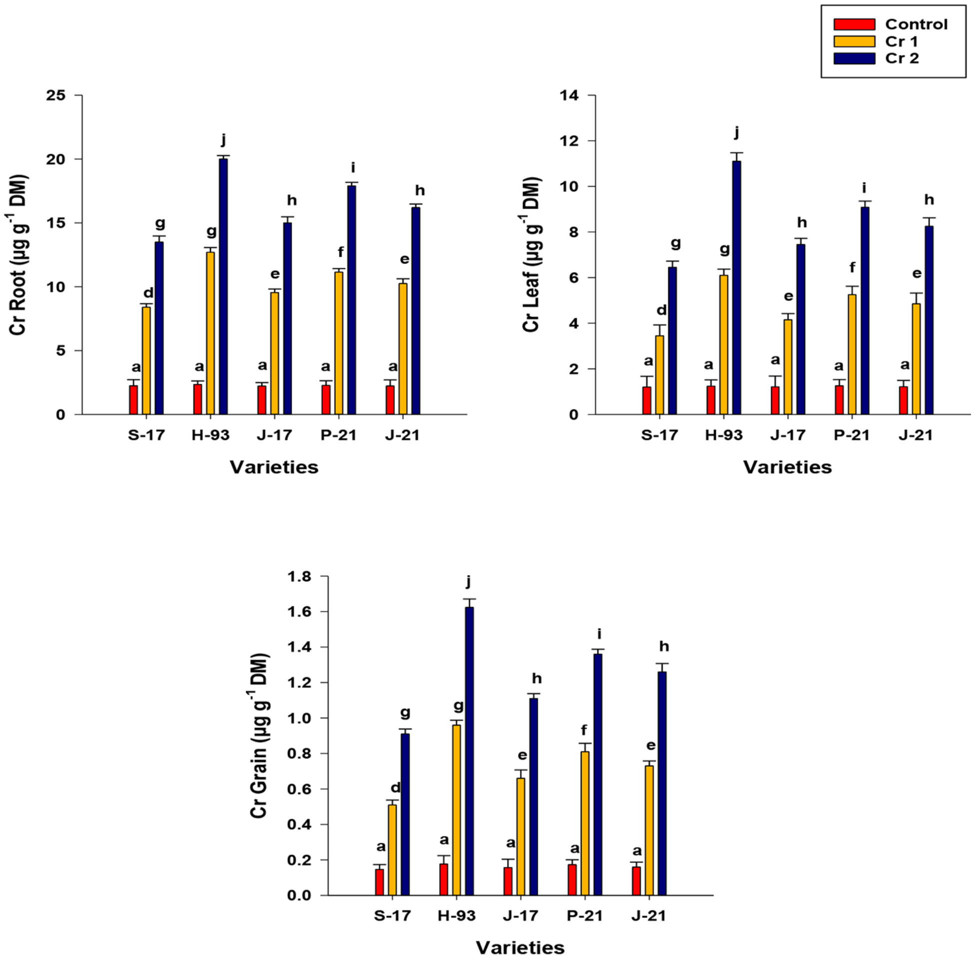

Figure 4. Accumulation of Cr content in different parts (root, leaf, grain) of different barley varieties. Where: S-17: Sultan-17, H-93: Haider-93, J-17: Jau-17, P-21: Pearl-21, J-21: Jau-21, Cr: Cr_1; 20 mg kg^{-1}, Cr_2; 40 mg kg^{-1}. Same letters on bars do not indicate significant difference among means. The means of three replicates accompanied by bars are indicating the mean standard deviation of three replicates and various letters on the same treatment above the bars indicate the significant differences at $p < 0.05$

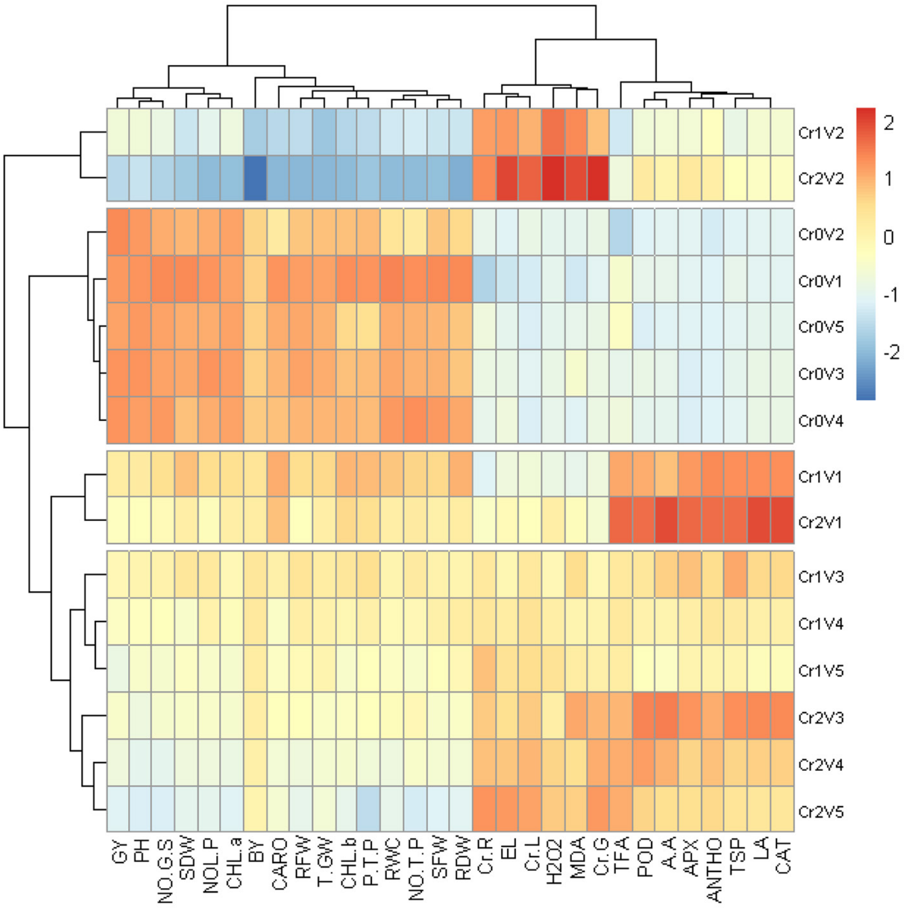

Figure 5. Heatmap with dendrogram between morpho-physiological, biochemical, yield and ionic parameters and treatments for different barley varieties under chromium stress. V1: Sultan-17, V2: Haider-93, V3: Jau-17, V4: Pearl-21, V5: Jau-21, Cr_1; 20 mg kg^{-1}, Cr_2; 40 mg kg^{-1}, PH; plant height, LA; leaf area, NOL/P; number of leaves per plant, SFW; shoot fresh weight, SDW; shoot dry weight, RFW; root fresh weight, RDW; root dry weight, CHL a; chlorophyll a, CHL b; chlorophyll b, CARO; carotenoids, RWC; relative water content, EL; electrolyte leakage, POD; peroxidase activity, APX; ascorbate peroxidase activity, CAT; catalase activity, ANTHO; anthocyanins, TSP; total soluble proteins, TFA; total free amino acids, A A; ascorbic acid, H_2O_2; hydrogen peroxide, MDA; malondialdehyde, NO T/P; number of tillers per plant, NO G/S; number of grains per spike, P T/P; productive tillers per plant, T GW; thousand grain weight, GY; grain yield per plant, BY; biological yield per plant, Cr L; chromium in leaves, Cr G; chromium in grains, Cr R; chromium in roots

difference of significance at ($P \leq 0.05$) according to HSD test. No T/P: No of tillers per plant, No G/S: No grains/spike, PT/P: Productive tillers/plant, B Yield: biological yield, G Y/P: Grain yield/pot, 1000-G W: 1000 grain weight. Cr1; 20 mg kg^{-1}, Cr2; 40 mg kg^{-1}. Also, Cr1V1 and Cr2V1 have a high positive relationship with characters such as TGW, CHL.b, and P.TP as shown by the dark red color. It shows that these parameters also effected by chromium stress. On the basis of the heatmap, the complete overview of the relations between particular treatments and their traits, the researchers will be able to select promising varieties with their desired traits and solve the problem in using a strategy of selective breeding to increase the yields and the quality of barley crop under stressful conditions.

Principal component analysis (PCA)

The first principle component (PC1) contributes 73.5% and the second principle component (PC2) gives 22.6% of the variance. A total of 96.1% is explained by the two components, which indicates that they take most information in the dataset (Figure 6). The variables are grouped into clear clusters whereby some variables are in small groups, which means that those variables have strong positive correlations (Figure 6). The close links given among some of the

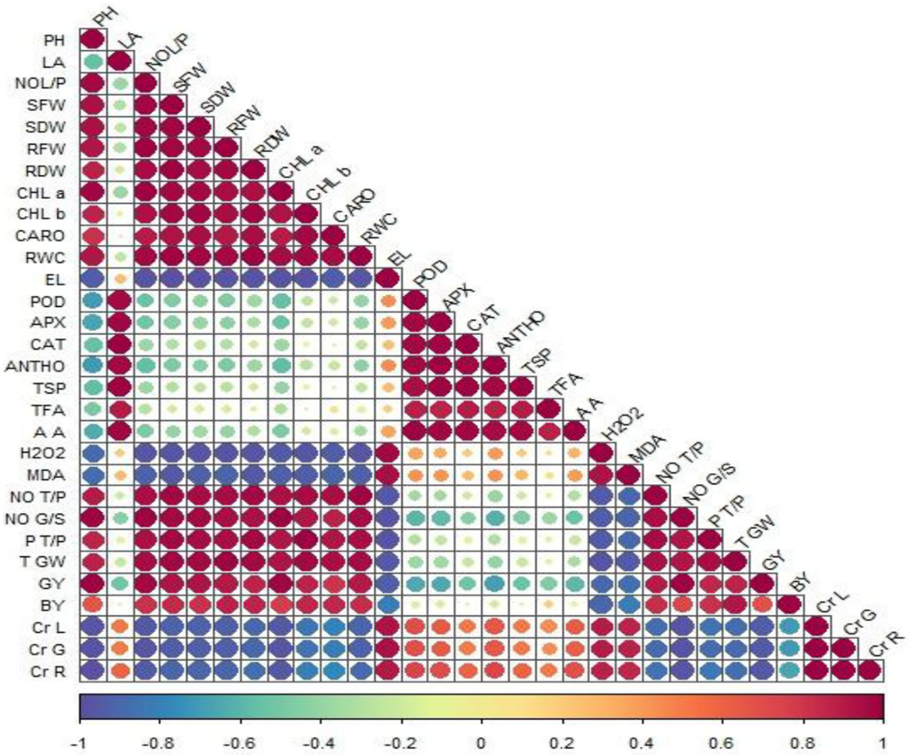

Figure 6. Pearson's correlation between morpho-physiological, biochemical, yield and ionic parameters for different barley varieties under chromium stress

variables indicate that the variables might be indicating similar constructs. The group of variables, which are associated with plant growth, shows that the variables are highly correlated and might be dependent on the same causes of events. The contributing variables will also most probably be influential factors of the patterns identified in the studied data (Figure 7).

DISCUSSION

The present study demonstrated that chromium stress significantly decreased barley growth and productivity, which aligns with the previous finding that heavy metal stress significantly reduces growth (Song et al., 2024). This study revealed that growth traits with more severe reduction was observe in Haider-93 (Table 1). Chromium stress caused a significant decrease in leaf area, which decreased the light harvesting and reduced assimilate production, contributing to a substantial decrease in plant growth (Iftikhar et al., 2025). Chromium stress also reduced root growth by inhibiting cell division as well as decreasing cell size in the elongated zone (Elkelish et al., 2024). This decrease in root growth affects limit nutrient and water uptake, ultimately suppressing overall plant development.

Chromium stress, has a significant impact on plant physiological and biochemical properties. The most obvious symptoms of Cr toxicity in cereals are chlorosis, which results in yellowing of leaves through inhibition of chlorophyll synthesis. Likewise, in this research, a significant decrease photosynthetic pigments was observed with increasing Cr concentrations which aligns with the previous study (Sallah-Ud-Din et al. 2017). Chromium stress inhibits water movement, decreases transpiration, influences the nutrient absorption, and inhibits various enzymatic processes within the plant, thereby causing a reduction in chlorophyll synthesis (Bukhari et al., 2015; Singh et al., 2021). Conversely, there were also varietal differences in the response to Cr stress. Sultan-17 showed the least reduction of pigment contents, which was attributed to its robust antioxidant defense and increased synthesis of osmolytes. Accumulation of chromium significantly disturbs the biochemical and physiological traits of the plant that ultimately leads to a decrease in productivity (Singh, 2020). The present study showed that Cr stress decreased RWC and enhanced EL, MDA, and H_2O_2 production. These findings are similar

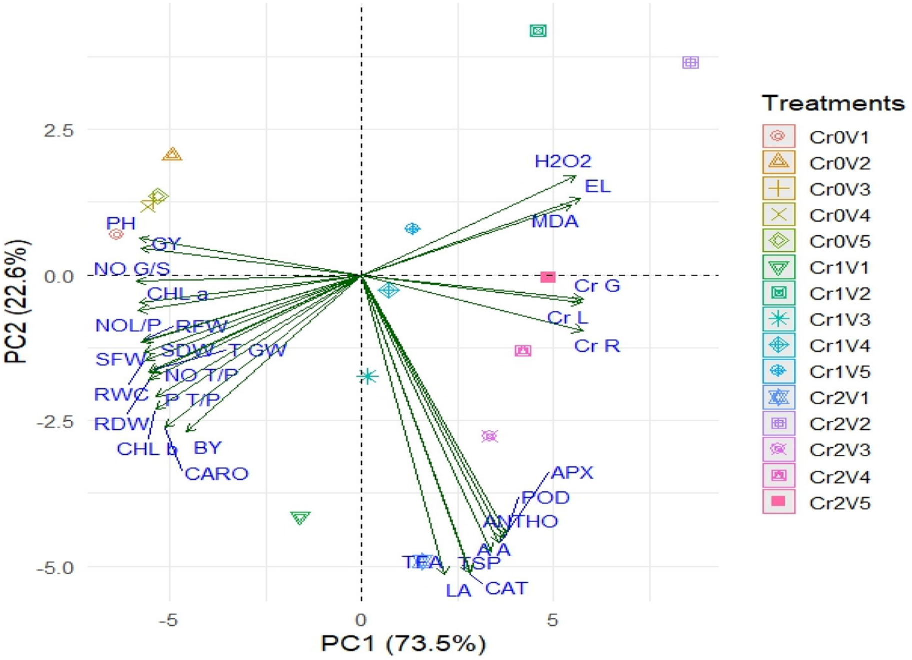

Figure 7. PCA between morpho-physiological, biochemical, yield and ionic parameters for different barley varieties under chromium stress. Where, V1: Sultan-17, V2: Haider-93, V3: Jau-17, V4: Pearl-21, V5: Jau-21, Cr1; 20 mg kg^{-1}, Cr2; 40 mg kg^{-1}

to previous reports stating Cr toxicity induces severe damage by increasing oxidative stress markers (Anjum et al., 2017: Li et al., 2018).

Cereal plants grown in the soils contaminated with heavy metals might result in smaller-sized and less-filled grains, which eventually translate to yield reduction (Aslam et al., 2021). The toxicity of heavy metals could largely affect the reproduction procedures of the cereal crops, leading to less flowering and adversely impacting the development of the fruit (Yang et al., 2022). The present findings depicted that Cr stress significantly decreased barley growth and yield. This decrease in yield was linked with a reduction in chlorophyll synthesis, RWC, and an increase in oxidative damage mediated by Cr accumulation in plant organs. The exposure to heavy metals may disrupt the production, translocation, and signaling of hormones; therefore, this could also be reasons of decrease in plant growth in the present study (Sharma et al., 2022). These findings aligns with previous reports stating that Cr stress significantly decreased growth and yield Anjum et al., 2017; Kumar et al., 2019; Singh et al., 2020).

The present findings support earlier reports that Cr accumulates in the edible parts of cereal crops, especially grains (Ohiagu, 2020; Figure 4). Heavy metals are absorbed by cereal crops from contaminated soils through a root system; however, the extent of uptake differs for various species and variety (Awino, 2022). The conducted study showed significant differences among barley varieties for Cr accumulation. Sultan-17 consistently reflected the lowest Cr accumulation, while Haider-93 reflected the highest accumulation. The uptake and distribution of Cr in plants is dependent on external Cr concentrations as well as varietal characteristics (Wakeel et al., 2020). The cultivar Haider-93 accumulated maximum Cr as compared to other cultivars, while Sultan-17 accumulated less Cr. This shows that Sultan-17 may efficiently bind the Cr to cell walls or chelate with organic acids and compartmentalize it in root vacuoles. This prevented the Cr accumulation in Sultan-17, while Haider-93 lacks these features, thus it accumulated more Cr. The other possible reasons can be the growth dilution effect. The cultivar Sultan-17 maintained better growth, which led to dilution of absorbed Cr in plant organs.

CONCLUSIONS

This study demonstrated that chromium stress significantly reduced barley growth and productivity by decreasing photosynthetic pigments and increasing stress, membrane damage, and chromium accumulation. Notably, the obtained results found that Sultan-17 was a chromium tolerant cultivar, while Haider-93 was a chromium sensitive cultivar. The higher tolerance in Sultan-17 was linked with its ability to maintain better relative water contents, chlorophyll synthesis, through robust antioxidant defense, and a decrease in Cr accumulation. Therefore, findings suggest that Sultan-17 can be recommendation for cultivation in chromium-contaminated soils. Furthermore, identification of antioxidant responses and ionic regulation in Sultan-17 can serve as important markers which can help in developing tolerant cultivars.

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